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GC-MS Profiling of Secondary Metabolites in Hexane and Ethyl Acetate Crude Extracts of Red Sea Soft Coral *Xenia macrospiculata*

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Abstract

Soft corals are macro-invertebrates found in marine ecosystems. The genus *Xenia* belongs to the family *Xeniidae* containing ninety-eight species, rich sources of bioactive natural products including terpenoids, steroids, and fatty acids. The hexane and ethyl acetate solvent extracts of soft coral *Xenia macrospiculata* Gohar, 1940 (*X. macrospiculata*) were investigated for the novel chemical constituents using Gas Chromatography-Mass Spectroscopy (GC-MS). Qualitative analysis of soft coral crude extracts has shown various compounds with high and low molecular weights. In the hexane extracts twenty-five compounds were identified including sesquiterpene derivatives (34.61%), steroid derivatives (30.76%), cyclic diterpenes (7.69%), oxygenated terpenes (7.69%), fatty esters (7.69%), mercaptans (3.84%), alcohols (3.84%), and bicyclic monoterpenes (3.84%). In ethyl acetate extract twenty compounds were identified, the major compounds are fatty acid derivatives (28.57%), element type sesquiterpenes (23.80%), steroid derivatives (19.04%), thio compounds (9.52%), alkaloids (4.76%), diterpenes (4.76%), alcohols (4.76%), Fluro-hydrocarbons (4.76%). GC-MS profiling of hexane and ethyl acetate extracts of *X. macrospiculata* revealed the presence of several potential bioactive compounds. The elemene, azulene, himachalene and aromadendrane sesquiterpenoids are unique compounds reported first in the soft coral *X. macrospiculata*. The available literature supports that these compounds have potential antioxidant, Tumour Necrosis Factor (TNF) inhibition and cytotoxic activity. Some compounds are used as tasteful natural sweeteners and some of the fatty ester derivatives are potential flavouring agents in food industries.

Keywords: Ethyl Acetate Extract, GC-MS Analysis, Hexane Extracts, *Xenia macrospiculata*

1. Introduction

Soft corals are a diverse group of widely distributed marine organisms belonging to the sub-class Octacorallia. These soft corals are found in shallow to deep waters. Soft corals found in marine environments are recognized for being abundant reservoirs of biologically fascinating natural compounds, showcasing a diverse array of structural features[1]. The soft corals live in hostile and highly competitive ecosystems, so they use chemical defence systems to defend themselves from predators[2]. More than 5800 secondary metabolites are produced by soft corals[3]. It is estimated that the newly recognized and isolated compounds from soft corals account for more

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than 20% of the newly isolated compounds from marine organisms[4].

Marine soft corals have prolific bioactive compounds sequestered within their tissues. These bioactive compounds with diversified structures include, unusual fatty acids and their derivatives, terpenoids, steroids, quinones, alkaloids, glycosides and other chemical compounds[5]. Compelling evidence shows that these novel compounds serve predator deterrence, antifoulant, competitor exclusion and reproductive functions[6]. The novel bioactive compounds isolated from marine soft corals have shown cytotoxic, anti-inflammatory, antimicrobial, antioxidant and other pharmacological activities[7]. Numerous secondary metabolites derived from soft corals are acknowledged for displaying cytotoxic, anti-inflammatory, antimicrobial, antioxidant, and various other pharmacological activities

The Northern Red Sea is an oligotrophic water body characterized by an epipelagic zone. In the shallow reef flats, the average sea surface temperature is 36°C and salinity is 40.5% across all seasons[8]. The long-term temperature exposure above 30°C affects the coral bleaching, in response to these bleaching episodes, the zooxanthellae communities and soft corals exhibit varying levels of adaptation to increasing sea surface water temperatures[9]. The seasonal fluctuation of air, seawater temperature, an eminent diverse marine biota and unparalleled coral reef systems favour the production of distinguished primary and secondary metabolites by the marine organisms[10].

The Red Sea is an epicenter for benthic coral communities which habitats around 40% of the soft coral species. *Xenia* is one of the most abundant genera of soft corals distributed in dense patches[11]. The genus *Xenia* belongs to the family *Xeniidae* and presently includes 49 valid species[12]. The colonies of the family *Xeniidae* are mostly small, soft and slippery to the touch due to the secretion of a lot of mucus[13]. *X. macrospiculata* is one of the most abundant xeniids distributed on the reef slopes at 15–30 m depth along the Red Sea, Madagascar and Indo-Pacific regions[14]. The adult colonies reach up to 30–50 mm in height, branched arising from the common base, light brown to yellowish with regular pulsation in live condition[15].

The genus *Xenia* is known for its productivity of terpenoids and steroidal derivatives[16]. Groweis and Kashman characterized the bicyclic diterpenoids xenicin, xeniculin, xeniaphyllenol-C, epoxyxeniaphyllenol-A, xeniaphyllenol-B and l4,15-xeniaphyllandiol-4,5-epoxide from the soft coral *X. macrospiculata* collected from the gulf of Eilat, Northern Red Sea[17,18]. According to the available literature, a total of 199 terpenes including 180 diterpenes, 14 sesquiterpenes and 5 steroids were reported[19]. Among the diterpenes, xenic ane diterpenes are predominant[20]. Most of the natural products isolated and identified from the genus *Xenia* proved to exhibit a wide range of biological activity including cytotoxic activity, TNF inhibition, and antimicrobial and anti-inflammatory activities. Given this, the present study planned to identify phytochemical constituents present in hexane and ethyl acetate extract of Red Sea Soft Coral *X. macrospiculata* utilizing GC-MS.

2. Experimental

2.1 Sample Site and Collection

The soft coral *X. macrospiculata* (Gohar, 1940) was collected at 15-20 meters depth using SCUBA diving around Sheik Sied Island (Green Island) at Massawa coast of Red Sea (15° 35'19.47"N; 39° 29'7.83"E), Eritrea during June 2023 (Figure 1(a) and 1(b)). The samples

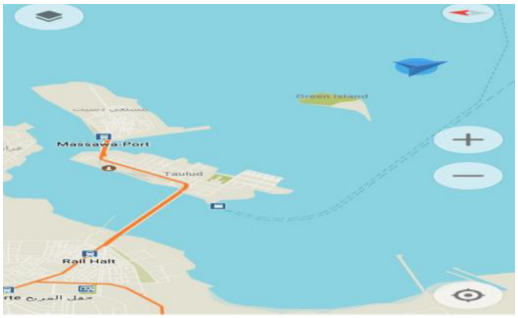


Figure 1(a). Sampling site.



Figure 1(b). Underwater photograph of *X. macrospiculata*.

were separated and cleaned with seawater and preserved in an ice box with ice cubes and a pinch of table salt. Later, samples were dried in the shade and a dry specimen approximate weight of 0.9kg was hermetically sealed and stored at -40°C for further extraction. A voucher specimen is on deposit at the Department of Chemistry, Acharya Nagarjuna University, Guntur, Andhra Pradesh, India.

2.2 Sample Extraction

100g of dry sample was subjected to sequential extraction using organic solvents with increasing polarity including hexane, ethyl acetate, methanol and water in Soxhlet apparatus. Crude extracts were evaporated and preserved in an airtight container at 4°C. The crude extracts of hexane and ethyl acetate were used in this experiment.

2.3 GC-MS Analysis

GC-MS analysis of hexane and ethyl acetate extracts of *X. macrospiculata* was conducted on Triple Quadrupole GCMS system (7000E model, Agilent, USA) with Agilent Mass-Hunter Workstation Software (version B.05.00) and flame ionizable detector. Extracts at 1 µL quantity were injected through an automatic liquid sampler (7693A, model, Agilent, USA) and maintained at 280°C and were resolved on DB-35 capillary (30m×250 µm×0.25 µm) column. Helium gas at 1.0mL/min flow was utilized as carrier gas and column temperature was initiated at 50°C for 1 minute, followed by an increase of 30°C/minute till 210°C for 1 minute, and ultimately elevated to 310°C with 5°C/minute raise rate, where it was maintained for 3 minutes. The total analysis was completed with a 30.33-minute run time.

The retention indices along with Wiley and NIST libraries (MS Interpreter Ver. 3.4.5) were employed for compound identification. The correlation of Wiley and NIST libraries data with instrumental results confirms compound details and were obtained results were tabulated.

3. Results and Discussion

GCMS profiling of hexane and ethyl acetate crude extracts of soft coral *X. macrospiculata* has shown unique phytochemical compounds with novel biological activity. The chromatograms of hexane and ethyl acetate extracts are given in Figures 2 and 3 respectively. Retention time, name of the identified compounds, molecular formula, molecular weight (g/mol) and peak area (%) of phytochemical constituents detected in hexane and ethyl acetate extracts of *X. macrospiculata* are given in Table 1 and 2 respectively. The structures of identified compounds are shown in Table 3.

In the hexane extract of the samples the following compounds were identified: *γ*-gurjunene, *β*-elemene, *γ*-himachalene, 7-tetracyclo [6.2.1.0(3.8)0(3.9)] undecanol, 4,4,11,11-tetramethyl-, alloaromadendrene oxide-(1),spathulenol,murolan-3,9(11)-diene-10-peroxy, 6-isopropenyl-4,8a-dimethyl- 1,2,3,5,6,7,8,8a-octahydro-naphthalen-2-ol, longifolenaldehyde, (-) spathulenol, bicyclo[4.4.0]dec-2- ene-4-ol, 2-methyl-9-(prop-1- en-3-ol-2-yl)-,1-heptatriacotanol,cholestan-3-ol,2-methylene-,

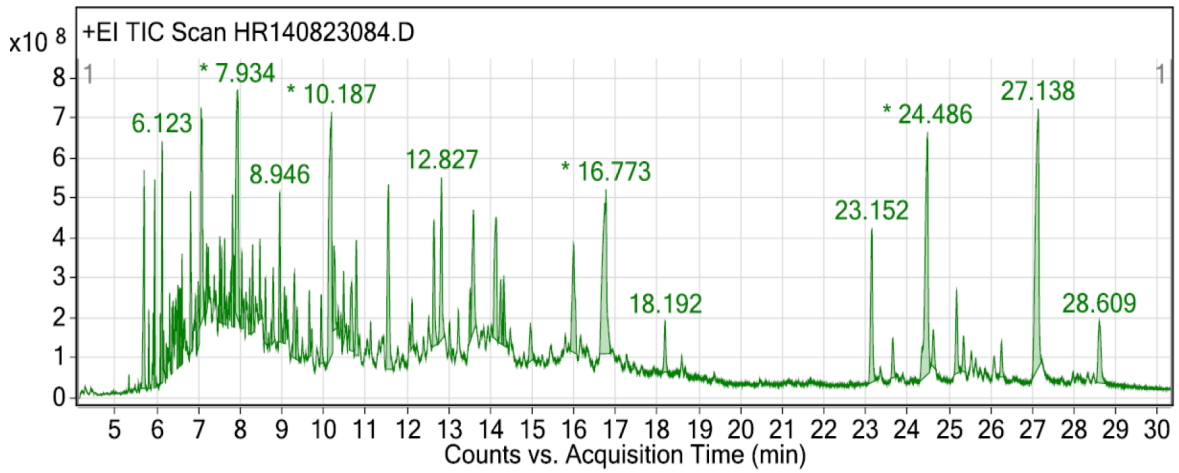


Figure 2. GC-MS chromatogram observed for hexane extract of *X. macrospiculata*.

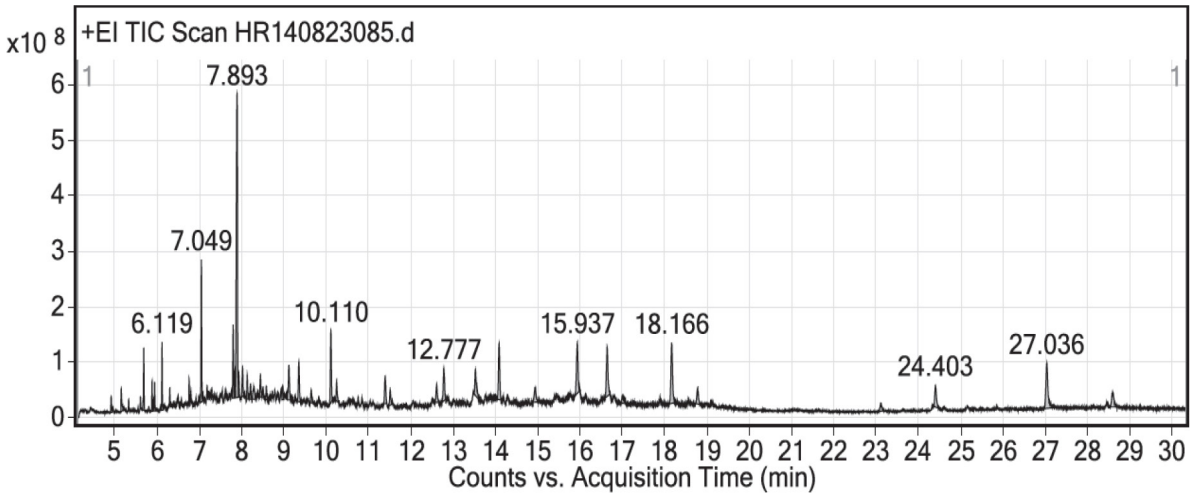


Figure 3. GC-MS chromatogram observed for ethyl acetate extract of *X. macrospiculata*.

Table 1. Constituents identified in hexane extract of *X. macrospiculata*

No	RT (min)	Compound name	Compound formula	Molecular weight (g/mol)	Area %	Nature of the compound
1	5.69	γ-Gurjunene	$C_{15}H_{24}$	204.35	29.88	Sesquiterpenoid
2	5.948	β-Elemene,	$C_{15}H_{24}$	204.35	16.79	Sesquiterpenoid
3	6.359	γ-Himachalene	$C_{15}H_{24}$	204.35	5.06	Sesquiterpenoid
4	6.502	7-Tetracyclo [6.2.1.0(3.8)0(3.9)] undecanol,4,4,11,11-tetramethyl-	$C_{15}H_{24}O$	220.36	7.1	Bicyclic monoterpenoid
5	6.565	Alloaromadendreneoxide-(1)	$C_{15}H_{24}O$	220.36	8.16	Sesquiterpenoid
6	6.806	Spathulenol	$C_{15}H_{24}O$	220.36	18.96	Sesquiterpenoid
	7.508				6.93	
	7.815				15.5	
7	7.192	Murolan-3,9(11)-diene-10-peroxy	$C_{15}H_{24}O_2$	236.35	6.89	Oxygenated terpene
8	7.551	6-Isopropenyl-4,8a-dimethyl-1,2,3,5,6,7,8,8a-octahydro-naphthalen-2-ol	$C_{15}H_{24}O$	220.36	5.99	Sesquiterpenoid
9	7.623	Longifolenaldehyde	$C_{15}H_{24}O$	220.36	7.16	Sesquiterpenoid
10	8.036	Bicyclo [4.4.0]dec-2-ene-4-ol,2-methyl-9-(prop-1-en-3-ol-2-yl)-	$C_{15}H_{24}O_2$	236.35	7.81	Sesquiterpenoid
11	8.227	1-Heptatriacotanol	$C_{37}H_{76}O$	537.0	5.1	Alcohol
	14.139				33.54	
12	8.471	Cholestan-3-ol,2-methylene-, (3-beta.,5.alpha.)-	$C_{28}H_{48}O$	400.7	7.16	Steroid
13	8.946	Thunbergol	$C_{20}H_{34}O$	290.5	18.22	Cyclic diterpene
	10.187				76.48	
14	9.3	Geranyllinalool	$C_{20}H_{34}O$	290.5	14.31	Diterpene
15	9.939	α-Cembrenediol	$C_{20}H_{34}O2$	306.5	9.26	Cyclic diterpene
16	13.595	10,12-Tricosadiynoicacid, methyl ester	$C_{24}H_{40}O_2$	360.6	29.29	Fatty esters
17	16.773	7-Methyl-Z-tetradecen-1-olacetate	$C_{17}H_{32}O_2$	254.41	76.92	Fatty esters

Table 1. Continued...

No	RT (min)	Compound name	Compound formula	Molecular weight (g/mol)	Area %	Nature of the compound
18	18.192	tert-Hexadecanethiol	$C_{16}H_{34}S$	258.5	9.42	Mercaptans
19	23.152	26-Nor-5-cholesten-3. beta. -ol-25-one	$C_{26}H_{42}O_2$	386.6	35.27	Steroid
20	24.627	Stigmasterol	$C_{29}H_{48}O$	412.69	7.5	Steroid
21	23.655	Ergost-5,8(14)-dien-3-ol	$C_{28}H_{46}O$	398.7	7.45	Steroid
22	24.486	Campesterol	$C_{28}H_{48}O$	400.7	82.31	Steroid
23	26.258	Estra-1,3,5(10)-trien-17. beta. -ol	$C_{18}H_{24}O$	256.38	6.54	Steroid
24	27.138	Gorgost-5-en-3-ol, (3. beta.)-	$C_{30}H_{50}O$	426.7	100	Steroid
25	28.609	Cholest-22-ene-21-ol,3,5-dehydro-6-methoxy pivalate	$C_{33}H_{54}O_3$	498.8	20.6	Steroid

Table 2. Constituents identified in ethyl acetate extract of *X. macrospiculata*

No	RT (min)	Compound name	Compound formula	Molecular weight (g/mol)	Area %	Nature of the compound
1	4.916	Dodecane,1-fluoro-	$C_{12}H_{25}F$	188.33	3.02	Fluro-hydrocarbon
2	5.155	Methyl 3-hydroxy-decanoate	$C_{11}H_{22}O_3$	202.29	4.84	Fatty acid
3	5.686	δ-Elemene	$C_{15}H_{24}$	204.35	10.3	Sesquiterpenoid
4	5.883	Trans-2-methyl-4-n-pentylthiane, S, S-dioxide	$C_{11}H_{22}O_2S$	218.36	3.87	Thiopyran
5	5.943	β-Elemene	$C_{15}H_{24}$	204.35	4.03	Sesquiterpenoid
6	6.119	γ-Elemene	$C_{15}H_{24}$	204.35	9.62	Sesquiterpenoid
7	6.757 10.244	cis-1-Chloro-9-octadecene	$C_{18}H_{35}Cl$	286.92	3.41 5.16	Fatty acid
8	7.049	Spathulenol	$C_{15}H_{24}O$	220.35	24.36	Sesquiterpenoid
9	7.841	Cholestan-3-ol,2-methylene-, (3.beta.,5.alpha.)-	$C_{28}H_{48}O$	400.7	5.43	Steroid
10	7.934 8.132	1-Heptatriacotanol	$C_{37}H_{76}O$	537.0	5.24 5.02	Alcohol
11	8.024	Bicyclo [4.4.0] dec-2-ene-4-ol,2-methyl-9(prop-1-en-3-ol-2-yl)-	$C_{15}H_{24}O_2$	236.35	6.75	Sesquiterpenoid
12	9.353	Hexadecanoic acid, ethyl ester	$C_{18}H_{36}O_2$	284.48	11.3	Fatty acid
13	10.11	Geranyllinalool	$C_{20}H_{34}O$	290.5	19.43	Diterpene
14	11.391	Methyl 2,8-dimethyltridecanoate	$C_{16}H_{32}O_2$	256.42	8.91	Fatty acid
15	13.526	10,12-Tricosadiynoic acid, methyl ester	$C_{24}H_{40}O_2$	360.6	10.83	Fatty acid
16	14.085	tert-Hexadecanethiol	$C_{16}H_{34}S$	258.5	20	Mercaptan
17	23.119	Cholesterol	$C_{27}H_{46}O$	386.7	3.81	Steroid
18	24.403	Campesterol	$C_{28}H_{48}O$	400.7	9.64	Steroid
19	27.036	Gorgost-5-en-3-ol, (3. beta.)-	$C_{30}H_{50}O$	426.7	25.87	Steroid
20	28.594	2,4,6-Cycloheptatrien-1-one,3,5-bis trimethylsilyl-	$C_{13}H_{22}OSi_2$	250.48	9.36	Alkaloid

(3.beta., 5.alpha.)-, thunbergol, geranyllinalool, α-cembrenediol, 1,5,9-trimethyl-12-(1- methylethyl)-, 10,12-tricosadiynoic acid, methyl ester, 7-methyl-z-tetradecen-1-ol acetate, tert-hexadecanethiol, 26-nor-5-cholesten-3.beta.-ol- 25-one, stigmasterol, ergost-5,8(14)-dien-3-ol, campesterol, estra-1,3,5(10)-trien-17.beta.- ol, gorgost-5-en-3-ol, (3.beta.)-, cholest-22-ene-21- ol, 3,5-dehydro-6-methoxy-, pivalate.

In the ethyl acetate solvent extract of the *X. macrospiculata* sample total of forty compounds were

Table 3. Structures of compounds identified from hexane and ethyl acetate extracts of *X. macrospiculata*

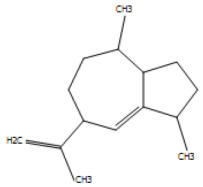
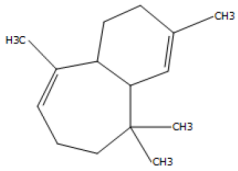
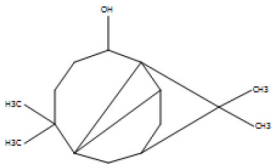
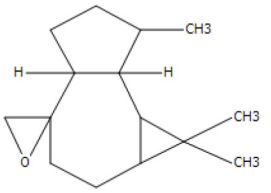
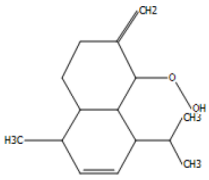
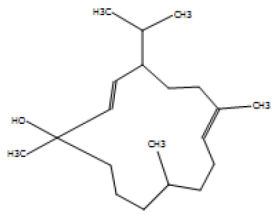
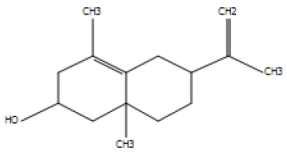
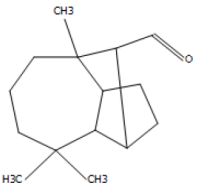
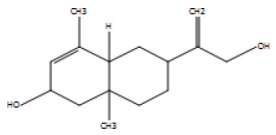
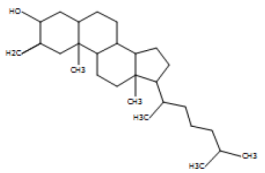
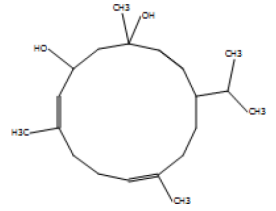
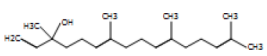
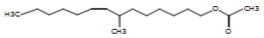
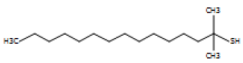
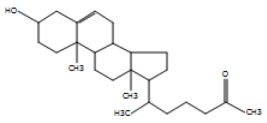
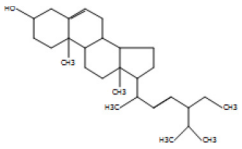
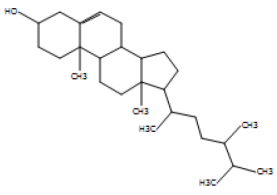
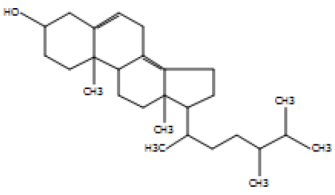
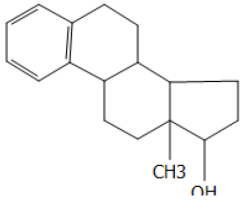
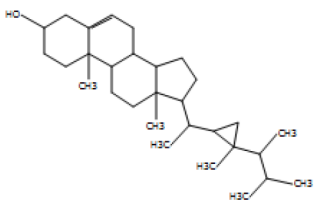
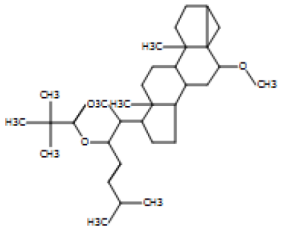
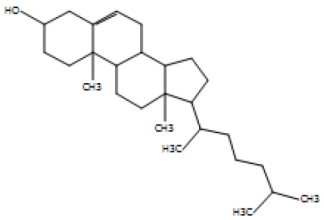
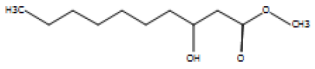
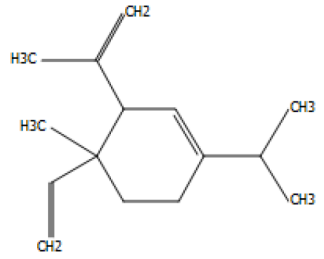
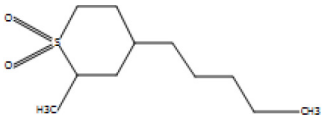
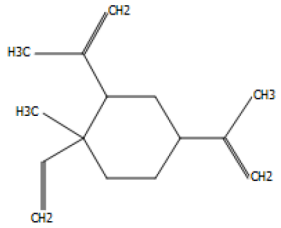
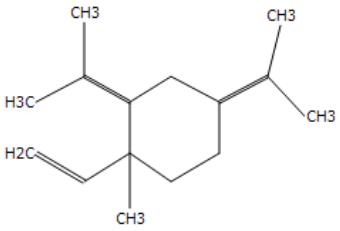
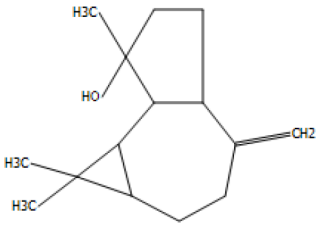
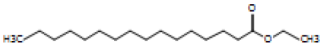
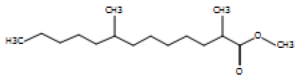
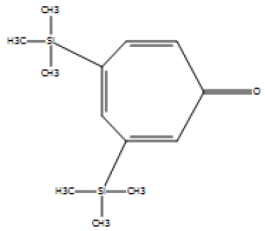
		
γ-Gurjunene	γ-Himachalene	Tetracyclo [6.2.1.0(3.8)0(3.9)] undecan ol,4,4,11,11-tetramethyl-
		
Alloaromadendreneoxide-(1)	Murolan-3,9(11)-diene-10-peroxy	Thunbergol
		
6-Isopropenyl-4,8a-dimethyl-1,2,3,5,6,7,8,8a-octahydro-naphthalen-2-ol	Longifolenaldehyde	6- Bicyclo [4.4.0] dec-2-ene-4-ol,2-methyl-9-(prop-1-en-3-ol-2-yl)-
		
1-Heptatriacotanol	Cholestan-3-ol, 2- methylene-, (3-beta, 5-alpha)	α-Cembrenediol
		
Geranyllinalool	10,12-Tricosadiynoicacid, methyl ester	7-Methyl-Z-tetradecen-1-olacetate
		
tert-Hexadecanethiol	26-Nor-5-cholesten-3 beta -ol-25-one	Stigmasterol
		
Campesterol	Ergost-5,8(14)-dien-3-ol	Estra-1,3,5(10)-trien-17 beta-ol

Table 3. Continued...

		
Gorgost-5-en-3-ol, (3.beta.)-	Cholest-22-ene-21-ol,3,5-dehydro-6-methoxy pivalate	Cholesterol
		
Dodecane,1-fluoro-	Methyl 3-hydroxy-decanoate	δ-Elemene
		
trans-2-methyl-4-npentylthiane, S, S dioxide	β-Elemene	γ-Elemene
		
cis-1-Chloro-9-octadecene	Spathulenol	Hexadecanoic acid, ethyl ester
		
Methyl 2,8-dimethyltridecanoate	2,4,6-Cycloheptatrien-1-one, 3,5-bis trimethylsilyl-	

detected from which below mentioned twenty compounds were identified: dodecane, 1-fluoro-, methyl 3-hydroxy-decanoate, δ-elemene, trans-2-methyl-4-n- pentylthiane, S, S-dioxide, β-elemene, γ-elemene, cis-1-hloro-9-octadecene, spathulenol, cholestan-3-ol, 2-methylene-, (3.beta.,5. alpha.)-, 1-heptatriacotanol, bicyclo[4.4.0]dec-2-ene-4-ol, 2-methyl-9-(prop-1-en-3-ol-2- yl)-, hexadecanoic acid, ethyl ester, geranyllinalool, methyl 2,8-dimethyltridecanoate, 10,12-tricosadiynoic acid, methyl ester, tert-hexadecanethiol, cholesterol, campesterol, gorgost-5-en-3-ol, (3.beta.)-, 2,4,6-cycloheptatrien-1-one, 3,5-bis-trimethylsilyl.

Gorgost-5-en-3-ol, (3. beta.), an ergosterol derivative, is present in the highest percent peak area in both hexane and ethyl acetate extracts of *X. macrospiculata*. These phytochemical constituents extracted from various samples have demonstrated potential in several areas, including anti-proliferative activity[21,22], phytotoxic activity[23], allelopathic effect on weed plants[24], anti-inflammatory activity[25], anti-hypercholesterolemic activity[26], immunomodulatory effects[27].

Thunbergol is a macrocyclic diterpene alcohol having a membered ring that was isolated from the oleoresin of Douglas fir (*Pseudotsuga menziesii*) which is also found in the essential oils of many terrestrial plants[28]. This compound has shown notable antimicrobial activity[29]. The essential oils containing Thunbergol have shown potential antimicrobial and hydroxy radical scavenger activity[30]. Spathulenol is a cycloaromadendrene sesquiterpenoid compound identified in both the extracts of the soft coral sample is a volatile metabolite found in several plants that were reported to have anaesthetic and vascodialator activity. Alloaromadendrene oxide-(1) is one of the potential bioactive compounds with analgesic, anti-inflammatory and antibiotic properties[31].

The phytosterols were found to be one of the dominant compounds in both the extracts of the soft coral sample, which have been proven to possess anti-diabetic and lipid-lowering effects[32]. Molecular docking studies confirmed that campsterol and stigmasterol interact with the amino acids at the binding sites of α-amylase and glycosidase inhibiting the enzyme activity. Similarly, elemenes are sesquiterpenoid compounds which have been shown to have antidiabetic activity inhibiting α- amylase and α- glycosidase activity[33]. Particularly, β-elemene is one of the novel compounds having potential antitumor ability by arresting the cell cycle, cell proliferation inhibition and inducing cell apoptosis[34]. The literature also confirmed that administration of stigmasterol improved insulin resistance, reduced the fasting glucose, induced β–cell regeneration and improved the glucose transporter type 4 (GLUT4) translocation[35].

4. Conclusion

Soft corals, belonging to the sub-class Octacorallia, are diverse marine organisms widely distributed in shallow to deep waters, renowned for their rich repository of biologically active compounds. These compounds play crucial roles in chemical defence mechanisms against predators and competitive interactions within their ecosystems. Hence, this study aims to identify the phytochemical constituents present in hexane and ethyl acetate extracts of the Red Sea Soft Coral *X. macrospiculata* utilizing GC-MS, contributing to the understanding of its chemical defence strategies and potential pharmacological applications. The hexane and ethyl acetate solvent extracts of the soft coral *X. macrospiculata* were found to be good sources of novel compounds. The extracts from *X. macrospiculata*, a soft coral species abundant in the Red Sea, revealed a rich array of compounds with potential pharmacological significance. In the hexane extract, compounds such as gamma-gurjunene, beta-elemene, and stigmasterol were identified among others, while the ethyl acetate extract contained delta-elemene, hexadecanoic acid ethyl ester, and campesterol. Notably, Gorgost-5-en-3-ol, (3. beta.), an ergosterol derivative, emerged as a prominent constituent in both extracts, suggesting its importance in the chemical defence mechanisms of *X. macrospiculata*. Further, Thunbergol, macrocyclic diterpene alcohol known for its antimicrobial activity, and spathulenol, recognized for its anaesthetic and vasodilator properties, were found in both extracts, underscoring the pharmacological diversity within these soft corals. Additionally, alloaromadendrene oxide-(1), identified in the extracts, holds potential as a bioactive compound with analgesic, anti-inflammatory, and antibiotic properties, further highlighting the therapeutic potential of soft coral-derived compounds. In addition, further research is required to investigate its bioactivity and toxicity profile.

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