IJPSR (2020), Volume 11, Issue 8 (Research Article)

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 08 September 2019; received in revised form, 23 February 2020; accepted, 19 April 2020; published 01 August 2020

SCREENING OF ANTI-BACTERIAL ACTIVITY OF BROWN SEAWEEDS FROM SOUTH EAST COAST OF INDIA

K. Kanmani Vennila [*1], P. S. Chitra [2], K. Hilda [2], S. Janarthanan [2] and P. Martin [3]

Department of Zoology [1], Quaid-e-millath College, Chennai - 600002, Tamil Nadu, India.
Department of Zoology [2], University of Madras, Guindy Campus, Chennai - 600025, Tamil Nadu, India.
Department of Zoology [3], Government Arts College for Men, Nandhnam, Chennai - 600035, Tamil Nadu, India.

Keywords:

Gram-positive, Gram-negative bacteria, Brown seaweeds, Solvents, GC-MS compounds

Correspondence to Author:
K. Kanmani Vennila

Assistant Professor,
Department of Zoology,
Quaid-e-millath College, Chennai - 600002, Tamil Nadu, India.

E-mail: kanmanivennila68@gmail.com

ABSTRACT: Three different species of seaweeds collected from south east coast of India such as *Sargassum plagiophyllum*, *Padina gymnospora* and *Turbinaria conoides* for evaluating anti-bacterial activity against selected gram-positive and gram-negative bacteria. Extracts prepared using solvents namely hexane, ethyl acetate, methanol, and water against human pathogenic strains of *Proteus vulgaris*, *Staphylococcus aureus*, *Klebsiella pneumonia*, *Enterobacter aerogenes*, *Salmonella typhimurium*, *Yersinia pestis*, *Shigella fiexneri*, *Staphylococcus epidermidis* and *Bacillus subtilis*. Gram-negative bacteria showed a lesser zone of inhibition than gram-positive bacteria with various extracts tested. All the three solvent extracts of *Turbinaria conoides* showed, zone of inhibition for the gram-positive bacteria, *Staphylococcus epidermidis* over the standard control, with a significant zone in hexane extract. Analysis of GC-MS of *Turbinaria conoides* hexane extract revealed 14 major and numerous minor phytochemical compounds that have to be fractionated and explored for specific anti-bacterial activity in order to isolate a potential anti-bacterial compound.

INTRODUCTION: Marine seaweeds produce an assorted collection of compounds that helps as a chemical defense system that facilitating their survival in extremely viable environments. Some of them used as fertilizers, medicines, and dietary chemicals [1]. Chemicals such as carbohydrates and proteins are vital for maintaining life processes, while others like alkaloids, phenolic and steroids are secondary metabolites, which have toxicological, pharmacological, and ecological significance [2].

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.11(8).3993-09

This article can be accessed online on www.ijpsr.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.11(8).3993-09

They considered as a source of bioactive compounds, since they produce a great variety of secondary metabolites with broad spectrum of biological activities, which possess pharmaceuticals, industrial, agricultural and bio-technological applications [3, 4]. Compounds with anti-oxidant, antiviral, antifungal, and antimicrobial activities have been isolated from various groups of brown, red, and green algae [5, 6, 7].

Epidemiological evidence suggests regular seaweed consumption may protect against a range of diseases of novelty. Extracts of marine algae are likely to exhibit potent anti-bacterial activity, which used as a potent anti-microbial agent [8]. More precisely, Southeast coast of India has an enormous amount of Chlorophyceae, Rhodophyceae, and Phaeophyceae seaweeds, which has anti-bacterial property [9].

Understanding the commercial value and therapeutic usage of these compound would create a solution to various clinical and public health problems caused due to antibiotic resistance and multi-resistant bacteria, which are difficult and sometimes impossible to treat [10]. Hence, the current study aimed to investigate the antimicrobial activities of brown marine macroalgae (*Turbinaria conoides*, *Padina gymnospora*, and *Sargassum plagiophyllum*) collected from coastal waters of Gulf of Mannar Coast of Indian Subcontinent.

MATERIALS AND METHODS:

Collection of Seaweeds: Seaweeds were collected by handpicking from Gulf of Mannar, South Indian coastal region of India which lies between 8° 47' to 9° 15' N latitude and 78° 12' to 79° 14' E longitude. The samples collected in the month of November 2017 to February 2018. They were washed with marine water thoroughly to remove epiphytes and debris and then with running water and rinsed with sterile water and was stored at room temperature until analysis. The collected seaweeds were identified in the Center for Marine Fisheries Research Institute, Mandapam, Tamil Nadu, and India. The identified species of seaweeds such as *Turbinaria conoides*, *Padina gymnospora* and *Sargassum plagiophyllum* screened for the evaluation of their antibacterial efficacy.

Selective Micro-organisms for Screening: The MTCC strains of *Proteus vulgaris* 1771, *Staphylococcus aureus* 96, *Klebsiella pneumonia* 109, *Enterobacter aerogenes* 111 *and Salmonella typhimurium* 1251, *Yersinia pestis* 840, *Shigella fiexneri* 1457, *Staphylococcus epidermidis* 3615 and *Bacillus subtilis* 441 were obtained from CSIR Institute of Microbial Technology, Chandigarh.

Preparation of Crude Seaweed Extract: The seaweeds were shade dried for 5-15 days, and the dried pieces were ground into powder by using electrical grinder. 100 g of powdered seaweeds were mixed with 300 ml of different solvents, *i.e.,* hexane (polarity index -0.1), ethyl acetate (polarity index-4.4), methanol (polarity index -10.2) and placed on rotary shaker at 100 rpm. The extracts then filtered using 11-µm pore size of Whatman no.1 filter paper. Then the extracts were concentrated using a rotary evaporator (Buchi-R420) at 40 °C under reduced pressure. Finally, the crude extracts weighed and stored at -20 °C until use. The extraction of aqueous fraction was by lyophilization from the dried powder.

Determination of Antimicrobial Activity: The antimicrobial activity was determined by agar well diffusion method, according to National Committee for Clinical Laboratory Standards (NCCLS). Briefly, Mueller-Hinton agar (Hi-Media, India) plates swabbed with inoculums containing 10^8 CFU /ml of each bacterial culture and inoculated with standardized inoculums of the test microorganism with sterile effusion. Wells of 10 mm in diameter punched into the seeded MH agar plates.

Different concentrations (150 mg/ml and 200 mg/ml) of crude extracts were prepared using 2% dimethyl sulphoxide (DMSO) for the analysis of the antibacterial activity against the bacterial pathogens 100 µl of each extract, which has a final concentration of 3 mg / 20 µl and 4 mg / 20 µl was loaded along with DMSO into the well for standard diffusion. Streptomycin disc (Hi-Media, India) acts as a positive control, and the plates were incubated in triplicates at 37 °C for 24 h. The diameter of the inhibition zone (mm) measured, and the activity index was calculated. DMSO acts as a negative control for all the experiments.

Determination of Minimum Inhibitory Concentration: MIC performed was by a serial dilution technique using 96-well microliter plates [11]. The extracts of the test samples were diluted with DMSO in different concentrations *viz.*, 20, 50, 100, 150, 200 mg/ml. and were allow diffusing thoroughly. Absorbance reading at 600 nm was determined after incubation for 18 h at 37 °C using a UV-1800 spectrophotometer.

RESULTS: The antimicrobial activities of the three species of seaweeds were calculated according to their zone of inhibition against various pathogens.

The activity of crude extract of *Sargassum plagiophyllum* against various microbes revealed that a zone of inhibition in ethyl acetate solvent extract against *Staphylococcus aureus, Staphylococcus epidermidis, Klebsiella pneumonia*, *Proteus vulgaris*, *Shigella flexneri*, and *Bacillus subtilis*.

Whereas, *Staphylococcus aureus* and *Proteus vulgaris* showed a zone of inhibition against hexane extraction in addition to ethyl acetate extract, on the contrary, *Enterobacter aerogenes* showed activity only against hexane extract of *Sargassum plagiophyllum.* The crude extract of *Sargassum plagiophyllum* was not effective against *Salmonella typhimurium,* and *Yersinia pestis* of all the extracts tested ethyl acetate extract of *Sargassum plagiophyllum* showed higher zone of inhibition activity only against *Staphylococcus epidermidis, Proteus vulgaris,* and *Bacillus subtilis* when compared to control in **Fig. 1** and **Table 1.**

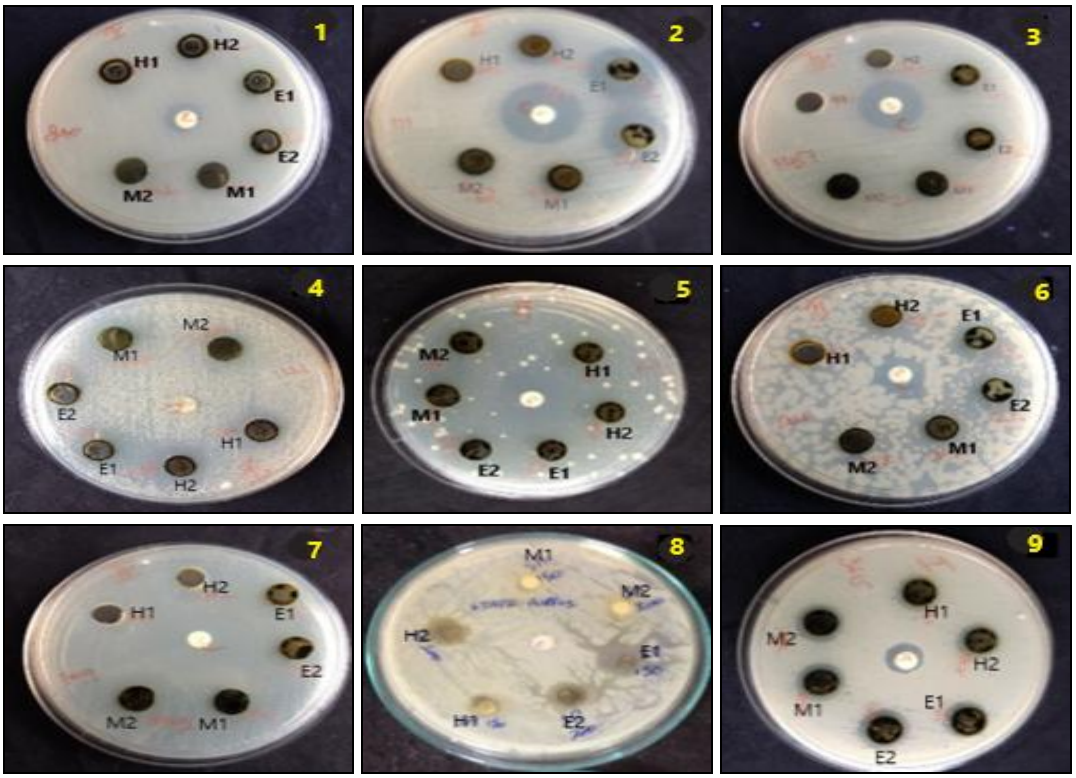


FIG. 1: PATHOGENEIC ACTIVITY OF *SARGASSUM PLAGIOPHYLLUM* 1. *Yersinia pestis* 840; 2. *Enterobacter aerogenes* 111; 3. *Shigella flexneri* 1457; 4. *Proteus vulgaris* 1771; 5. *Salmonella typhimurium* 1251; 6. *Bacillus subtilis* 441; 7. *Klebsiella pneumonia* 109; 8. *Staphylococcus aureus* 96; 9. *Staphylococcus epidermidis* 3615

TABLE 1: PATHOGENEIC ACTIVITY OF *SARGASSUM PLAGIOPHYLLUM*

Crude extract	Pathogens	H1 150 mg/ml	H2 200 mg/ml	E 150 mg/ml	E2 200 mg/ml	M1 150 mg/ml	M2 200 mg/ml	Aqueous 150 200 mg/ml	Control 10 mg
Sargassum plagiophyllum	*Salmonella typhimurium* 1251	-	-	-	-	-	-	-	12
	Staphylococcus aureus 96	15 ± 0.25	20 ± 0.25	11 ± 0.24	16 ± 0.27	-	-	-	25
	Staphylococcus epidermidis 3615	-	-	12 ± 0.26	15 ± 0.27	-	-	-	13
	Enterobacter aerogenes 111	10 ± 0.25	11 ± 0.27	-	-	-	-	-	13
	Yersinia pestis 840	-	-	-	-	-	-	-	10
	Klebsiella pnuemoniae 109	-	-	11 ± 0.25	14 ± 0.27	-	-	-	20
	Proteus vulgaris 1771	-	11 ± 0.26	13 ± 0.25	17 ± 0.24	-	-	-	15
	Shigellafiexneri 1457	-	-	11 ± 0.26	15 ± 0.24	-	-	-	20
	Bacillus subtilis 441	-	-	11 ± 0.27	16 ± 0.24	-	-	-	14

H1, H2 - Hexane, E1, E2 - Ethyl acetate, M1, M2 - Methanol, 1 (150 mg/ml), 2 (200 mg/ml)

Ethyl acetate extract of *Padina gymnospora* showed activity against *Staphylococcus epidermidis*, *Proteus vulgaris*, *Bacillus subtilis*, *Shigella flexneri*, *Enterobactera erogenes*, *Salmonella typhimurium* and *Klebsiella pneumonia*, whereas it fails to show the activity in all the other solvents tested of these results, the higher zone of inhibition was against *Staphylococcus epidermidis*, *Proteus vulgaris*, and *Bacillus subtilis* when compared to control, whereas *Yersinia pestis* does not show any zone of inhibition against any of the solvents tested in **Fig. 2** and **Table 2.**

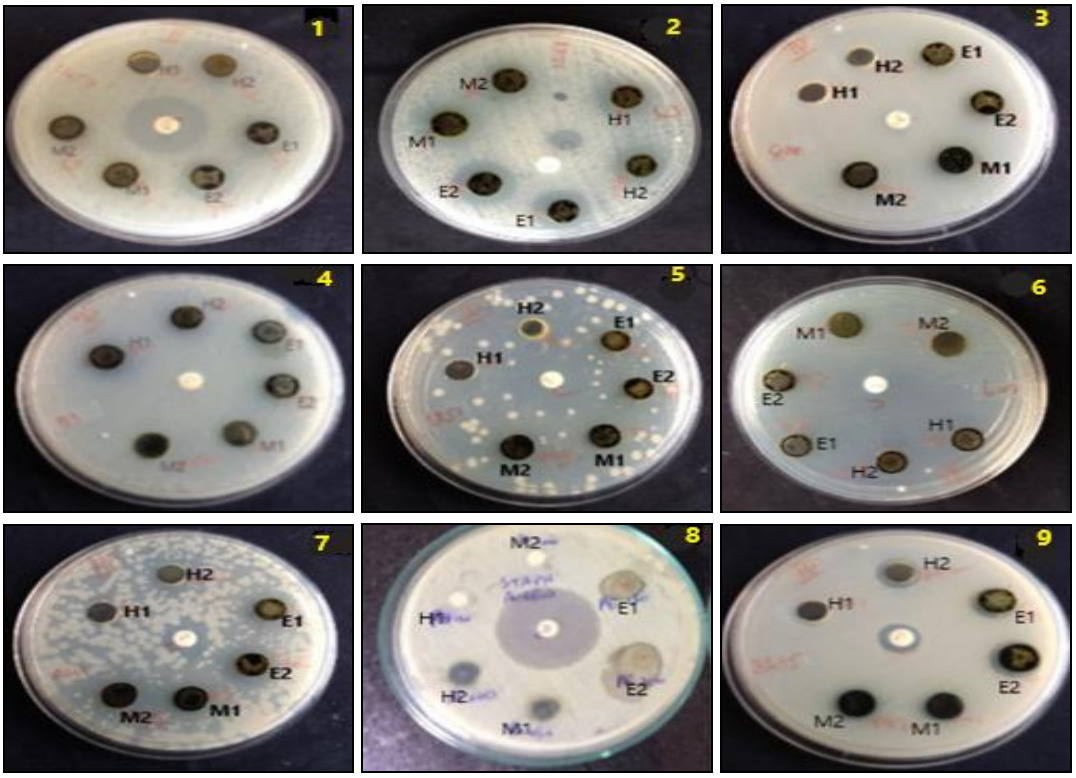


FIG. 2: PATHOGENEIC ACTIVITY OF *PADINA GYMNOSPORA* 1. *Yersinia pestis* 840; 2. *Enterobacter aerogenes* 111; 3. *Shigella flexneri* 1457; 4. *Proteus vulgaris* 1771; 5. *Salmonella typhimurium* 1251; 6. *Bacillus subtilis* 441; 7. *Klebsiella pneumonia* 109; 8. *Staphylococcus aureus* 96; 9. *Staphylococcus epidermidis* 3615

TABLE 2: PATHOGENEIC ACTIVITY OF *PADINA GYMNOSPORA*

Crude extract	Pathogens	H1 150 mg/ml	H2 200 mg/ml	E1 150 mg/ml	E2 200 mg/ml	M1 150 mg/ml	M2 200 mg/ml	Aqueous 150 200 mg/ml	Control 10 mg
Padina gymnospora	*Staphylococcus aureus* 96	11 ± 25	13 ± 24	-	-	-	-	-	14
	Staphylococcus epidermidis 3615		-	11 ± 0.27	14 ± 0.25	-	-	-	13
	Yersinia pestis 840	-	-	-	-	-	-	-	11
	Proteus vulgaris 1771	-	-	13 ± 0.27	17 ± 0.24	-	-	-	15
	Bacillus subtilis 441	-	-	11 ± 0.26	16 ± 0.27	-	-	-	15
	Shigella fiexneri 1457	-	-	11 ± 0.25	16 ± 0.28	-	-	-	20
	Enterobacter aerogenes 111	-	-	12 ± 0.27	16 ± 0.23	-	-	-	16
	Salmonella typhimurium 1251	-	-	11 ± 0.27	15 ± 0.26	-	-	-	16
	Klebsiella pnuemoniae 109	-	-	9 ± 0.26	13 ± 0.26	-	-	-	20

H1, H2 - Hexane, E1, E2 - Ethyl acetate, M1, M2 - Methanol, 1 (150 mg/ml), 2 (200 mg/ml)

Pathogenic activity of crude extract of *Turbinaria conoides* showed very significant against *Staphylococcuse pidermidis* for hexane, ethyl acetate and methanol extracts, of which 200 mg/ml of hexane, ethyl acetate and methanol extract showed very high zone of inhibition of 17 ± 0.24, 15 ± 0.27 and 13 ± 0.28 respectively when compared to control. A maximum zone of inhibition of about 17 ± 0.24 mm recorded for hexane solvent extract when compared to the control (12 mm).

Gram-negative bacteria like *Enterobactera erogenes, Salmonella typhimurium*, *Yersinia pestis, Proteus vulgaris* and *Shigella flexneri* failed to show any zone of inhibition against various solvents tested, but *Klebsiella pneumoniae* shows the zone of inhibition against hexane extract.

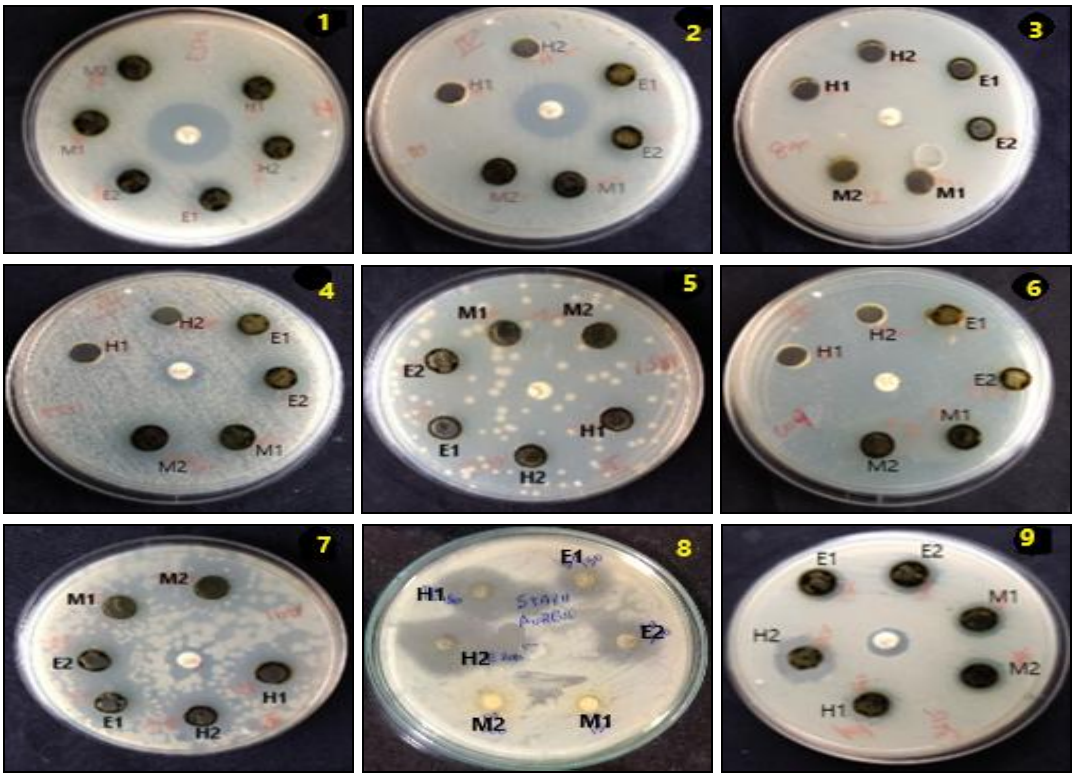


FIG. 3: PATHOGENEIC ACTIVITY OF *TURBINARIA CONOIDES* 1. *Yersinia pestis* 840; 2. *Enterobacter aerogenes* 111; 3. *Shigella flexneri* 1457; 4. *Proteus vulgaris* 1771; 5. *Salmonella typhimurium* 1251; 6. *Bacillus subtilis* 441; 7. *Klebsiella pneumonia* 109; 8. *Staphylococcus aureus* 96; 9. *Staphylococcus epidermidis* 3615

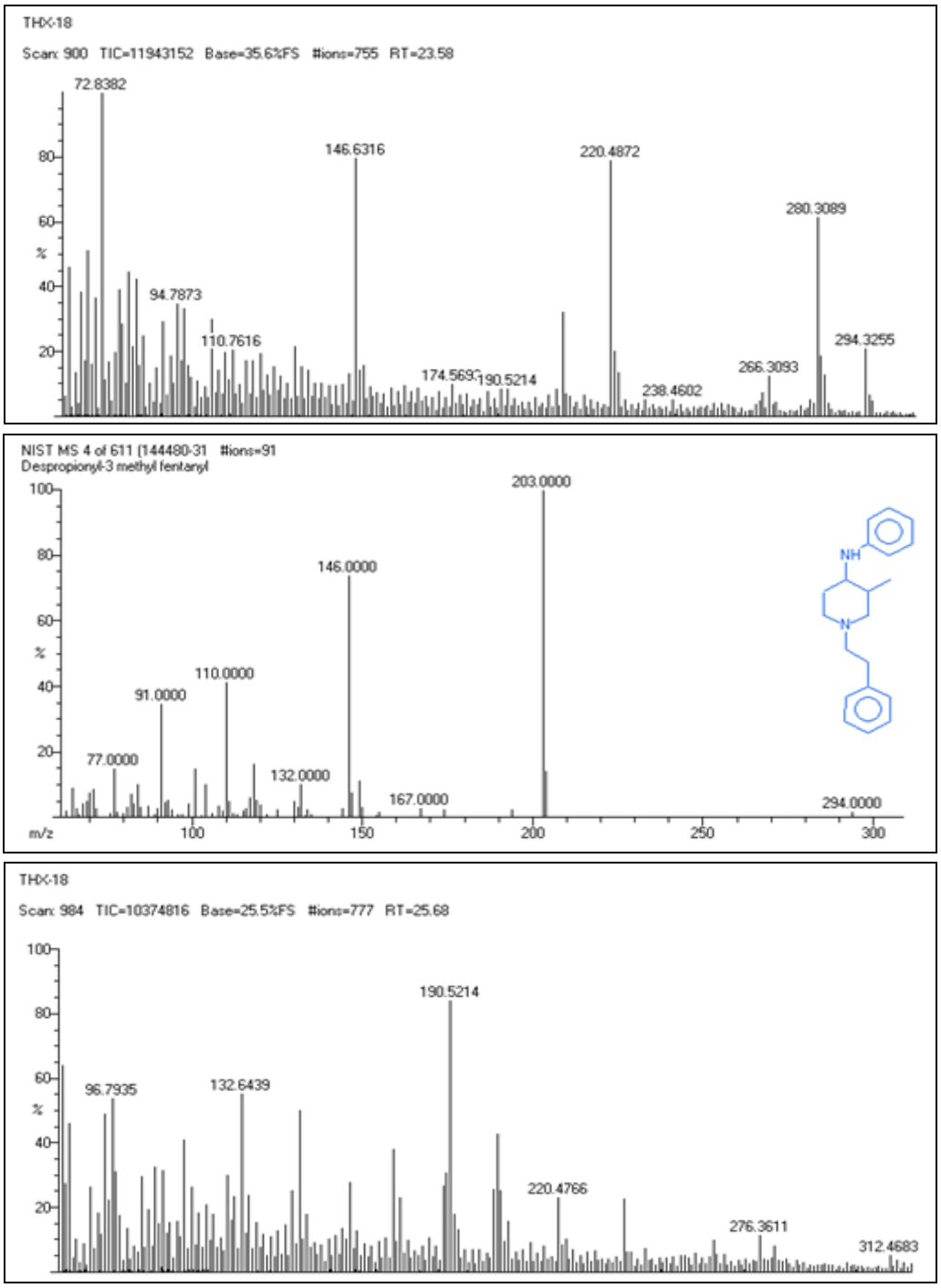
TABLE 3: PATHOGENEIC ACTIVITY OF *TURBINARIA CONOIDES*

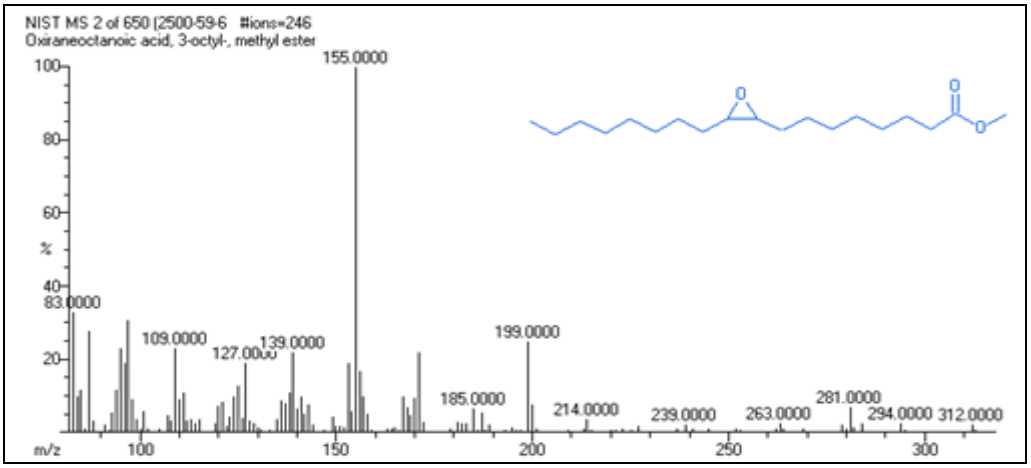
Crude extract	Pathogens	H1 150 mg/ml	H2 200 mg/ml	E1 150 mg/ml	E2 200 mg/ml	M1 150 mg/ml	M2 200 mg/ml	Aqueous 150 200 mg/ml	Control 10 mg
Turbinaria conoides	*Enterobacter aerogenes* 111	-	-	-	-	-	-	-	14
	Klebsiella pnuemoniae 109	11 ± 0.28	15 ± 0.25	-	-	-	-	-	19
	Staphylococcus aureus 96	22 ± 0.26	29 ± 0.25	18 ± 0.23	24 ± 0.26	-	-	-	25
	Salmonella typhimurium 1251	-	-	-	-	-	-	-	16
	Staphylococcus epidermidis 3615	15 ± 0.26	17 ± 0.24	14 ± 0.26	15 ± 0.27	9 ± 0.25	13 ± 0.28	-	12
	Yersinia pestis 840	-	-	-	-	-	-	-	11
	Proteus vulgaris 1771	-	-	-	-	-	-	-	15
	Shigella fiexneri 1457	-	-	-	-	-	-	-	19
	Bacillus subtilis 441	-	-	11 ± 0.24	16 ± 0.25	-	-	-	14

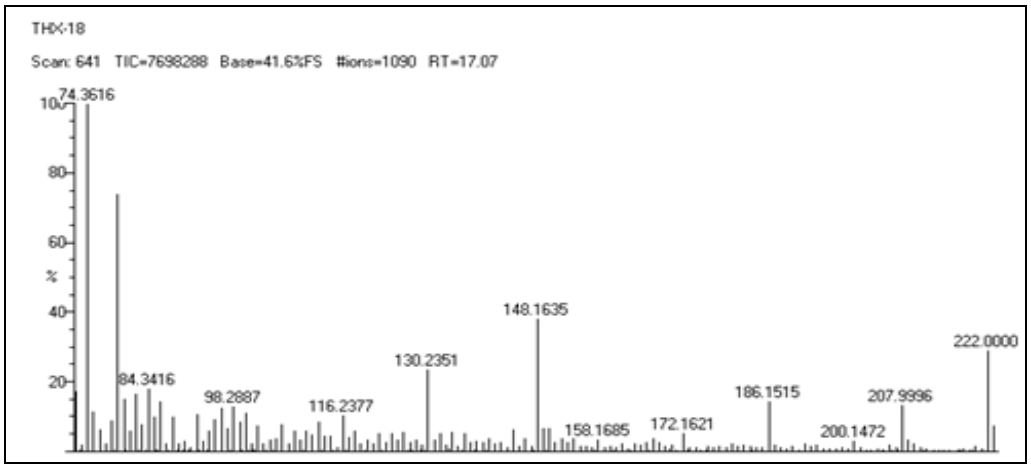
H1, H2 - Hexane, E1, E2 - Ethyl acetate, M1, M2 - Methanol, 1 (150 mg/ml), 2 (200 mg/ml)

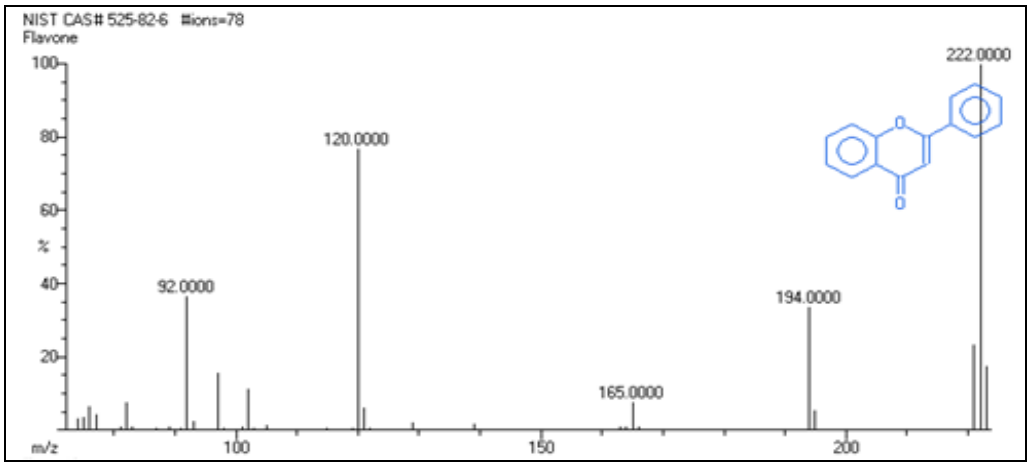
Gram-positive bacteria, *Bacillus subtilis* showed the zone in ethyl acetate solvent alone, but *Staphylococcu saureus* showed the zone of inhibition both in hexane and in ethyl acetate solvents, where the zone of inhibition was high in hexane solvent (29 ± 0.25 mm) **Fig. 3** and **Table 3**. Aqueous extracts of all the three species of seaweeds *Sargassum plagiophyllum, Padina gymnospora, Turbinaria conoides,* shows no zone of inhibition in all the three gram-positive and six gram-negative bacteria. Among the three species of seaweeds, *Turbinaria conoides* showed the maximum zone of inhibition. The chromatogram is showing the GC-MS profile of crude hexane extract of *Turbinaria conoides* **Fig. 4**, which shows 14 major compounds with numerous minor compounds.

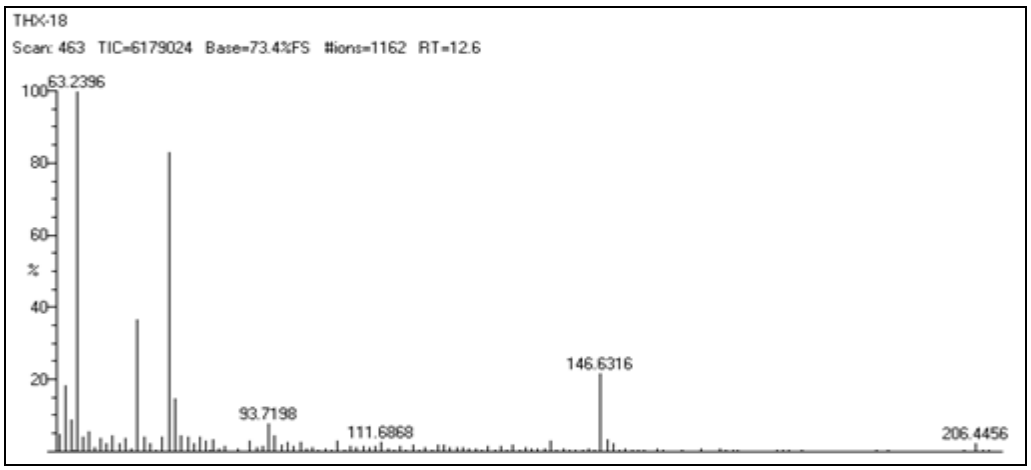
The various phytochemical components were compared with the spectra of known compounds stored in the NIST library by the peak area, molecular weight, molecular formula, chemical structure, and retention time (RT) in **Table 4**.

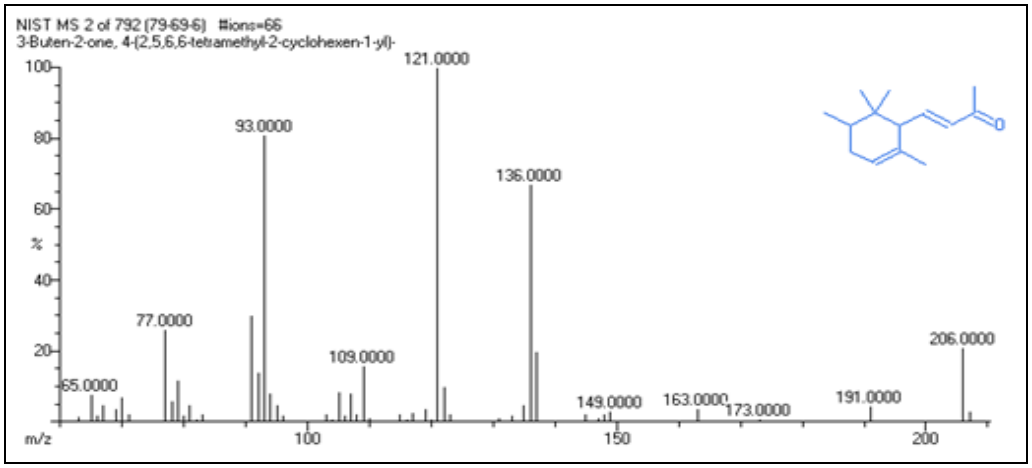


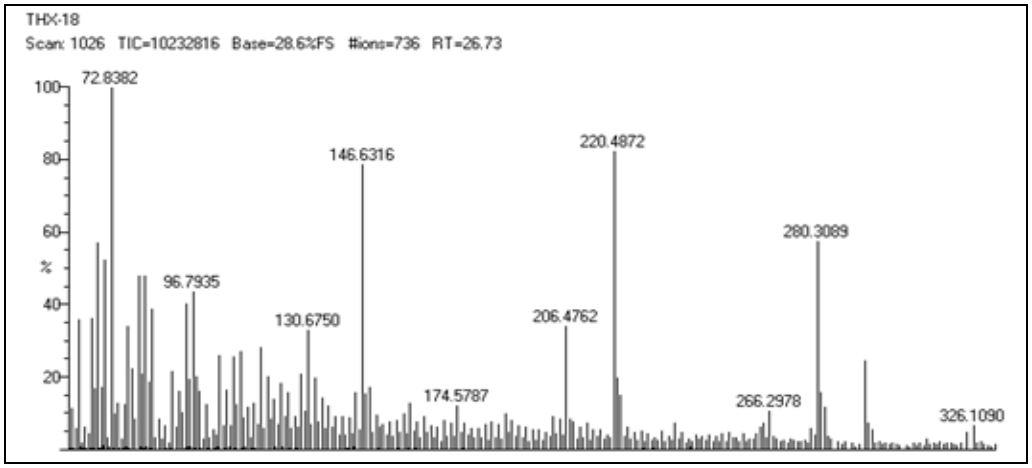

NIST MS 2 of 650 (2500-59-6 #ions=246
Oxiraneoctanoic acid, 3-octyl-, methyl ester
155.0000
83.0000
109.0000
127.0000
139.0000
199.0000
185.0000
214.0000
239.0000
263.0000
281.0000
294.0000
312.0000
m/z

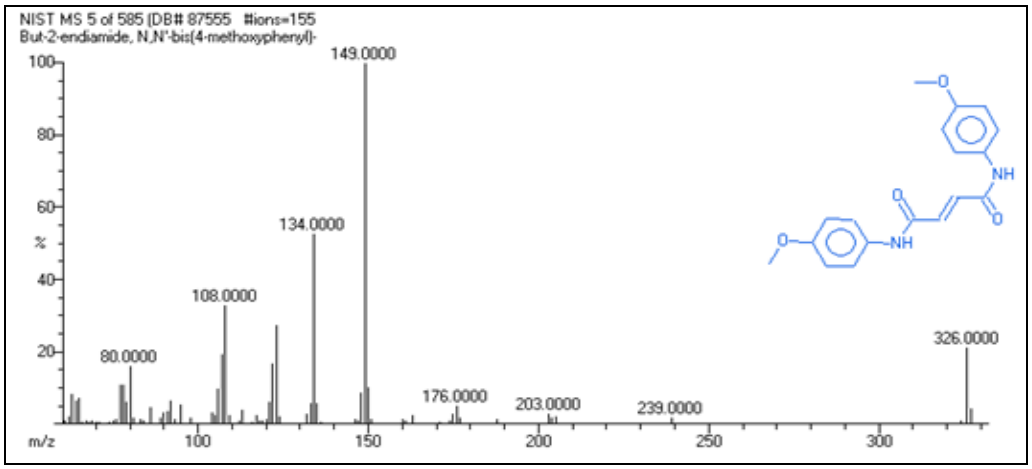

THX-18
Scan: 641 TIC=7698288 Base=41.6%FS #ions=1090 RT=17.07
74.3616
84.3416
98.2887
116.2377
130.2351
148.1635
158.1685
172.1621
186.1515
200.1472
207.9996
222.0000

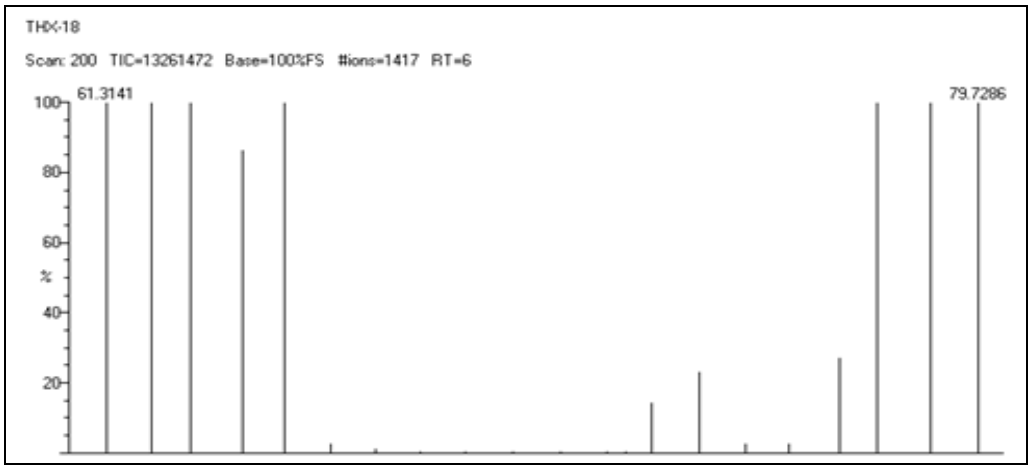

NIST CAS# 525-82-6 #ions=78
Flavone
222.0000
120.0000
92.0000
194.0000
165.0000
m/z

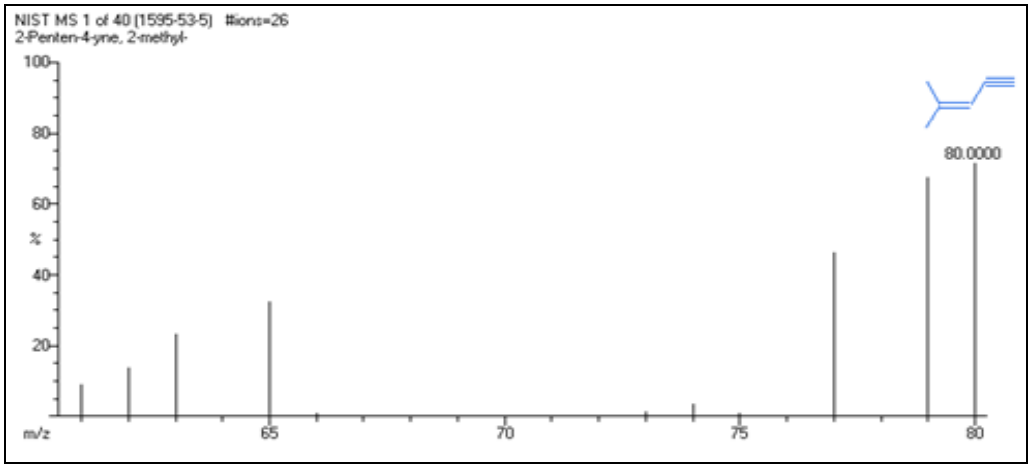

THX-18
Scan: 463 TIC=6179024 Base=73.4%FS #ions=1162 RT=12.6
63.2396
146.6316
93.7198
111.6868
206.4456

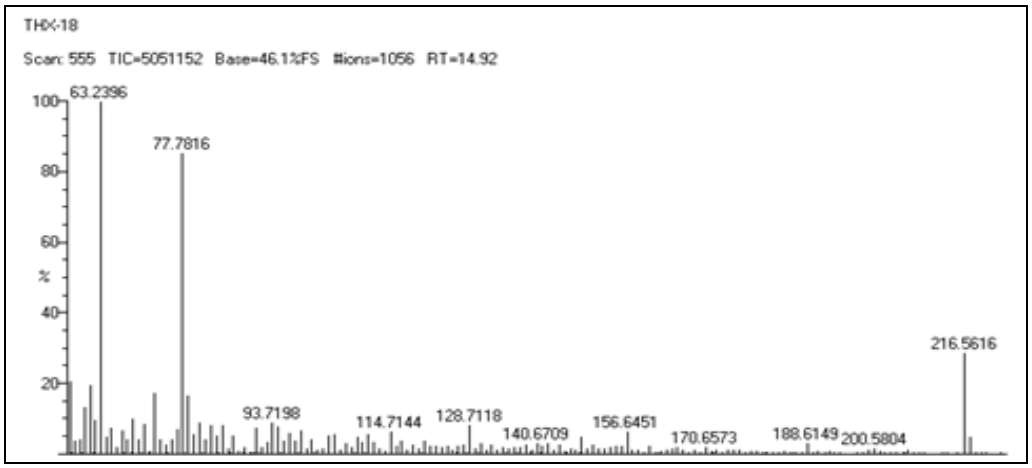

NIST MS 2 of 792 (79-69-6) #ions=66
3-Buten-2-one, 4-(2,5,6,6-tetramethyl-2-cyclohexen-1-yl)-
121.0000
93.0000
136.0000
77.0000
109.0000
65.0000
149.0000
163.0000
173.0000
191.0000
206.0000
%
m/z

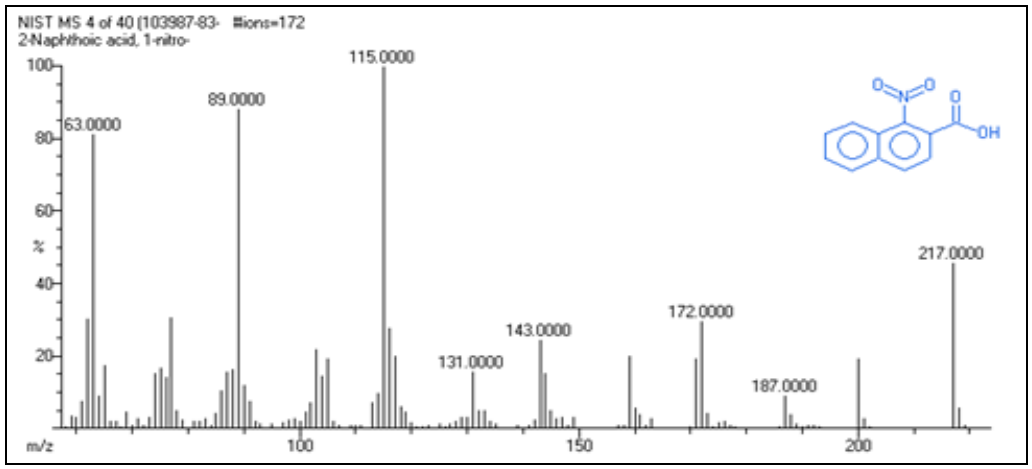

THX-18
Scan: 1026 TIC=10232816 Base=28.6%FS #ions=736 RT=26.73
72.8382
146.6316
220.4872
280.3089
96.7935
130.6750
206.4762
174.5787
266.2978
326.1090
%

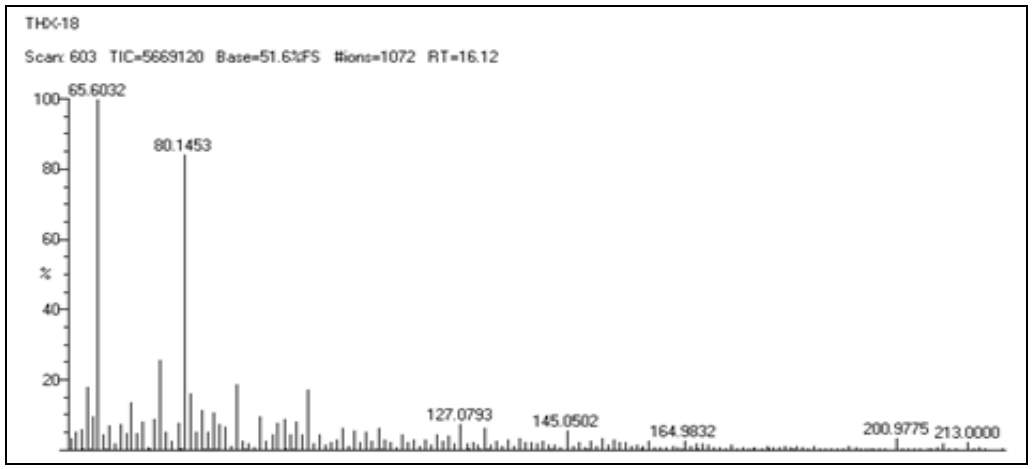

NIST MS 5 of 585 (DB# 87555 #ions=155
But-2-enediamide, N,N'-bis(4-methoxyphenyl)-
149.0000
134.0000
108.0000
80.0000
176.0000
203.0000
239.0000
326.0000
NH
NH
%
m/z

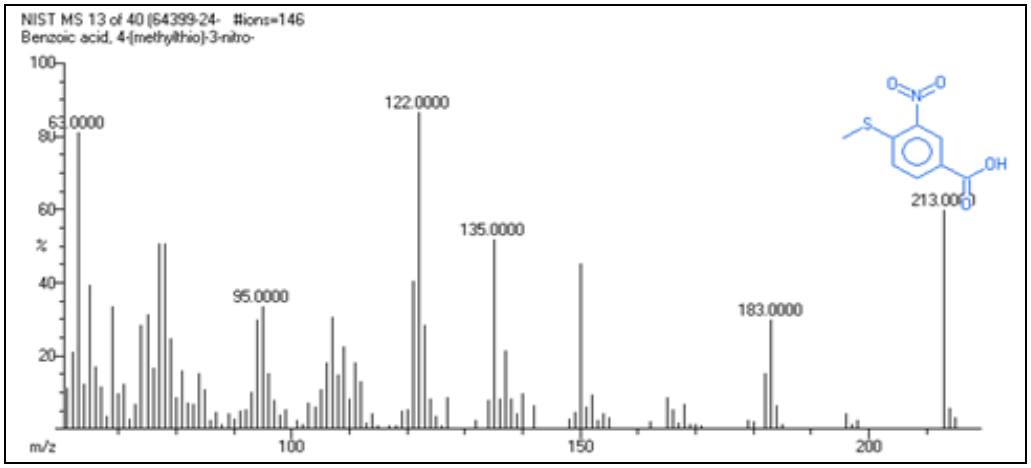

THX-18
Scan: 200 TIC=13261472 Base=100%FS #ions=1417 RT=6
61.3141
79.7286
%

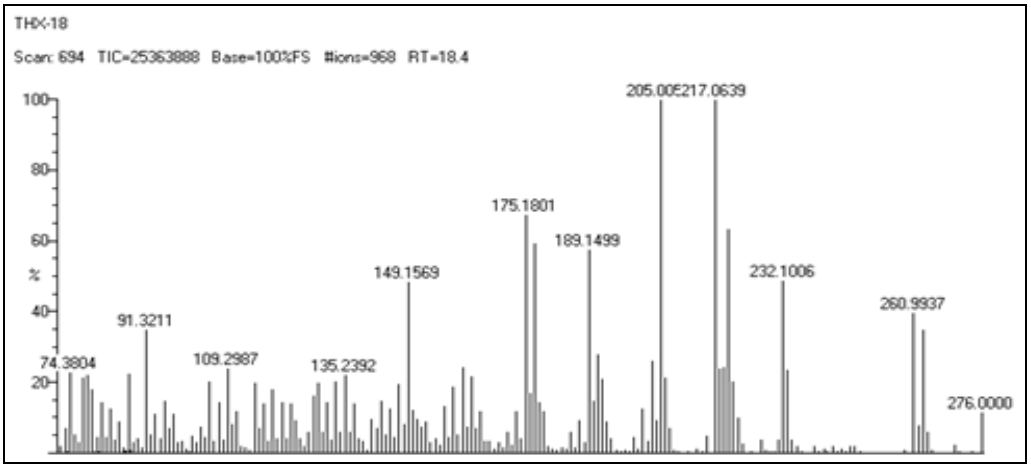

NIST MS 1 of 40 (1595-53-5) #ions=26
2-Penten-4-yne, 2-methyl-
80.0000
%
m/z

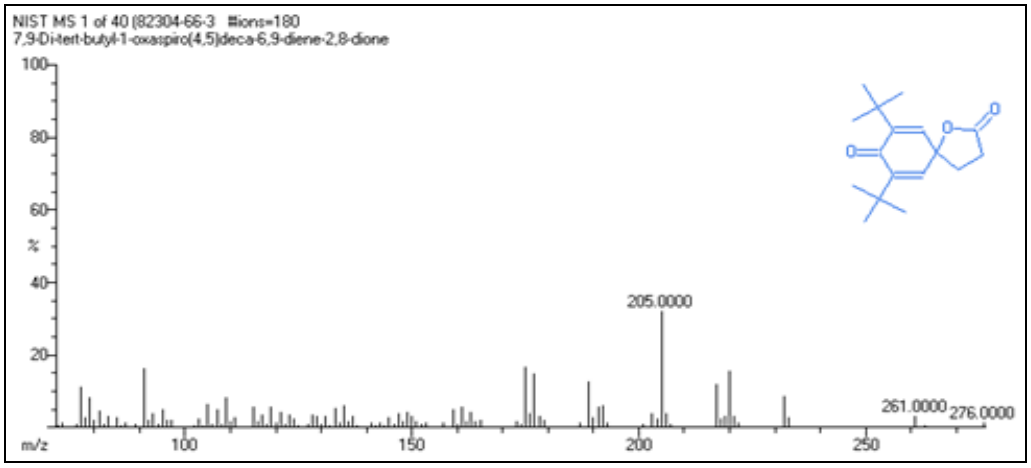

THX-18
Scan: 555 TIC=5051152 Base=46.1%FS #ions=1056 RT=14.92
63.2396
77.7816
216.5616
93.7198
114.7144
128.7118
140.6709
156.6451
170.6573
188.6149
200.5804
%

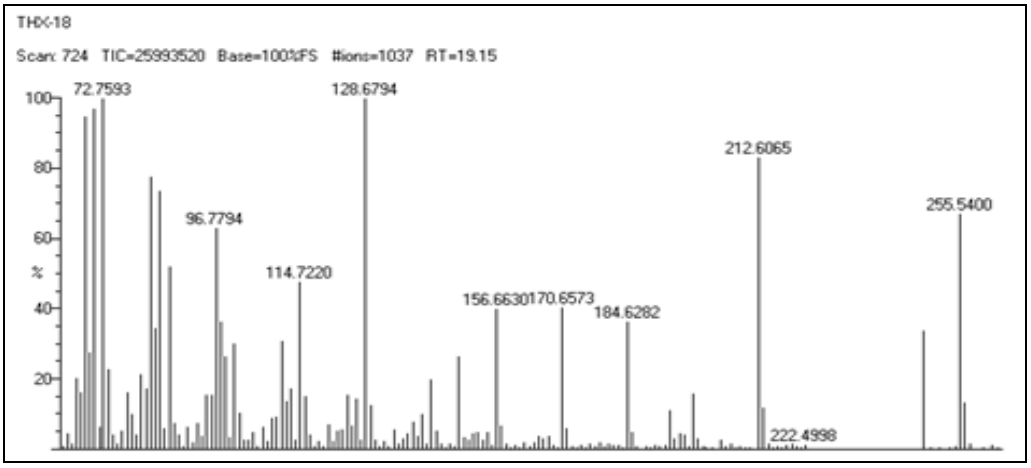

NIST MS 4 of 40 (103987-83- #ions=172
2-Naphthoic acid, 1-nitro-
115.0000
89.0000
63.0000
217.0000
172.0000
143.0000
131.0000
187.0000
%
m/z

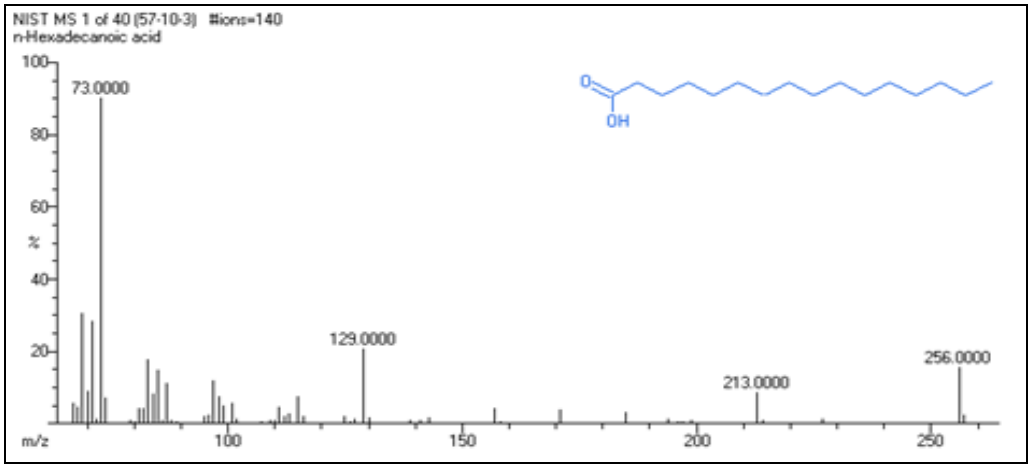

THX-18
Scan: 603 TIC=5669120 Base=51.6%FS #ions=1072 RT=16.12
65.6032
80.1453
127.0793
145.0502
164.9832
200.9775
213.0000
%

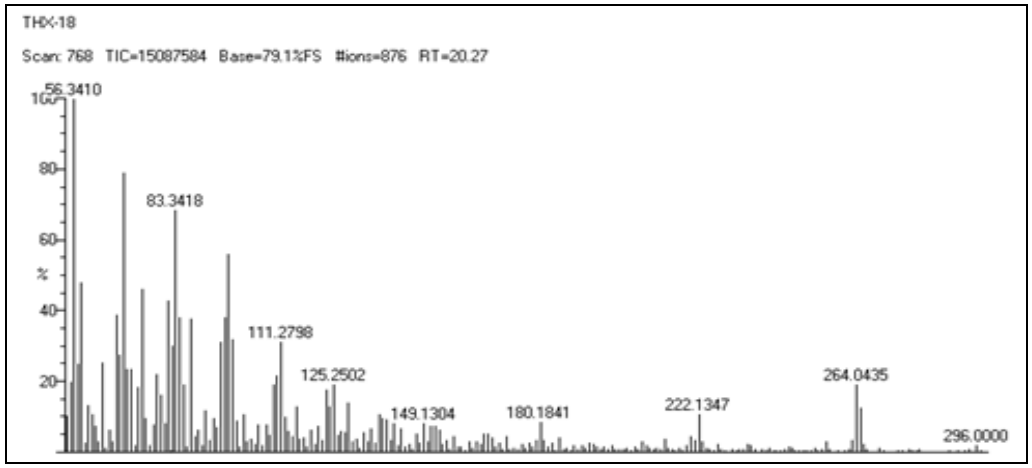

NIST MS 13 of 40 (64399-24- #ions=146
Benzoic acid, 4-(methylthio)-3-nitro-
63.0000
122.0000
135.0000
95.0000
183.0000
213.0000
m/z

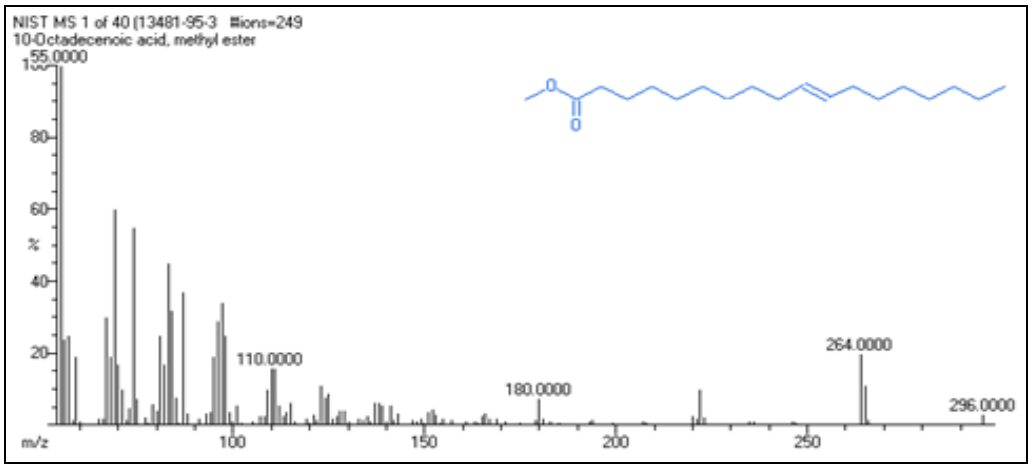

THX-18
Scan: 694 TIC=25363888 Base=100%FS #ions=968 RT=18.4
205.0052
217.0639
175.1801
189.1499
149.1569
232.1006
260.9937
91.3211
74.3804
109.2987
135.2392
276.0000

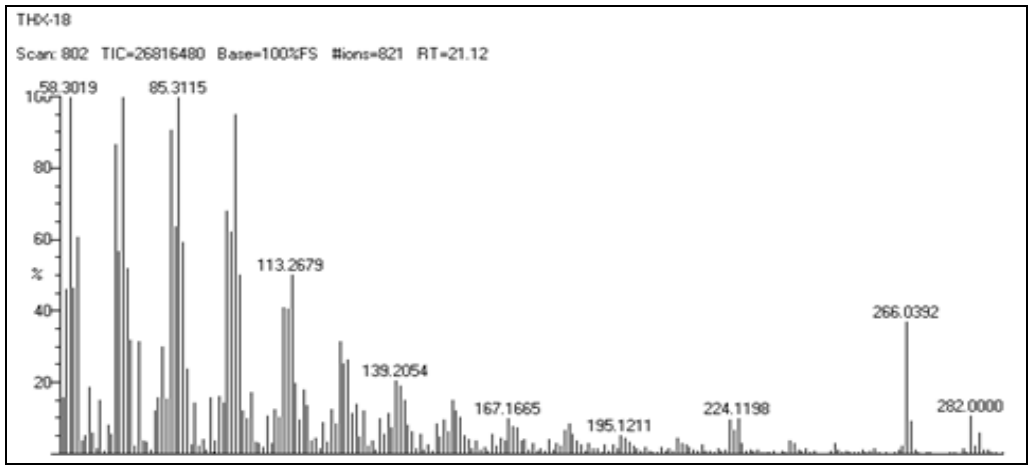

NIST MS 1 of 40 (82304-66-3 #ions=180
7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione
205.0000
261.0000
276.0000
m/z

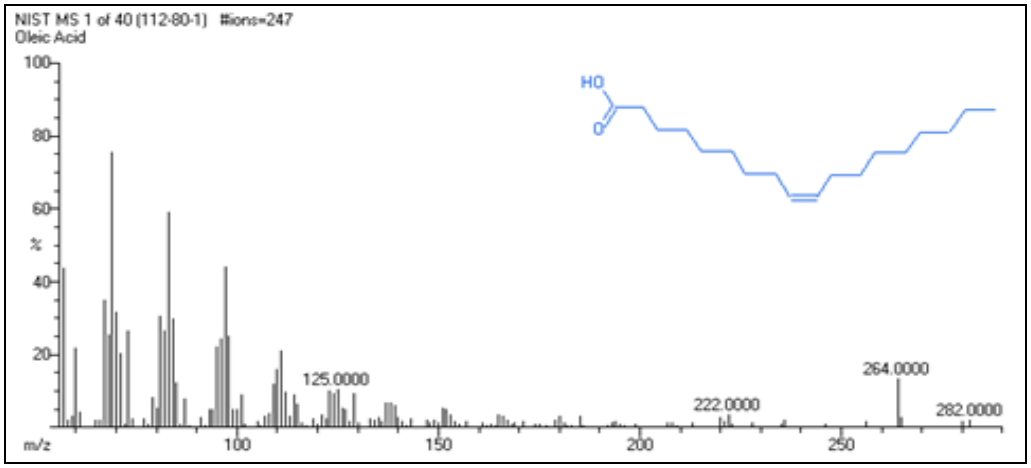

THX-18
Scan: 724 TIC=25993520 Base=100%FS #ions=1037 RT=19.15
72.7593
128.6794
212.6065
255.5400
96.7794
114.7220
156.6630
170.6573
184.6282
222.4998

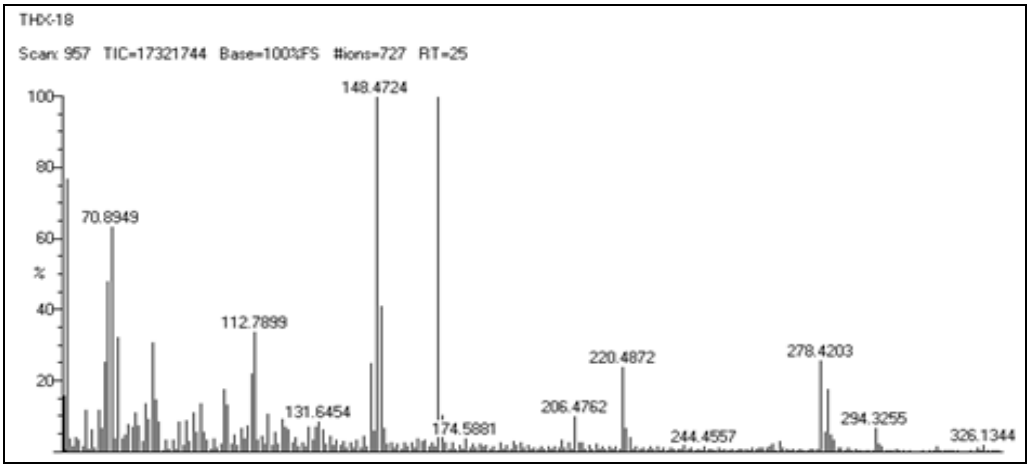

NIST MS 1 of 40 (57-10-3) #ions=140
n-Hexadecanoic acid
73.0000
129.0000
213.0000
256.0000
m/z

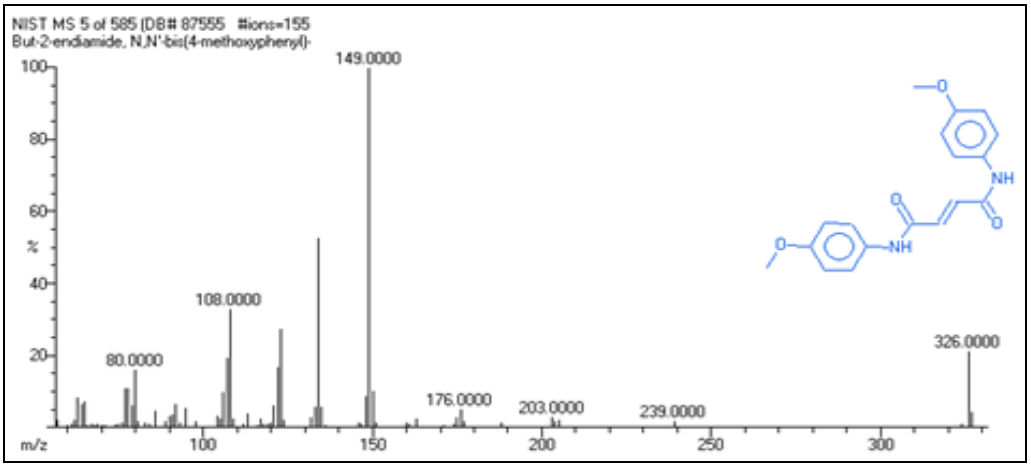

THX-18
Scan: 768 TIC=15087584 Base=79.1%FS #ions=876 RT=20.27
56.3410
83.3418
111.2798
125.2502
149.1304
180.1841
222.1347
264.0435
296.0000

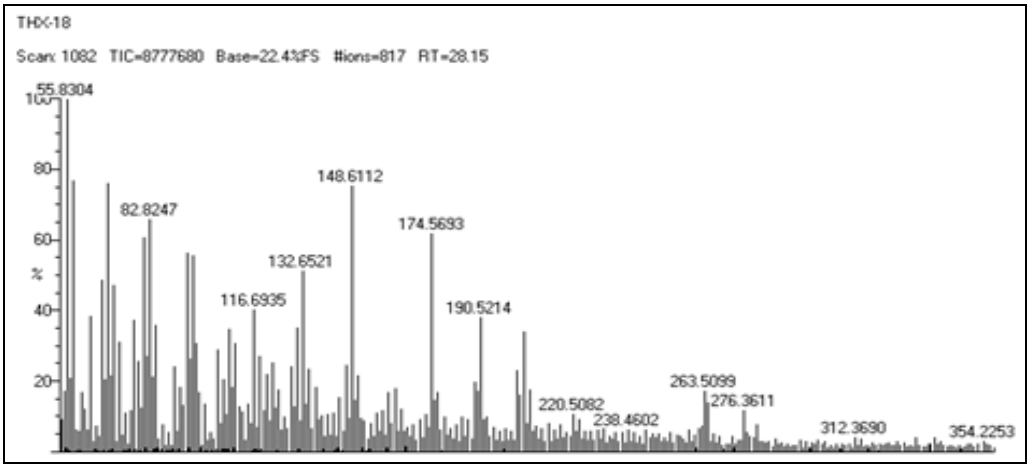

NIST MS 1 of 40 (13481-95-3 #ions=249
10-Octadecenoic acid, methyl ester
55.0000
110.0000
180.0000
264.0000
296.0000
m/z


THX-18
Scan: 802 TIC=26816480 Base=100%FS #ions=821 RT=21.12
58.3019
85.3115
113.2679
139.2054
167.1665
195.1211
224.1198
266.0392
282.0000


NIST MS 1 of 40 (112-80-1) #ions=247
Oleic Acid
HO
O
100
80
60
%
40
20
125.0000
222.0000
264.0000
282.0000
m/z
100
150
200
250


THX-18
Scan: 957 TIC=17321744 Base=100%FS #ions=727 RT=25
148.4724
70.8949
112.7899
131.6454
174.5881
206.4762
220.4872
244.4557
278.4203
294.3255
326.1344
100
80
60
%
40
20


NIST MS 5 of 585 (DB# 87555 #ions=155
But-2-enediamide, N,N'-bis(4-methoxyphenyl)-
149.0000
108.0000
80.0000
176.0000
203.0000
239.0000
326.0000
O
NH
O
O
NH
O
100
80
60
%
40
20
m/z
100
150
200
250
300


THX-18
Scan: 1082 TIC=8777680 Base=22.4%FS #ions=817 RT=28.15
55.8304
148.6112
82.8247
174.5693
132.6521
116.6935
190.5214
263.5099
276.3611
220.5082
238.4602
312.3690
354.2253
100
80
60
%
40
20

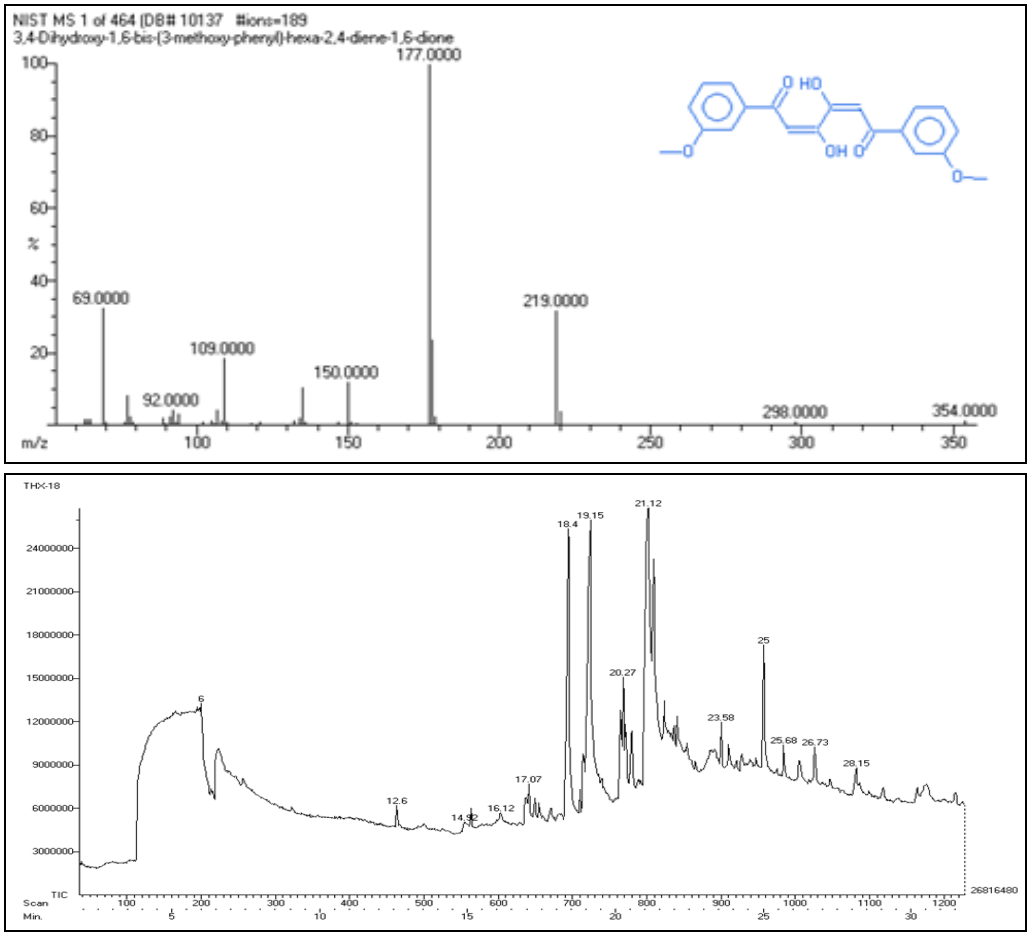
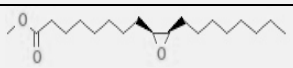
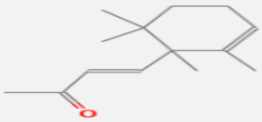
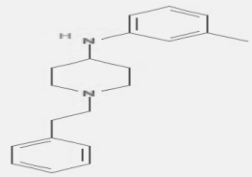
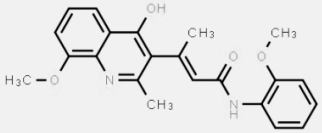
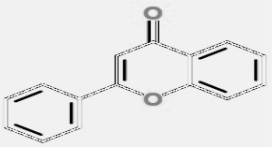
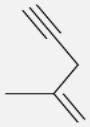
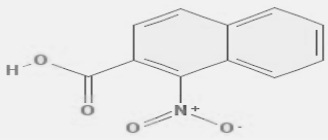
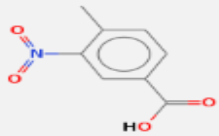
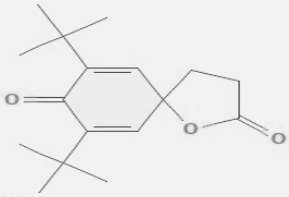
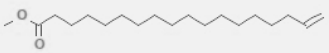
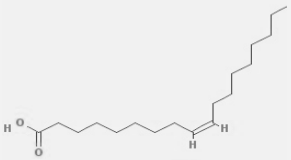
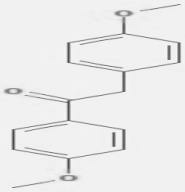
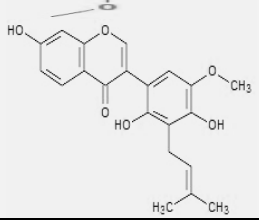


FIG. 4: GC-MS PROFILE OF COMPOUNDS IN TURBINARIA CONOIDES CRUDE EXTRACT (HEXANE)

TABLE 4: GC-MS ANALYSIS OF COMPOUNDS IN TURBINARIA CONOIDES CRUDE EXTRACT (HEXANE)

Phytochemical compound	RT(min)	Formula	Molecular Wt	Chemical structure
Oxiraneoctanoic acid, 3-octyl, methyl ester	25.68	$C_{19}H_{36}O_3$	312.494 g/mol	
3 buten 2-one 4 (2,5,6,6-tetramethyl-2-cyclohexen-1-yl)	12.6	$C_{14}H_{22}O$	206.3239 g/mol	
despropionyl-3 methyl fentanyl	23.58	$C_{20}H_{26}N_2$	294.442 g/mol	
but-2-enamide, N, N'-bis (4-methoxyphenyl)	26.73	$C_{24}H_{28}N_2O_6$	440.496 g/mol	
Flavone	17.07	$C_{15}H_{10}O_2$	222.243 g/mol	
2-penten-4-yne 2-methyl	6	C5H6	66.10110 g/mol	

2-naphthoic acid 1-nitro	14.92	$C_{11}H_7NO_4$	217.18 g/mol	
benzoic acid, 4- (methyl thio) 3-nitro	16.12	$C_8H_7NO_4$	181.1455 g/mol	
7, 9-di-tert-butyl-1-oxaspiro (4, 5) deca-6 9-diene-2 8-dione	18.4	$C_{17}H_{24}O_3$	276.376 g/mol	
N-hexadecanoic acid	19.15	$C_{16}H_{32}O_2$	256.43 g/mol	
10-Octadecenoic acid, methyl ester	20.27	$C_{19}H_{36}O_2$	296.4879 g/mol	
Oleic acid	21.12	$C_{18}H_{34}O_2$	282.468 g/mol	
But-2-endiamide, N, N'-bis (4-methoxyphenyl)-	25	$C_{16}H_{16}O_3$	256.301 g/mol	
3, 4-dihydroxy-1, 6-bis (3-methoxy-phenyl) hexa-2, 4-diene-1, 6-dione	28.15	$C_{21}H_{20}O_6$	354.2 g/mol	

DISCUSSION: A higher percentage of the population across the world prefers to use natural products for their various alignments as they produce fewer side effects on humans. One among them is the marine algae, which is a reservoir of many secondary metabolites, which serves various purposes. Different groups of marine algae known to contain active compounds that used as remedies for many diseases [12, 13]. Previous investigations on the production of antibiotic substances by aquatic organisms form a rich and varied source of antibacterial and antifungal agents. Over 15000 novel compounds have been chemically determined. Focusing on bio-products, recent trends in drug research from natural sources suggest that algae are a promising group to furnish novel biochemically active substances [14]. The present study focuses on the screening of various extracts of marine seaweeds for its antibacterial activity against gram-positive and gram-negative pathogens. The results revealed that all the extracts are potent antimicrobials against the micro-organisms tested.

From the previous studies, a variety of solvents used, based on its polarity, for antimicrobial activity screening, but still uncertain what kind of solvent is most effective for the extraction of seaweeds. Earlier studies showed the extraction of *Turbinaria conoides* with n-hexane, cyclohexane, methanol and ethanol: water (1:1) depicted with their antibacterial activities by disc diffusion method. In the present study, with increasing polarity, three organic solvents like hexane < ethyl acetate < methanol along with the water used for the extraction of crude extracts from three different

species of marine algae. In the case of *Turbinaria conoide* highest antibacterial activity was exhibited in ethanol extract, which was comparable to the standard, Streptomycin against *Bacillus subtilis, Enterococcus faecalis and Pseudomonas aeruginosa* [15]. There was no anti-bacterial, activity shown in Methanol extract of *Sargassum vulgare* against *Escherichia coli* and *Staphylococcus aureus* [16].

The methanol extract of marine brown algae *Padina gymnospora* and *Sargassum wightii* showed no activity against the bacterial species of *Bacillus subtilis, Staphylococcus aureus, Klebsiella pneumonia, Pseudomonas aeruginosa,* and *Salmonella typhus* [17]. In the present study, there is no zone of inhibition from methanol extract of *Sargassum plagiophylum* and *Padina gymnospora* against the human pathogens studied. The organic solvent, which shows an antibacterial activity against the pathogens, in one species of algae, is not effective in other algae. Earlier reports, against the effectiveness of extraction methods, explain that methanol extraction yields higher antimicrobial activity than hexane and ethyl acetate [18, 19], whereas another study gives better result in chloroform than methanol and benzene [20]. In another study, extract of acetone exhibited a strong antimicrobial activity against bacteria when compared to other extracts [21].

Higher antibacterial activity in brown algae extracts may be due to the presence of secondary metabolites like phenolic compounds of *Turbinaria conoides*, which may affect the growth as an inhibitory effect and the metabolism of bacteria, based on their chemical constitutions and concentrations [22]. The present study is in concur with the study of [23] reports in which cyclohexane extract was possessed a broad array of anti-bacterial property over the other extracts. It was also evident that organic solvents provide a higher efficiency in extracting compounds for anti-microbial activities compared to water-based methods. The highest antibacterial activity shown in organic solvents compared to aqueous extract [24, 25]. Previous literature does not pertain to a single organic solvent of extract for the effectiveness of antibacterial activity from marine algae. For the present study, the solvents chosen on the polarity gradient from Hexane to water, where the solvent extract of *Turbinaria conoides* has significant result in all the solvent extract, whereas the other species, *Sargassum plagiophyllum* and *Padina gymnospora* shown antibacterial activity in ethyl acetate, but the methanol extract fails to show activity in any of those pathogens studied. Aqueous extracts of the marine algae studied shown no result in all the human pathogens experimented for anti-bacterial activity and so no tabulation here.

Earlier experiments reveal that the dried samples accumulated more amounts of the antibiotic principle rather than the fresh samples, where the high water content diluted and leads to dissipated activity. It is also evident that the degradation of bioactive metabolites may not occur during shade drying [26]. In addition to earlier literature, the present investigation shows that *Turbinaria conoides*, the species of Phaeophyta showed the strongest activities against the human pathogenic bacteria in all the solvents. A study [27] on antimicrobial activity from the three families of Rhodophyta, Chlorophyta and Phaeophyta against *Bacillus subtilis*, *Staphylococcus aureus*, *E. coli*, *Klebsiella pneumonia,* showed, *Padinagymnospora* and *Sargassum wightii*, which belongs to the family Phaeophyta, were more active than *C. linum* of Chlorophyta, against human pathogenic bacteria using the disc diffusion method.

Four species experimented [28] from brown seaweed (*Sargassum plagiophyllum, Sargassum flavellum*, *Sargassum binderi* and *Padina australis*) in Malaysia, where the crude extracts showed stronger antimicrobial activity against the gram-positive bacteria (*Bacillus subtilis* and *Staphlococcus aureus*) than the gram-negative bacteria (*P. aeruginosa* and *E. coli).* Susceptibility is more in gram-positive organisms than gram-negative bacteria against the seaweed extracts, and this susceptibility is due to the difference in their cell wall structure and their composition [29]. Earlier studies among the species tested, *Staphlococcus aureus* and *Vibrio spp*. were the most susceptible pathogens reported [30].

The methanol extract of *Sargassum vulgare* does not show effective result of antibacterial activity against *E. coli*, and *Staphlococcus aureus* pathogens reported [31]. However, the report of *Sargassum fusiforme* diethyl ether extract showed a positive activity against *Staphlococcus aureus* [32].

Among the three different species of seaweeds, crude extracts of *Turbinaria conoides* showed anti-bacterial activity against *Staphylococcus epidermidis* for all the solvent tested. *Staphylococcus epidermidis* is a facultative anaerobic bacterium, Coagulase-negative staphylococci (CoNS) which causes nosocomial infections in humans [33]. In addition, it was evident from previous research that, *Staphylococcus epidermidis* shows an increased resistance against various antibacterial agents, when the bacteria attached to plastic surface [34] and also they are often resistant to antibiotics, including rifamycin, fluoroquinolones, gentamicin, tetracycline, clinda-mycin, and sulfonamides [35].

CONCLUSION: In the present study, the effect of crude extract of three seaweed species, upon the human pathogens shows a remarkable antibacterial activity against both gram-positive and gram-negative bacteria. Out of the solvents used like hexane, ethyl acetate, methanol, and water, methanol extract shows no activity in both *Sargassum plagiophyllum* and *Padina gymnospora* for all the pathogens. Ethyl acetate solvent extract showed the zone of inhibition both in gram-positive and gram-negative bacteria. Comparatively, gram-negative bacteria showed lesser activity than Gram-positive bacteria.

In *Turbinaria conoides* all three solvents showed the zone of inhibition for *Staphylococcus epidermidis*. The hexane extract of *Turbinaria conoides* showed the zone of inhibition in all the positive bacteria, where it shows a significant zone of inhibition in *Staphylococcus epidermidis* compared to control. Further, the hexane extract analyzed by GC-MS to find the phytochemical compounds responsible for the activity.

ACKNOWLEDGEMENT: The work is done completely supported by the Gunidy campus University laboratory. The author greatly thanks everyone, who rendered help for the work.

CONFLICTS OF INTEREST: Nil

REFERENCES:

1. Babu PA, Suneetha G, Boddepalli R, Lakshmi VV, Rani TS, Rambabu Y and Srinivas K: A database of 389 medicinal plants for diabetes. Bioinformation 2006; 1(4): 130-71.
2. Oceans CS: A store house of drugs- a review. J Pharm Res 2010; 3: 1293-96.
3. Armstrong E, Yan L, Boyd KG, Wright PC and Burgess JG: The symbiotic role of marine microbes on living surfaces. Hydrobiologia 2001; 461: 37-40.
4. Penesyan A, Tebben J, Lee M, Thomas T, Kjelleberg S, Harder T and Egan S: Identification of the antibacterial compound produced by the marine epiphytic bacterium Pseudovibrio sp. D323 and related sponge-associated bacteria. Mar Drugs 2011; 9: 1391-02.
5. Yuan VY and Bone DEF: Carrington antioxidant activity of dulse (Palmaria palmata) extract evaluated *in-vitro*. Food Chemistry 2005; 91: 485-94.
6. Bansemir A, Blume M, Schroder S and Lindequist U: Screening of cultivated seaweeds for antibacterial activity against fish pathogenic bacteria. Aquaculture 2006; 252: 79-84.
7. Chew Y, Lim Y, Omar M and Khoo K: Antioxidant activity of three edible seaweeds from two areas in South East Asia. LWT-Food Sci Technol 2008; 41: 1067-72.
8. Ely R, Supriya T and Naik CG: Antimicrobial activity of marine organisms collected off the coast of South East India. J Exper Mar Biol Ecol 2004; 309: 121-27.
9. Kandhasamy M and Arunachalam KD: Evaluation of *in-vitro* antibacterial property of seaweeds of southeast coast of India. Afr J Biotechnol 2008; 12: 1958-61.
10. Levy S: Factors impactingon the problems of antibiotic resistance. J Antimicrob Chemother 2002; 49: 25-30.
11. Akinyemi KO, Oladapo O, Okwara CE, Ibe CC and Fasure KA: Screening of crude extracts of six medicinal plants used in South-West Nigerian unorthodox medicine for antimethicillin resistant *S. aureus* activity. BMC Comp Alt Med 2005; 5(6): 1-7.
12. Cox S, Abu-Ghannam N and Gupta S: An assessment of the antioxidant and antimicrobial activity of six species of edible Irish seaweeds. Int Food Res J 2010; 17: 205-20.
13. Tyagi N and Bhora A: Screening of phytochemicals of fruit plant and antibacterial potentials against *Pseudomonas aeruginosa*. Bioc Cell Arch 2002; 2: 21-24.
14. Blunt JW, Copp BR, Munro MHG, Northcote PT and Prinsep MR: Marine natural products. Natural Product Reports 2005; 22: 15-61.
15. Ponnan A, Ramu K, Marudhamuthu M, Marimuthu A, Siva K and Kadarkarai M: Antibacterial, antioxidant and anticancer properties of *Turbinaria conoides*. Clinical Phytoscience International Journal of Phytomedicine and Phytotherapy 2017; 3: 5.
16. Chiheb I, Hassane R, Martinez L, José DSJ Francisco GVJ, Antonio B, Hassan and Mohamed K: Screening of anti-bacterial activity in marine green and brown macroalgae from the coast of Morocco. Afr J Biotechnol 2009; 8(7): 1258-62.
17. Rangaiah SG, Lakshmi P and Manjula E: Antimicrobial activity of seaweeds Gracillaria, Padina and Sargassum sps. on clinical and phyto pathogens. Int J Chem Anal Sci 2010; 1: 114-17.
18. Rosell KG and Srivastava LM: Fatty acids as antimicrobial substances in brown algae. Hydrobiolo 1987; 151: 471-75.
19. Sastry VM and Rao GR: Antibacterial substances from marine algae: successive extraction using benzene, chloroform and methanol. Bot Mar 1994; 37: 357-60.
20. Febles CI, Arias A, Gil-Rodriguez MC, Hardisson A and Lopez SA: *In-vitro* study of antimicrobial activity in algae (Chlorophyta, Phaeophyta and Rhodophyta) collected from the coast of Tenerife (in Spanish). Anuariodel Instituto de Estudios Canarios 1995; 34: 181-92.
21. Kayalvizhi K, Subramanian V, Anantharaman P and Kathiresan: Antimicrobial activity of seaweeds from the Gulf of Mannar. Inl J of Pharma Appli 2012; 3(2): 306-14.

22. Reguant C, Bordons A, Arda L and Roze N: Influence of phenolic compounds on the physiology of *Oenococcus oeni*. J Appl Microbiol 2000; 88: 1065-71.
23. Jyh-Horng S, Guey-Horng W, Ping-Jyun S and Chang-Yih D: New cytotoxic oxygenated fucosterols from the brown Alga Turbinaria conoides. J Nat Prod 1999; 62(2): 224-7.
24. De compos-Takaki M, Koenong L and Periera GC: Screening the marine algae from Brazilian northeastern coast of antimicrobial activity. Bot Mar 1988; 31: 375-77.
25. Lima-Filho JVM, Carvalho AFFU, Freitas SM and Melo VMM: Antibacterial activity of extracts of six macroalgae from the northeastern Brazilian coast. Brazilian Journal of Microbiology 2002; 33: 311-13.
26. Tuney I, Cadirci BH, Unal D and Sukatar A: Antimicrobial activities of the Extracts of marine algae from the coast of Urla (Izmir, Turkey). Turkish Journal of Biology 2006; 30: 171-75.
27. Osman MEH, Abushady AM and Elshobary ME: *In-vitro* screening of antimicrobial activity of extracts of some macroalgae collected from Abu-Qir bay Alexandria, Egypt. Afr J Biotechnol 2010; 9: 7203-08.
28. Jaswir I, Tope AHT, Raus RA, Monsur HA and Ramli N: Study on anti-bacterial potentials of some Malaysian brown seaweeds. Food Hydrocoll 2014; 42: 275-79.
29. Taskin E, Ozturk M and Krut O: Antibacterial activities of some marine algae from the Aegean Sea (Turkey). Afr J Biotechnol 20016; 2746-51.
30. Padmakumar K and Ayyakkannu K: Seasonal variation of antibacterial and antifungal activities of the extracts of marine algae from southern coasts of India. Bot Mar 1997; 40: 507-15.
31. Ibtissam C, Radio H, Martinez-Lopez J, Dominguez SJF, Gomez VJA, Bouziane H and Kadiri M: Screening of antibacterial activity in marine green brown macroalgae from the coast of Morocco. Afr J Biotechnol 2009; 8: 1258-62.
32. Shimaa M and Shafay EL: Antimicrobial activity of some seaweeds species from red sea, against multidrug resistant bacteria. The Egyptian Journal of Aquatic Research 2016; 42(1): 65-74.
33. Rogers KL, Fey PD and Rupp ME: Coagulase-negative staphylococcal infections. Infect Dis Clin North Am 2009; 23(1): 73-98.
34. Sheth NK, Franson T and Sohnle PG: Influence of bacterial adherence to intravascular catheters on *in-vitro* antibiotic susceptibility. Lancet 1985; 2: 1266-68.
35. Otto M: *Staphylococcus epidermidis* - the "accidental" pathogen. Nature Reviews Microbiol 2010; 7(8): 555-67.

How to cite this article:
Vennila KK, Chitra PS, Hilda K, Janarthanan S and Martin P: Screening of anti-bacterial activity of brown seaweeds from South East Coast of India. Int J Pharm Sci & Res 2020; 11(8): 3993-09. doi: 10.13040/IJPSR.0975-8232.11(8).3993-09.