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Original Article

Gas Chromatography-mass spectrometry Analysis of Methanol Extracts from Marine Red Seaweed *Gracilaria corticata*

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ABSTRACT

Introduction: The objective of the work is to analyse the methanol extract of marine red macro algae species *Gracilaria corticata* using Gas chromatography-Mass spectrometry (GC-MS) to reveal the presence of various secondary metabolites and bioactive compounds present in the algae and study its diverse properties. **Methods:** *Gracilaria corticata* was collected along the shore of Mandapam and was identified and authenticated. The methanol extract of the algae was prepared and analysed using GC-MS Perkin-Elmer, Clarus 680 model to reveal the various bioactive present in the algae. **Results:** The analysis revealed several bioactive compounds:undecane; 2-decyloxirane (2.023%); Methy n-tridecanoate;n-hexadecanoic acid (74.198%); eicosanoic acid (2.262%); nonanoic acid (2.084%); oleic acid (6.609%); oleic acid (4.156%); pentadecanoic acid (2.176%); bicycle [3.2.1] oct-3-en-2-one,3,8-dihydroxy-1-1methoxy-7-(7-methoxy-1, 3 benzodioxol-5-yl)-6-methyl-5 (2.901%);N-(5-chloro-2-hydroxy-phenyl) dodecanamide (2.048%); and cholesta-8,24-dien-3-ol,4-methyl (1.542%). The bioactive compounds from methanol extract of algae after GC-MS analysis and their essential medicinal properties were studied in this research work. **Conclusion:** *Gracilaria corticata* has potential against bacteria, fungi, free radical scavenging, etc and can used in the drug discovery and development sector.

Key words: *Gracilaria corticata*, Bioactive compounds, GC-MS, Medicinal properties, Secondary metabolites.

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INTRODUCTION

The marine ecosystem is a home for nearly 80% of the world's animal and plant species.[1] Approximately 150,000 species of seaweeds are found along the intertidal zone. The seaweeds are a rich source of various natural products and find an important place in the pharmaceutical, cosmetics and drug development industries.[2] Seaweeds occur in areas of seashore such as coastal areas of Mandapam, Ramanathapuram district. Seaweeds grow in the shallow waters in the intertidal zones of the marine ecosystem. The seaweeds possess the unique feature that promote their survival in the salty marine ecosystem. The osmolarity in their cytoplasm is adjusted with respect to the osmolarity of the salty sea water. This adaptation does not allow the desiccation to take place. The seaweeds lack true leaves, stems and roots. They have modified stems, roots and leaves to sustain in the marine ecosystem.[3]

Seaweeds are one of the predominant and promising sources of various bioactive secondary metabolites. Their discovery has augmented in the past few decades.[4] Their discovery is essential for mankind. They are a good source of proteins, carbohydrates, minerals, vitamins, polysaccharides and steroids. They are used as a predominant protein rich-food for the scientists working at International space station.[4] The brown and red macroalgae are used against hyperthyroidism.[5-6] *Gracilaria corticata* alcoholic extracts have shown potential against AIDS.[7] The poly-unsaturated lipids are used for the treatment of diverse cardiovascular pathogens.[8] *Gracilaria corticata* synthesize a diverse variety of essential compounds such asxanthophylls, chlorophylls, proteins, minerals, vitamins, carrageenan, alginate, agar, fucoidan, laminarin, galactosyl glycerol, proteoglycans, amino acids, poly-unsaturated fatty acids and antioxidants like polyphenols, terpenoids, halogens and alkaloids.[9-19] *In-vivo* studies on the toxicity of *Gracilaria corticata* was studied on mice models and were reported to be non-toxic.[8]

Studies on *Gracilaria corticata* and their phyto-chemicals, bioactive compounds and medicinal properties are less revealed when compared to other species of *Gracilaria*. Therefore, this research work illuminates on the various bioactive compounds and their properties from GC-MS analysis of *Gracilaria corticata*.

MATERIALS AND METHODS

Collection of algae

The *Gracilaria corticata* was collected from the intertidal shallow zone of Mandapam, Ramanatha-

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puram district. The algae were obtained from the coat of Mandapam (Lat.: 9°16'32.56"N and Lon.: 79°7'25.03"E) along the southern regions of Tamilnadu. The harvested algae were washed with water to remove sand particles and epiphytes and packed in a polythene bag and was brought to the laboratory. The algae were further washed with distilled water to remove traces of salts and other contaminants, shade dried and stored.

Authentication of algae

The collected algae were authenticated by Dr. M. Ganesan, Senior Scientist, CSIR- Central Salt Marine Chemical Research Institute (CSIR-CSMCRI), Ramanathapuram district, Tamilnadu, India. The algae were identified as *Gracilaria corticata* and were used for the research work.

Preparation of powder and extract

The shade dried algae were crushed to small pieces using mortar and pestle and further powdered using an electric blender. The extract of was prepared by adding 2g of the powdered algae sample with 20ml of methanol. The mixture was then incubated overnight in an orbital shaker at 32°C. The extract was then filtered using No.1 Whatmann filter paper and a funnel. The extract was collected in a 100 mL conical flask. The collected extract was dried using a rotary vacuum evaporator to evaporate the solvent to obtain the crude extract of the algae. The collected extract was then used for GC-MS analysis.

Gas Chromatography-mass Spectrometry (GC-MS) analysis of *Gracilaria corticata*

The methanolic extract of *Gracilaria corticata* was subjected to GC-MS analysis. The analysis was performed in Sophisticated Instrumentation Facility (SIF), Chemistry Division for NMR and GC-MS Analysis, Vellore Institute of Technology, Vellore. The GC-MS analysis was performed using the Perkin-Elmer, Clarus 680 model using Clarus 600 (Electron Ionization) as the source for ionization.

GC-MS analysis

The Clarus 680 equipped with fused silica column packed with Elite-5MS (5% biphenyl 95% dimethyl polysiloxane, 30 m × 0.25 mm ID × 250µm df) was used. The active components were separated using the inert gas helium as carrier gas at the flow rate of 1 mL/min. The injector temperature was set at 260°C during the run. One µL of the sample extract (*Gracilaria* methanol extract) was injected into the GC-MS instrument and the oven temperature set were as follows: 60°C for 2 min; followed by 300°C at the rate of 10°C per min; and held for 6 mins at temperature of 300°C. The mass detector process was as follows: transfer line temperature of 240°C; ion source temperature of 240°C and 70 eV of electron ionization impact. The scan interval of 0.1 sec and a scan time of 0.2 secs with a scan range of 50 to 600 Daltons were set. The spectrum for the GC-MS analysis was obtained using the Turbo Massver 5.4.2 software and was compared with the National institute of standards and technology-2008 (NIST-2008) standard library database.

RESULTS AND DISCUSSION

A total of thirteen bioactive compounds were obtained from the peaks of methanolic extracts of *Gracilaria corticata* by GC-MS analysis. The details of the bioactive compounds were tabulated (Table 1). Figure 1 shows the chromatogram of the compounds detected using GC-MS. Out of thirteen compounds, only ten compounds were detected as major peaks in the chromatogram with their retention time and peak area (Figure 2 to 12). The mass spectra of the compounds were compared with the standard library data bases and were characterized and identified.

There were three compounds whose peaks were too small to be detected in the chromatogram (Figure 13 to 15), the table below give the details of the predicted compounds with their retention time, the peak area for the compounds were not calculated but were analysed and predicted through mass spectrum (Table 2).

The Table 3 represent the ten bioactive compounds from methanol extracts of *Gracilaria corticata* with their structure, molecular formula and molecular weight with high peak areas and intensities.

Table 4 denotes the diverse properties of all the bioactive compounds detected using GC-MS.

Phytochemicals are secondary metabolites from plants that are essential for the plant defence against grazing animals and other predators. The secondary metabolites are those that are not directly involved in the growth of the plant, instead they constitute a vital protection and resistance. The methanol extract of *Gracilaria corticata* after GC-MS analysis showed several bioactive compounds including fatty acids like n-hexadecanoic acid, eicosanoic acid, nonanoic acid, oleic acid and

Table 1: Details of Phytochemicals Identified through GC-MS Analysis of Methanol Extract.

Peak	Retention time (min)	Name of the compound	Scan	Height	Area (Intensity*sec)	Area (%)	Norm %
1	17.345	Oxirane, decyl-	2908	21,133,272	2,632,621.2	2.023	2.73
2	18.795	n-hexadecanoic acid	3198	128,331,784	96,576,664.0	74.198	100.00
3	20.126	Eicosanoic acid	3464	24,689,006	2,944,463.2	2.262	3.05
4	20.306	Nonanoic acid	3500	20,235,054	2,713,160.8	2.084	2.81
5	20.716	Oleic acid	3582	24,858,102	8,602,715.0	6.609	8.91
6	20.956	Oleic acid	3630	19,378,138	5,409,151.5	4.156	5.60
7	21.731	Pentadecanoic acid	3785	12,568,093	2,832,692.0	2.176	2.93
8	22.862	Bicyclo[3.2.1]oct-3-en-2-one, 3,8-dihydroxy-1-methoxy-7-(7-methoxy-1,3-benzodioxol-5-yl)-6-methyl-5	4011	18,728,876	3,775,650.5	2.901	3.91
9	25.373	N-(5-chloro-2-hydroxyphenyl)dodecanamide	4513	15,933,098	2,665,857.5	2.048	2.76
10	27.294	Cholesta-8,24-dien-3-ol, 4-methyl-, (3.beta.,4.alpha.)-	4897	10,175,952	2,007,103.9	1.542	2.08

Min: minute, sec: second, Da: Daltons.

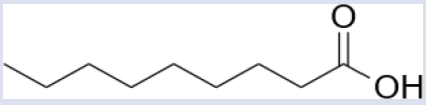
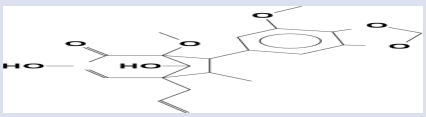
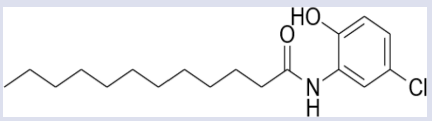
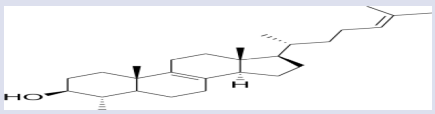
There were three compounds whose peaks were too small to be detected in the chromatogram, the table below give the details of the predicted compounds with their retention time, the peak area for the compounds were not calculated but were analysed and predicted through mass spectrum.

Table 2: Details of Compounds Identified through GC-MS without Peak Area.

Peak	Retention time (min)	Name of the compound	Structure	Molecular formula	Molecular weight (Da)
1	15.899	2-methyl, Undecane		$C_{12}H_{26}$	170
2	16.939	Hexadecanal		$C_{16}H_{32}O$	240
3	18.030	Triacontanoic acid, methyl ester		$C_{31}H_{62}O_2$	466

The table represent the methanolic extracts of ten bioactive compounds from *Gracilaria corticata* with their structure, molecular formula and molecular weightwith high peak areas and intensities.

Table 3: Essential Details of Phytochemicals from Methanol Extract.

S. No	Name of the compound	Structure	Molecular formula	Molecular weight (Da)
1	Oxirane, decyl-		$C_{12}H_{24}O$	184.32
2	n-hexadecanoic acid		$C_{16}H_{32}O_2$	256.43
3	Eicosanoic acid		$C_{20}H_{40}O_2$	312.53
4	Nonanoic acid		$C_9H_{18}O_2$	158.23
5	Oleic acid		$C_{18}H_{34}O_2$	282.47
6	Oleic acid		$C_{18}H_{34}O2$	282.47
7	Pentadecanoic acid		$C_{15}H_{30}O_2$	242.39
8	Bicyclo[3.2.1]oct-3-en-2-one, 3,8-dihydroxy-1-methoxy-7-(7-methoxy-1,3-benzodioxol-5-yl)-6-methyl-5		$C_{21}H_{24}O_7$	388.41
9	N-(5-chloro-2-hydroxyphenyl)dodecanamide		$C_{18}H_{28}ClNO_2$	325.87
10	Cholesta-8,24-dien-3-ol, 4-methyl-, (3.beta.,4. alpha.)-		$C_{28}H_{46}O$	398.00

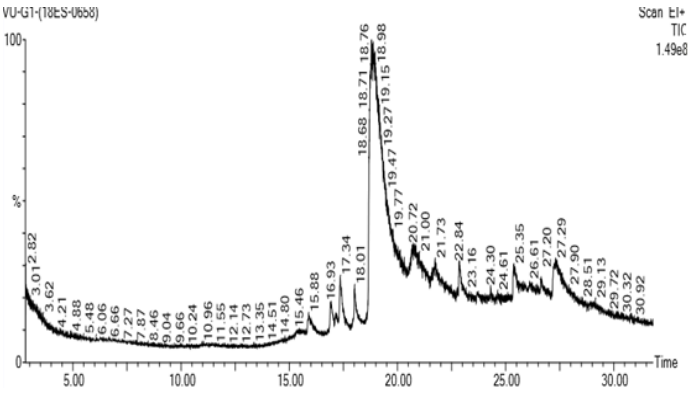


Figure 1: Qualitative report of GC-MS analysis (Area % vs Retention time) of methanol extract of *Gracilaria corticata*.

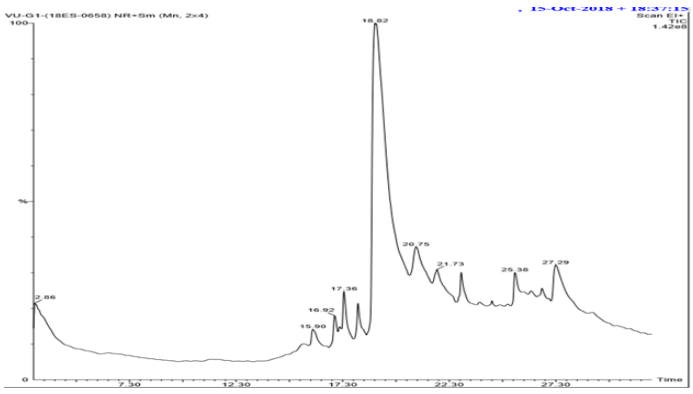


Figure 2: Chromatogram of methanol extract of *Gracilaria corticata*.

Figure 3: Spectrum of Oxirane, decyl-.

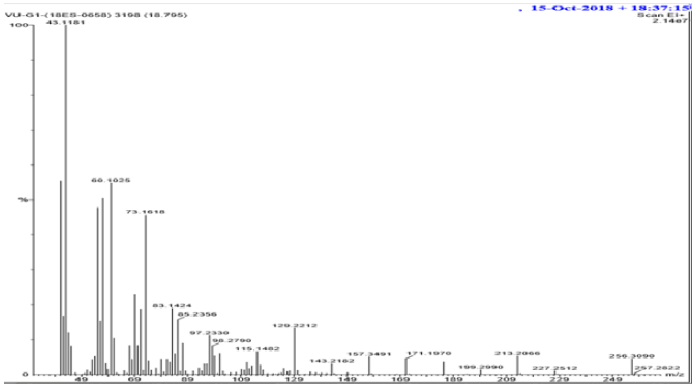


Figure 4: Spectrum of n-hexadecanoic acid.

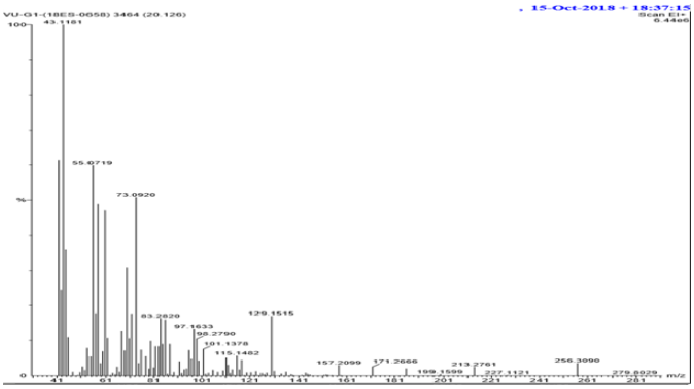


Figure 5: Spectrum of eicosanoic acid.

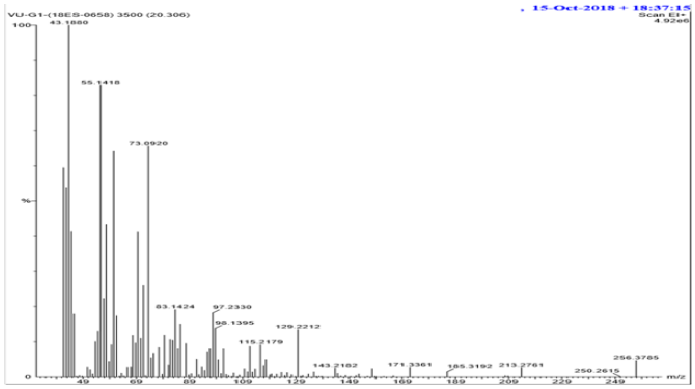


Figure 6: Spectrum of nonanoic acid.

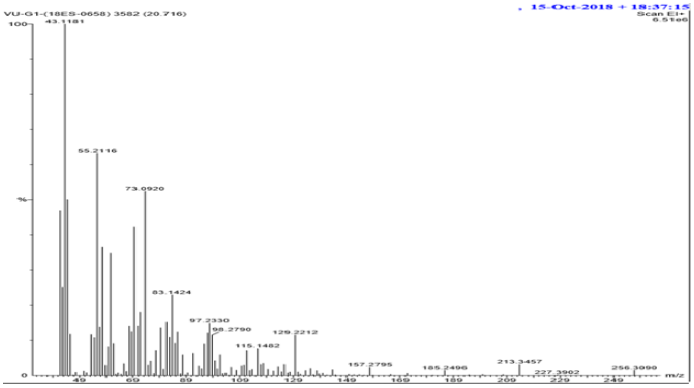


Figure 7: Spectrum of oleic acid.

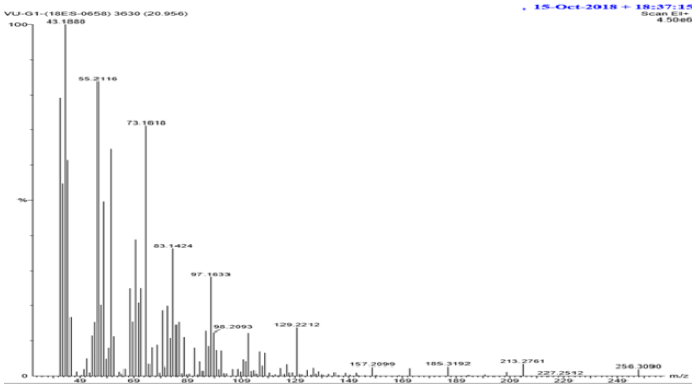


Figure 8: Spectrum of oleic acid.

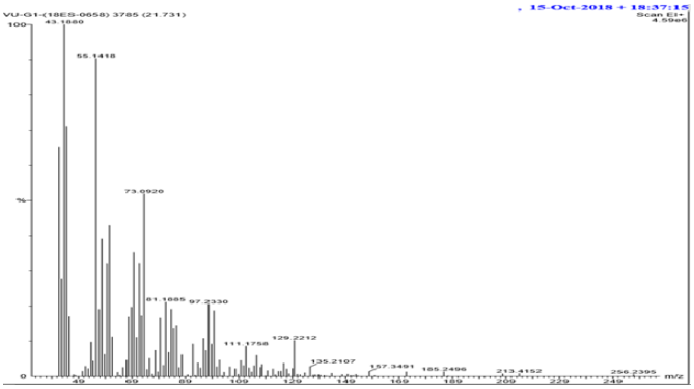


Figure 9: Spectrum of pentadecanoic acid.

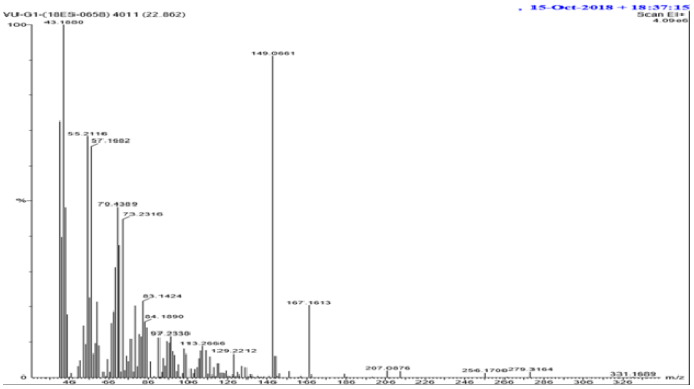


Figure 10: Spectrum of Bicyclo[3.2.1]oct-3-en-2-one, 3,8-dihydroxy-1-methoxy-7-(7-methoxy-1,3-benzodioxol-5-yl)-6-methyl-5.

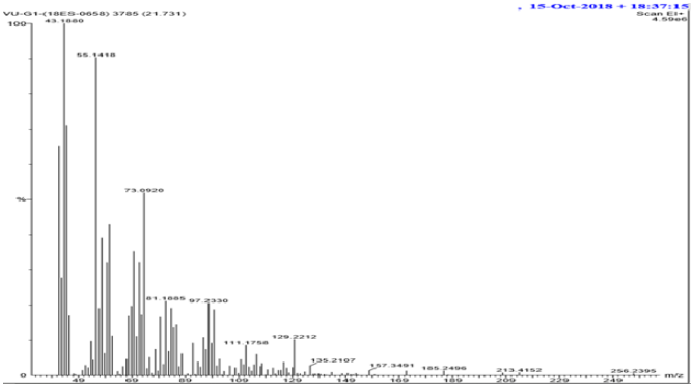


Figure 11: Spectrum of N-(5-chloro-2-hydroxyphenyl) dodecanamide.

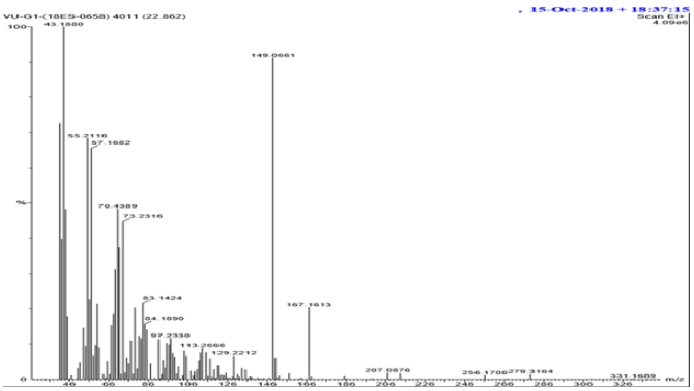


Figure 12: Spectrum of Cholesta-8,24-dien-3-ol, 4-methyl-, (3.beta.,4.alpha)-.

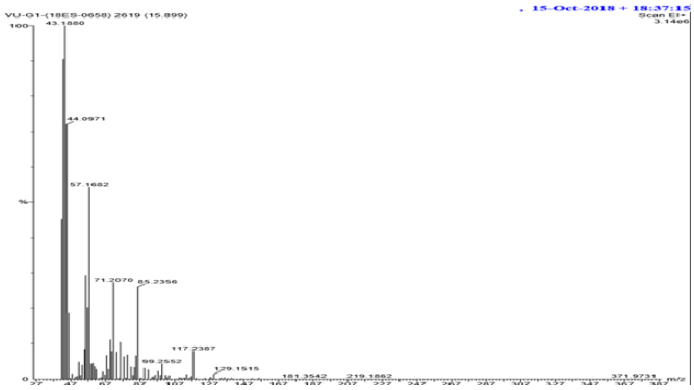


Figure 13: Spectrum of undecane, 2-methyl-.

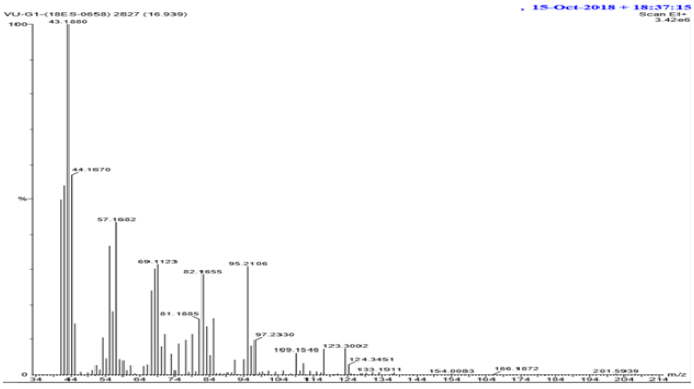


Figure 14: Spectrum of hexadecanal.

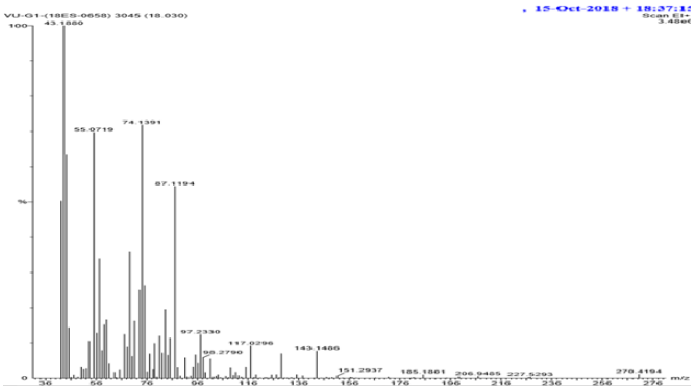


Figure 15: Spectrum of triacontanoic acid, methyl ester.

pentadecanoic acid that have reported to possess high antibacterial, antifungal and antioxidant properties (Sermakkani and Thangapandian, 2012; Elizabeth and Arumugam, 2014).[20-28] Methyl esters such as n-hexadecanoic acid (Palmitic acid), eicosanoic acid (Arachidic acid), oleic acid and pentadecanoic acid have shown potential to inhibit various bacterial pathogensand polymorphic fungal species such as *Bacillus pumilis, Escherichia coli, Micrococcus luteus, Klebsiella pneumoniae, Staphylococcusaureus, Pseudomonas aeruginosa, Candida albicans, candida tropicalis* and *Candida krusei*.[33] *Candida albicans*is a pathogenic fungal strain that causes various life-threatening skin infections, candidiasis and oral infections[34] were inhibited by methyl esters. Free saturated fatty acids like n-hexadecanoic acid, eicosanoic acid, oleic acid and pentadecanoic acid have shown immense potential to inhibit the growth of gram-positive bacteria such as *Streptococcus mutans* and polymorphic fungi

Table 4: Activity of phytochemicals from methanol extracts of *Gracilaria corticata*

S.No	Name of the compound	Reference	Reference number	Property
1	Oxirane, decyl-	-	-	-
2	n-hexadecanoic acid	Sermakkani *et al.*, 2012; Elezabeth *et al.*, 2014; Zheng *et al.*, 2005	20-22	Preservative, antioxidant, nematicide, pesticide, lubricant, antipsychotic, antiandrogenic
3	Eicosanoic acid	Pinto *et al.*, 2017; Sahin *et al.*, 2006	23, 24	Antibacterial, antifungal, antioxidant
4	Nonanoic acid	ORHAN *et al.*, 2011	25	Antibacterial
5	Oleic acid	Walter *et al.*, 2004; El-Din and El-Ahwany, 2016; Liu *et al.*, 2017	26-28	Antibacterial, antiviral, antioxidant, antifungal
6	Oleic acid	Walter *et al.*, 2004; El-Din and El-Ahwany, 2016; Liu *et al.*, 2017	26-28	Antibacterial, antiviral, antioxidant, antifungal
7	Pentadecanoic acid	Liu *et al.*, 2017	28	Antioxidant
8	Bicyclo[3.2.1]oct-3-en-2-one, 3,8-dihydroxy-1-methoxy-7-(7-methoxy-1,3-benzodioxol-5-yl)-6-methyl-5	Geethalakshmi *et al.*, 2013	29	Antifungal, antiparasitic
9	N-(5-chloro-2-hydroxyphenyl) dodecanamide	-	-	-
10	Cholesta-8,24-dien-3-ol, 4-methyl-, (3.beta.,4. alpha.)-	-	-	-
11	Undecane, 2-methyl-	Iauk *et al.*, 2015	30	Antimicrobial
12	Hexadecanal	Kumar *et al.*, 2015	31	Antibacterial, antioxidant
13	Triacontanoic acid, methyl ester	Sermakkani and Thangapandian, 2012	32	Antibacterial

Candida albicans that are an important causative agent for oral infections such as dental caries, gingivitis and periodontitis in humans. These studies were performed *in-vitro* through MIC studies.[35] Fortunately, the fatty acids have shown to inhibit the methicillin-resistant *Staphylococcus aureus*, *Helicobacter pyroli* and *Candida albicans*.[36-37] The fatty acids have shown potential to inhibit without any side effects. Bicyclo[3.2.1]oct-3-en-2-one, 3,8-dihydroxy-1-methoxy-7-(7-methoxy-1,3-benzodioxol-5-yl)-6-methyl-5 also known as medioresinol has shown inhibition against leishmanias is by producing Reactive Oxygen Species (ROS) and free radicals and causing the death of the parasite. Medioresinol also has antifungal property. The endophytic fungi *Candida albicans* responsible for skin and oral infections were killed.[38] The medioresinol acts by producing reactive oxygen species inside the cell and inhibit the cell cycle regulation and increase the chance for apoptosis. Medioresinol induces the ROS resulting in the increased oxidative stress inside the cell and ultimately leading the mitochondrial dysfunction and activating the pro-apoptotic factors. The pro-apoptotic factor proteins like BAK and BAX causes pores in the mitochondria resulting in the release of cytochrome c and cause apoptosis. This results in the DNA fragmentation causing the lysis of the fungi.[38] Hexadecanal, also known as palmitaldehyde have shown to resist the growth of pathogenic microorganisms such as *Staphylococcus aureus, Bacillus subtilis* and *Pseudomonas aeruginosa*. Ethyl acetate root extract of the plant *Dorema ammoniacum* contained high levels of hexadecanal and also has antioxidant properties in both the DPPH and FRAP assays.[39]

CONCLUSION

Marine seaweed *Gracilaria corticata* has several immense properties against pathogenic bacteria, fungi, viruses and parasites. The presence of essential bioactive compounds from the algae are responsible for these properties. Novel antimicrobial cream and pharmaceutical products are developed day to day using this species. The algae possess antioxidant potential in the free radicals scavenging. The presence of diverse essential components such as amino acids, polysaccharides, vitamins, minerals, carrageenan makes them more suitable to be included in the diet. The presence of carrageenan, sulphated polysaccharides and agar make them to find a spot in the cosmetics and food industries. The presence of pigments such as phycoerythrin and phycocyanin are added benefit for their application in the food colour industry. The presence of negatively charged functional groups such as sulphates, phosphates, carboxylic acids, phenols and alcohols on the surface of the algae makes the algae to be best suited for the bio sorption process. The presence of negatively charged functional group senable them to attract the positive charged cationic dyeslike crystal violet, methylene blue and toxic heavy metals like Cd^{2+}, Pb^{2+}, As^{2+}, Hg^{2+}, Cr^{+6} and Ti.[40-43] Hence, the algaecan be employed in the dye decolorization process and sewage treatment sector. Hence, the *Gracilaria corticata* are the nature's gift to mankind.

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CONFLICT OF INTEREST

No conflict of interests declared from the author's side.

ABBREVIATIONS

GC-MS: Gas chromatography-mass spectrometry, **CSIR-CSMCRI:** Central Salt Marine Chemical Research Institute, **SIF:** Sophisticated instru-

mentation facility, **NMR:** Nuclear magnetic resonance, **NIST:** National institute of standards and technology-2008, **mL:** Millilitre, **min:** minute, **sec:** second, **Da:** Daltons.

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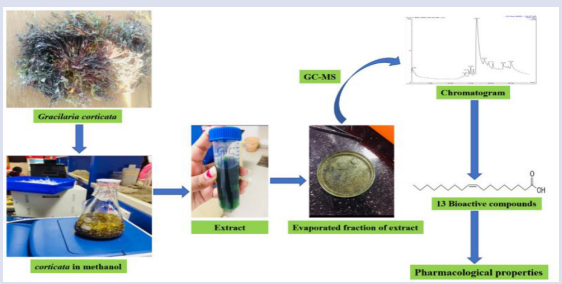
GRAPHICAL ABSTRACT



SUMMARY

- The main motive of the work was to reveal the various bioactive compounds from red macroalgae *Gracilaria corticata*. The methanolic extracts of *Gracilaria corticata* was analysed using the GC-MS (Perkin Elmer, Clarus 680). The analysis revealed the presence of thirteen bioactive compounds. The present work reported the thirteen compounds along with their potential activities like antibacterial, antifungal, antioxidant, antiparasitic etc. Hence, *Gracilaria corticata* can be used in the drug discovery and development sector towards the development of novel therapeutics.

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