Profiling of Bioactive Constituents of *Sargassum wightii* Greville ex J. Agardh by GC-MS Analysis

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ABSTRACT
The work is to profile and characterize the bioactive phytochemical compounds in the marine brown seaweed, *Sargassum wightii* by GC-MS analysis. The three different solvents such as methanol, ethyl acetate and chloroform were used for the extraction of compounds from the seaweed. More number of bioactive compounds was identified from all the three extracts. The methanol extract showed maximum number of compounds followed by ethyl acetate and chloroform extracts. The identified compounds from the extracts comprised of alkaloids, phenols, terpenoids, flavonoids, fatty acids, ketone, benzimidazole derivatives, ethylene analogues, aminopyrimidine derivatives, benzofuran derivatives, synthetic non-steroidal estrogen, akuammiline derivatives, benzoxazepine derivatives and cyclic organic compounds.

Keywords: Bioactive compounds, Extract, GC-MS analysis, Retention Time, Seaweed.

INTRODUCTION

Active phytochemical constituents synthesized by the marine seaweed are used in traditional and complementary medicine (Neelamathi and Kannan, 2015). Even though bioactive secondary metabolite exists in various plant species, a small proportion of them have been examined and sustained as a significant source of bioactive agents. Phytochemicals such as flavonoids, tannins, saponins, alkaloids and terpenoids are known to have several biological properties which includes antioxidant, anti-inflammatory, anti-diarrhea, anti-ulcer and anticancer activities (Starlin *et al*., 2019).

Thus, phytochemicals are vital in pharmaceutical industry for development of new drugs and for the preparation of therapeutic agents (Nisha *et al*., 2011). The screening of plant extracts is a new approach to find therapeutically active compounds in various plant species (Gopalakrishnan and Udayakumar, 2014; Starlin *et al*., 2019). For the search for new compounds, development of suitable screening methods is very important (Keskes *et al*., 2017). The initial screening of medicinal plants by spectrometric and chromatographic methods provides basic information on chemical and pharmacological activities, which help to select the biologically active plants (Juszczak *et al*., 2019).

Gas Chromatography and Mass Spectrometry (GC-MS) has commonly been employed for detection of functional groups and identification of various bioactive therapeutic compounds that are present in plants (Satapute *et al*., 2019; Fan *et al*., 2018). It plays an essential role in the phytochemical analysis and chemotaxonomic studies (Hethelyi *et al*., 1987), and it can be an interesting tool for testing the amount of active principles for preparing cosmetics, drugs, pharmaceutical or food industry, environmental and forensic applications (Uma *et al*., 2009). It is one of the best, fast and accurate techniques to detect various compounds (Razack *et al*., 2015) and requires a small volume of plant extracts. Gas chromatography separates the components of the mixture and mass spectroscopy analyzes each of the components separately.

Marine seaweeds are a rich source of functionally diversified bioactive compounds that play an active role in human nutrition and health (Palani *et al*., 2022). Seaweeds are considered as a vital component in the marine ecosystem providing shelter, nursery grounds and food sources for various organisms (Moghadamtousi *et al*., 2014; Cotas *et al*., 2020; Arguelles and Sapin, 2021). Due to the diverse chemical compounds reported from seaweeds more attention has been given to the potential of these organisms as novel sources of bioactive compounds for food, agriculture and pharmaceutical applications (Arguelles, 2022). The bioactive phytochemical compounds obtained from the different solvent extracts of *Sargassum wightii* were previously reported from various coastal regions. So the aim of this work is to find out the bioactive secondary metabolites from *Sargassum wightii* that was collected from the coastal region of Arockiapuram, Kanniyakumari.

MATERIALS AND METHODS

Collection, Identification and Preparation of Seaweed Extracts

Fresh samples of *Sargassum wightii* were collected from the coastal region of Arockiapuram, Kanniyakumari District and identified by algal experts. The samples were initially washed with sea water and then rinsed thoroughly in distilled water. The washed samples were air dried and ground to fine powder and stored in an airtight container for further analysis.
The dried powdered samples (25gm) were immersed separately in 50 ml of different organic solvents (methanol, ethyl acetate and chloroform) in a separate airtight conical flask for 2 days for the successive extraction of solvent extracts. The extracts were filtered and the filtrate was collected into sterile airtight bottle and stored in a refrigerator for further use (Arokiyaraj *et al*., 2009; Rebecca *et al*., 2012). The stored extract was then subjected to GC-MS analysis.

GC-MS Analysis for Phytochemical Constituents

A high resolution mass spectrum equipped with a data system in combination with Gas Chromatography was used for the chemical analysis of seaweeds. GC-MS analysis of the extracts were carried out by the following method of Hema *et al*., (2010) using a GC-MS Clarus 500 Perkin Elmer system and gas chromatography interfaced to mass spectrometer (GC-MS). The detection of the compounds was employed with the NIST (National Institute of Standards and Technology). The relative % amount was calculated by comparing its peak area to the total areas. Software adopted to handle mass spectra and chromatogram was Turbomass (Version 5.2).

Identification of Phytocompounds

Interpretation on mass spectrum of GC-MS analysis was done using the database of National Institute of Standard and Technology (NIST). The mass spectrum of the unknown compounds was compared with the spectrum of the known compounds stored in the NIST Library (Version, 2005).

RESULT AND DISCUSSION

Phytochemicals are secondary metabolites from plants that are essential for the plant defence against grazing animals and other predators (Ragunathan *et al*., 2019). These active constituents synthesized by the marine seaweeds are used in traditional and complementary medicine for curing illness as these claimed to produce fewer side effects (Tyagi and Bohra, 2002). GC-MS technique provides the identification and quantification of chemical compounds based on their characteristic fragmentation patterns at specific retention times (Ragunath *et al*., 2020). Active components responsible for various biological activities could be evaluated by investigating the chemical composition of each extract using GC-MS analysis (Nazarudin *et al*., 2020).
In the present investigation, the bioactive compounds present in the three different solvent extracts of *Sargassum wightii* were identified by GC-MS analysis. The GC-MS analysis of methanol, ethyl acetate and chloroform extracts revealed the presence of different bioactive compounds. A total of 14 peaks were observed with different retention time was identified in methanol extract followed by ethyl acetate and chloroform extracts. The compounds present in the extracts were identified after the comparison of the Mass Spectra with NIST Library. The identification of active principles was assured by observing their retention time (RT), molecular formula, molecular weight and peak area percentage are presented in Table (1, 2 & 3) and Figure (1, 2 & 3).

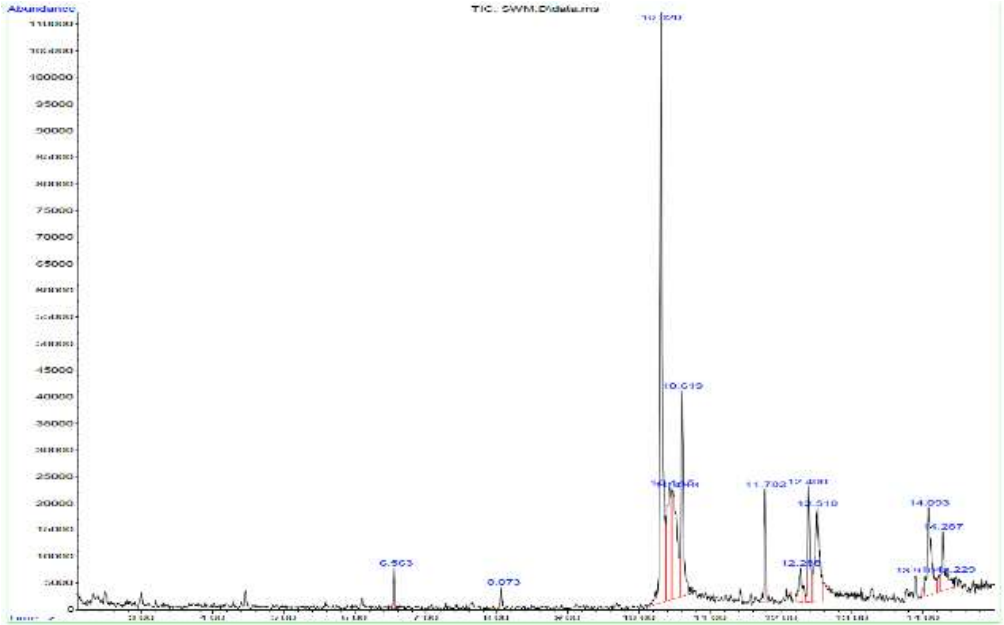

Figure.1. GC-MS Chromatogram of Methanol Extract of *Sargassum wightii*

Table.1. Bioactive Compounds Identified in the Methanol Extract of *Sargassum wightii*

S.No.	Retention Time (Min.)	Name of the Compound	Molecular Formula	Molecular Weight (m/z) (g/mol)	Peak Area (%)
1.	6.563	Cycloheptanol, 2-methylene	$C_8H_{14}O$	126.20 g/mol	1.22
2.	8.073	2(4H)-Benzofuranone, 5,6,7,7a-tetrahydro- 4,4,7a-trimethyl-, (R)-	$C_{11}H_{16}O_2$	180.2435 g/mol	0.90
3.	10.320	Bicyclo [4.3.0] nonan-2-one, 8-isopropylidene-	$C_{12}H_{18}O$	178.27 g/mol	29.88
4.	10.445	9H-Fluorene, 1-methyl-	$C_{14}H_{12}$	180.24 g/mol	9.73
5.	10.483	Diethylstilbestrol	$C_{18}H_{20}O_2$	268.3 g/mol	12.52
6.	10.619	3(2H)- Benzofuranone, 7-hydroxy-2,2-dimethyl-	$C_{10}H_{10}O_3$	178.18 g/mol	10.55
7.	11.782	4′-(Trifluoromethyl)acetophenone	$C_9H_7F_3O$	188.15 g/mol	3.94
8.	12.288	Pyrimidine, 2,4-diamino-6-ethyl-5-phenyl	$C_{12}H_{14}N_4$	214.27 g/mol	2.42
9.	12.400	Tridecanoic acid	$C_{13}H_{26}O_2$	214.34 g/mol	5.68
10.	12.518	Phenytoin	$C_{15}H_{12}N_2O_2$	252.27 g/mol	9.39
11.	13.903	3-Hydroxymethylene-1,7,7-trimethylbicyclo [2.2.1]heptan-2-one	$C_{11}H_{16}O_2$	180 g/mol	0.99
12.	14.093	1-Hexyl-2-nitrocyclohexane	$C_{12}H_{23}NO_2$	213.32 g/mol	7.42
13.	14.229	1H-1,3-Benzimidazole-2-carboxylic acid, 1-methyl-	$C_9H_8N_2O_2$	176.17 g/mol	0.75
14.	14.287	2-Hydroxyethyl vinyl sulfide	C_4H_8OS	104.17 g/mol	4.60

Figure.2. GC-MS Chromatogram of Ethyl Acetate Extract of *Sargassum wightii*

Table.2. Bioactive Compounds Identified in the Ethyl Acetate Extract of *Sargassum wightii*

S.No.	Retention Time (Min.)	Name of the Compound	Molecular Formula	Molecular Weight (m/z) (g/mol)	Peak Area (%)
1.	11.668	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256.42 g/mol	13.29
2.	13.356	Cyclopentane, cyclopropylidene	C_8H_{12}	108.18 g/mol	22.92
3.	13.391	Cyclohexa-2,5-diene-1,4-dione, 2-methyl-5-(4-morpholinyl)-	$C_{11}H_{13}NO_3$	207.23 g/mol	7.23
4.	14.802	Propiophenone, 2'-(trimethylsiloxy)-	$C_{12}H_{18}O_2Si$	222.35 g/mol	12.21
5.	14.901	2-Ethylacridine	$C_{15}H_{13}N$	207.27 g/mol	4.38
6.	14.942	2-Methyl-7-phenylindole	$C_{15}H_{13}N$	207.27 g/mol	5.49

7.	15.064	5H-Cyclohepta[b]pyridine-3-carbonitrile, 6,7,8,9-tetrahydro-2-amino-4-(2-fluorophenyl)-	$C_{11}H_{12}N_2$	172 g/mol	4.97
8.	18.832	Benz[b]-1,4-oxazepine-4(5H)-thione, 2,3-dihydro-2,8-dimethyl-	$C_{11}H_{13}NOS$	207.29 g/mol	29.51

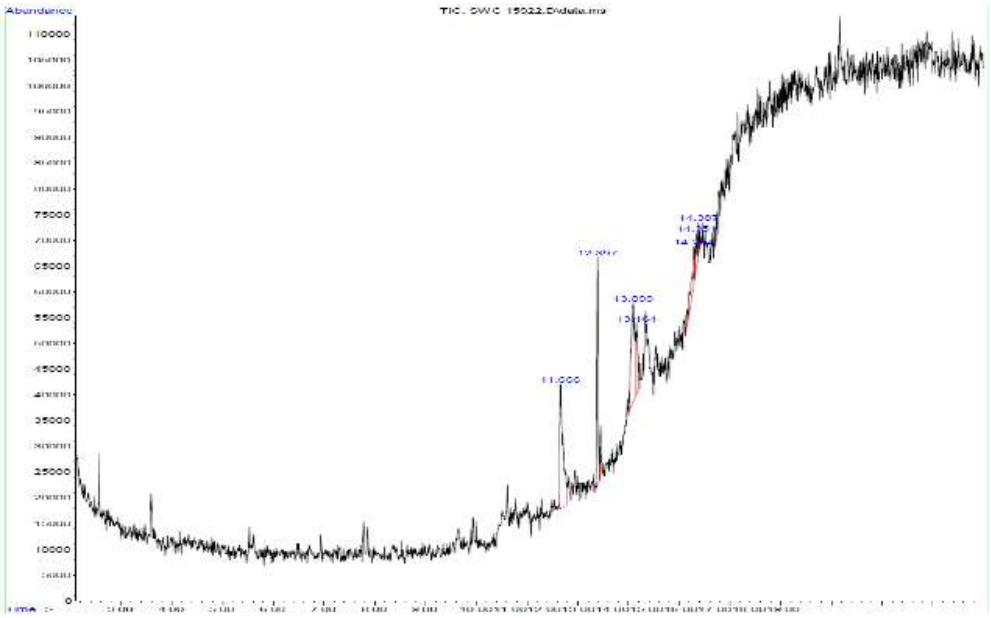

Figure.3. GC-MS Chromatogram of Chloroform Extract of *Sargassum wightii*

Table.3. Bioactive Compounds Identified in the Chloroform Extract of *Sargassum wightii*

S.No.	Retention Time (Min.)	Name of the Compound	Molecular Formula	Molecular Weight (m/z) (g/mol)	Peak Area (%)
1.	11.666	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256.42 g/mol	28.70
2.	12.397	1,4,8-Dodecatriene, (E,E,E)-	$C_{12}H_{18}$	162.27 g/mol	19.86
3.	13.099	4-Dehydroxy-N-(4,5-methylenedioxy-2-nitrobenzylidene)tyramine	$C_{16}H_{14}N_2O_4$	298.29 g/mol	26.90
4.	13.164	Akuammilan-16-carboxylic acid, 17-(acetyloxy)- methyl ester, (16R)-	$C_{23}H_{26}N_2O_4$	394.47 g/mol	9.59
5.	14.294	Indole-2-one, 2,3-dihydro-N-hydroxy-4-methoxy-3,3-dimethyl-	$C_{11}H_{13}NO_3$	207.23 g/mol	8.56
6.	14.351	2-Methyl-7-phenylindole	$C_{15}H_{13}N$	207.27 g/mol	4.08
7.	14.387	2-Ethylacridine	$C_{15}H_{13}N$	207.27 g/mol	2.32

The maximum numbers of phytochemical constituents were identified from the methanol extract of *Sargassum wightii* followed by ethyl acetate and chloroform extracts. Previous studies also reported the presence of more number of bioactive compounds from the methanol extract of *Sargassum wightii* (Rajeswari & Jeyaprakash, 2018; Deepak *et al*., 2017). Anitha *et al*. (2019) stated that the methanol extract was seemed to be effective in extractions as it could extract the bioactive substances from the seaweed efficiently which supports with the present investigation. From the earlier reports, the identified active phytocompounds were already proven to possess different pharmacological activities (Deepika, 2019; Pandurangan *et al*., 2010; Eluvakkal *et al*., 2010; Celikler, 2008).

The identified bioactive compounds from the studied seaweed extracts was previously found to show numerous bioactive phytochemical compounds belonging to various classes such as alkaloid, phenolic compound, terpenoids, flavonoids, fatty acid, ketone, benzimidazole derivative, ethylene analogues, aminopyrimidine derivative, benzofuran derivatives, synthetic non-steroidal estrogen, akuammiline derivatives, benzoxazepine derivatives and cyclic organic compounds. Previous researchers also reported that the seaweeds are tremendous source of compounds such as polysaccharides, tannins, flavonoids, phenolic acids, bromophenols, carotenoids and these compounds were responsible for different biological activities (Sheela and Uthayakumari, 2013; Mahabaleshwara *et al*., 2016; Zaha *et al*., 2016; Rodriguez *et al*., 2010).

Generally, the bioactive compounds obtained from the seaweeds reported to have many uses in different fields of agriculture, pharmaceuticals, biotechnology and industrial fields (Michalak and Chojnacka, 2015; Guiheneuf *et al*., 2016). The phytochemical compounds obtained from the solvent extracts of *Sargassum wightii* were already proved to have various biological activities (Muthukrishnan *et al*., 2022; Govindan, 2021; Vijayakumari and Raj, 2019; Kavitha and Mohideen, 2017). Hence, the compounds identified in the study may also have these biological activities.

CONCLUSION

From the present research investigation, it was concluded that the methanol extract of *Sargassum wightii* revealed the presence of more number of bioactive phytochemical compounds compared to other solvent extracts and it was identified as the most appropriate solvent to extract the bioactive compounds that was confirmed by GC-MS analysis. Therefore, the bioactive secondary metabolites may be used as a potential drug in pharmaceutical industries.

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