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Cycloheptasiloxane, tetradecamethyl identified from petroleum ether fraction of *Sargassum wightii*by Gas Chromatography – Mass Spectrometry Analysis.

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Abstract

In the present study, the phytoconstituents from *Sargassum wightii* were isolated, identified and characterized by FTIR and Gas Chromatography –Mass Spectrometry. The individual isolated phytochemicals were identified by comparing their mass spectrometric data and retention times of published data. Saponins and flavonoids were predominantly identified in aqueous extract of *Sargassum wightii*. Flavonoids have been widely employed as anticancer, antibacterial, antiviral, antiangiogenic, antimalarial, antioxidant, neuroprotective, antitumor, and anti-proliferative compounds.Flavonoids are an essential component in a variety of nutraceutical, pharmacological, medical, and cosmetic uses and are linked to a wide range of health-promoting properties. This paper presents FTIR analysis and complete GCMS profile and MS spectrometric data of brown algae (*Sargassum wightii).*Cycloheptasiloxane, tetradecamethyl identified from petroleum ether fraction of *Sargassum wightii.*

Key words: GC-MS,Cycloheptasiloxane, tetradecamethyl, *Sargassum wightii*, FTIR.

Introduction

In many Asian countries, seaweed has historically been utilized as a food source, while elsewhere it is widely employed as a source of biochemicals for culinary, pharmaceutical, and cosmetic purposes [1]. The Seaweeds are an important source of biologically active compounds including polysaccharides, carotenoids, phycobilins, fatty acids, vitamins, sterols, tocopherol and phycocyanins among others [2]. Seaweeds have become popular since they do not use arable land or fresh water, and generate high quantities of biomass without lignin components [2]. Since 2014, seaweed aquaculture, which corresponds to 97% of its production, generated approximately USD 5.6 trillion, with Asian countries being the main producers [2]. Brown seaweed is a potentially renewable resource in the marine environment. It is one of the significantly farmed algae species along the Asia coast and used as traditional medicine [3].Seaweeds are commercially available macroalgae known for its higher number of bioactive secondary metabolite content [4]. These are used as a raw material for the production of agar, algin and carrageenan in industries[4]. Agar is a jelly-like substance made of polysaccharides that are produced from the cell walls of some species of red algae.Brown seaweeds contain a particular kind of carbohydrate called algin. Algin is a food ingredient.Red edible seaweeds contain a family of naturally occurring linear sulfated polysaccharides called carrageenans. It is grow in light and high oxygen rich environment [4]. It contain several bioactive components such as phenolic compounds, carotenoids, sulphated polysaccharides, dietary fiber, vitamins, minerals and proteins[4].These possess properties such as anti-viral, anti-bacterial, anti-allergic, anti-coagulant[5].It also contain lower content of lipids and higher content of dietary fibers and thus helps to aid chronic gastrointestinal (GI) diseases and metabolic diseases[5]. This is because of their higher dietary content which provides bulk to low calorie foods[5].There are a lot of new bioactive secondary metabolites that are isolated from seaweeds and these compounds are usually tested using different biological assays to determine their anticancer, anti-inflammatory, antiviral, antihypertensive, antibacterial, or antidiabetic properties [6,7,8]. A type of fucoserich sulfated hetero-polysaccharide compounds termed fucoidan, which is found in several marine invertebrates and the cell walls of brown seaweed, have long been used as dietary fiber in many Asian nations [10]. Plants growing on land do not contain fucoidan [11].

A large genus of brown sea algae in the family Sargassaceae is called sargassum. Meroterpenoids, phlorotannins, fucoidans, sterols, and glycolipids are a few of the well-known bioactive substances found in sargassum. It is said to have anti-inflammatory, anticancer, antimicrobial, neuroprotective, hypolipidemic, hypoglycemic, anticoagulant, antimelanogenic, and hepatoprotective properties. *Sargassum wightii*, one of the species belonging to the brown algae contains antioxidant and hence are used as feed for animals, ingredient for food and fertilizer in agriculture[4].The marine brown alga *Sargassum wightii* (*S. wightii*) is widely distributed along the Indian coastline. It is highly branched, dark brown, and 21–40 cm tall. The midrib is spherical to ellipsoidal, 5-8 mm long, and 2-4 mm wide.This seaweed is thought to be edible, and its dry material contains substantial levels of carbohydrates (30–60%), including cellulose, fucoidan, laminaran, and alginic acid, as well as relatively low amounts of fat (5%), protein (15.8%) and ash (27.5%) [21,22]. This seaweed is used by residents of Jeju Island (Republic of Korea) to make salads, soups (Mom-Guk), and side dishes [23,24,25].This species contains various phytochemical constituents such as tannins, flavonoids, terpenoids, triterpenoids, steroids, saponins, alkaloids, polyphenols, glycosides, carbohydrates and proteins[9]. *Sargassum wightii* has several medicinal properties such as antibacterial, anti-alzheimer, anti-asthmatic and anti-tumor properties[26].

In the present study, the phytochemical constituents from *Sargassum wightii* is obtained in high purity and characterized using column chromatography, HPLC, FT-IR and GC-MS.

Materials and Methods

Samples

The *Sargassum wightii* was collected from the Ramaeshwarm, Tamil Nadu, India. The plant was identified and authenticated (No.BSI/SRC/5/23/2011-12/Tech.1486) by Botanical survey of India (BSI), Tamil Nadu Agricultural University (TNAU), Coimbatore, India. The bran was collected and was stored at -20°C.

Sample extraction

The powdered seaweed samples were soaked with ethanolsolvent at room temperature for 48 h and filtered through Whatman filter paper under vacuum. Thecrude extracts were stored at -20°C until analyzed.

Column Chromatography

Using a silica gel column with a diameter of 40 mm, 2 g of the seaweed sample was purified. The concentrated filtrates were then loaded onto silica column (1.5 x 40 cm)(sigma). In ascending order of polarity, petroleum ether (100%), chloroform (100%), ethyl acetate (100%), and methanol (100%) were used to elute phytoconstituents in different fractions. [27].

Thin – Layer Chromatography

When placed onto the activated silica gel TLC plates (20 cm x 20 cm), the fractions 1-6thand 16–18thfielded one distinct spots. By subjecting the plate to iodine vapors, a single spot was discoveredunder UV light, which was further subjected to characterizationby FTIR and GC-MS analyses[27].

Fourier Transform Infrared Spectroscopy

The soxhlet apparatus was used to prepare the *S. wightii* powder samples for FTIR, with 70% ethanol serving as the organic solvent for extraction. For the extraction process, 50g of the sample and 500ml of ethanol were used, and concentrated for about 7 hours to produce the concentrated extract of *S. wightii*. 400 mg of potassium bromide of FTIR grade was combined with 4 mg of the material to form a pellet. The Perkin Elmer System One: FTIR at room temperature was used to record the infrared reflectance vibrational spectra in the 4000-450 cm-1 region while the pellet was instantly placed in the sample container [38].

Gas Chromatography and Mass Spectrometry

Vacuum filtration was adopted to filter the *S. wightii* extract using a Durapore-HV membrane filter disc with a 2.5 cm diameter and 0.45 m pore size. After that, the filtrate was transferred into a 1.5 ml eppendorf tube and put in liquid nitrogen to freeze. Once samples were frozen, they were kept at 80 °C until metabolite extraction. The filter was submerged in 1 ml of 90% (v/v) methanol containing 0.1 g mL^{-1} U-13C-sorbitol, vortexed for about 5 seconds, then withdrawn from the eppendorf tube and the remaining solution centrifuged at 20,000g for 5 minutes at 4°C to extract the metabolites and dried by usinga vacuum concentrator (SpeedVac concentrator, Thermo, Waltham, MA). Using a JEOL GCMS equipment, a gas chromatography-mass spectrometry (GC-MS) analysis was carried out (JMS-GCMATE II, Japan). Helium was employed as the carrier gas at a concentration of 1.51 mL for 1 min in a fused hyperSep silica capillary column (30 m X 0.25 mm X 0.25 m) GC column. At a voltage of 70 eV, the mass spectrometer was run in the electron impact mode. The programmed oven temperature was initially 70°C for three minutes, rising to 250°C for the final 14 minutes, while the injector temperature was 250°C. The start and end times for the GCMS were 5 and 35 minutes, respectively. The mass spectra obtained were compared with NIST libraries (NIST 11- Mass Spectral Library 2011 edition), with an acceptance requirement of a match above a critical factor of 80%, to identify the molecules based on the peak in the crude *Sargassum wightii* extract [38].

Results

Column Chromatography

The active fractions were isolated and separated by column chromatography by ascending order of polarity, petroleum ether (100%), chloroform (100%), ethyl acetate (100%), and methanol (100%) were used to elute different fractions. (Table I). Around 62 fractions were collected from the silica column, two active fractions from petroleum ether and chloroform solvents were received. The two active fractions sample 1 and sample 2 were characterized by FTIR and GCMS analyses.

Table:I Separation of active components from column chromatography

S.No.	Solvent system	Fraction No.
1.	Petroleum ether	1-9
2.	Chloroform	10-30
3.	Ethyl acetate	31-60
4.	Methanol	61 and 62

Fourier Transform Infrared Spectroscopy

The FTIR spectrum analysis is used to identify the functional groups corresponding to its absorption frequencies. The spectrum obtained between 400 - 3000 cm^{-1} can be used to analyse the structural features of petroleum ether extract of *Sargassum wightii* including glycosidic bonds and functional groups. The FTIR analysis of petroleum ether extract of *Sargassum wightii* showed (Figure I) bands at2954.40, 2848.16, 1730.75, 1462.40,1377.07,1273.11,1073.49, 719.31cm-1 (Table II). The FTIR analysis of chloroform extract of *Sargassum wightii* (Figure II) showed bands at 3565.11, 2955.92, 2922.71, 2161.36, 1599.00, 1379.40, 1366.37, 1279.93, 1173.16, 1122.62, 1072.50, 1040.07, 1016.84,862.02, 771.31 cm-1 (Table III).

Figure:I FTIR analysis of petroleum ether extract of *Sargassum wightii*

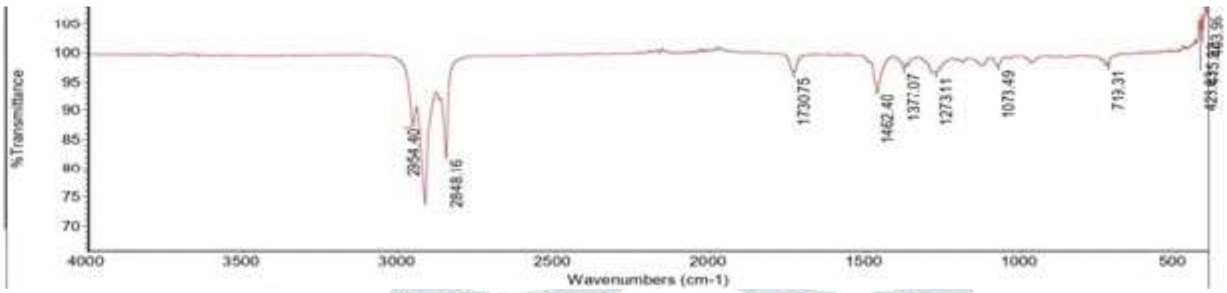

Table II: Functional group identification using FTIR absorption frequencies for petroleum ether extract of *Sargassum wightii*

S. No	Absorption characters	Vibration type	Functional groups
1	2954.40	Stretch	Alkane (C-H)
2	2848.16	Stretch	Alkane (C-H)
3	1730.75	Stretch	Carbonyl (C=O)
4	1462.40	Stretch	Aromatic (C=C)
5	1377.07	Bending	Alkane (-C-H)
6	1273.11	Stretch	Amine (C-N)
7	1073.49	Stretch	Alcohol (C-O)
8	719.31	Bending	Alkene (=C-H)

Figure:II FTIR analysis of choloform extract of *Sargassum wightii*

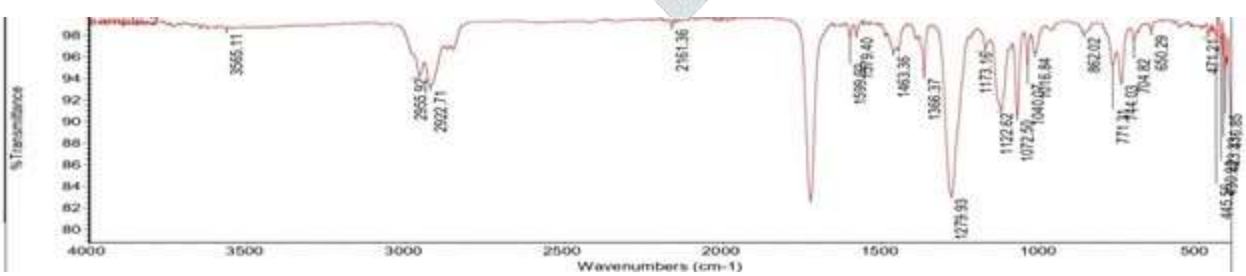

Table III: Functional group identification using FTIR absorption frequencies for chloroform extract of *Sargassum wightii*

S.no	Absorption frequency (cm^{-1})	Vibration type	Functional group
1	3565.11	Stretch, H-bonded	Alcohol (O-H)
2	2955.92	Stretch	Alkane (C-H)
3	2922.71	Stretch	Acid (O-H)
4	2161.36	Stretch	Alkyne (-C=C-)
5	1599.00	Stretch	Aromatic (C=C)
6	1379.40	Bending	Alkane (-C-H)
7	1366.37	Bending	Alkane (-C-H)
8	1279.93	Stretch	Amine (C-N)
9	1173.16	Stretch	Amine (C-N)
10	1122.62	Stretch	Amine (C-N)
11	1072.50	Stretch	Alcohol (O-H)
12	1040.07	Stretch	Ester (C-O)
13	1016.84	Stretch	Ester (C-O)
14	862.02	Bending	Alkene (=C-H)
15	771.31	Bending	Alkene (=C-H)

Gas Chromatography and Mass Spectrometry Analysis

Gas Chromatography and Mass Spectrometry analysis for the compounds isolated from *Sargassum wightii*(brown seaweed) of petroleum ether fraction wereDecane, 4-ethyl-; Hexadecane, 7,9-dimethyl-;Decane, 3,7-dimethyl-;Heptadecane, 2,6,10,14-tetramethyl; Benzene, 1,3-bis(1,1-dimethylethyl;2,4-Dimethyldodecane; Tridecane, 1-iodo-;Cyclohexasiloxane, dodecamethyl-;Dodecane;Pentadecane; Cycloheptasiloxane, tetradecamethy;Octane, 2,4,6-trimethyl-; Heptadecane, 9-octyl- by interpreting the GC-MS spectrum (Figure III) using the NIST library (Table IV). Gas Chromatography and Mass Spectrometry analysis for the compounds isolated from *Sargassum wightii* (brown seaweed) of chloroform fraction were Trichloromethane; 2-Octen-4-one, 2-methyl-;3-Methylbut-2-enoic acid, tetrahydropyran-2-yl ester;Benzene, 1,3-bis(1,1-dimethylethyl)-;Trichloroacetic acid, undecyleste;1-Decanol, 2-methyl-;Undecane, 4-ethyl-; Phenol, 2,4-bis(1,1-dimethylethyl);Ethyl 2-(methylamino)-1-phenyl-3-c yclohexene-1-carboxylate;7-Hexadecene, (Z)- by interpreting the GC-MS spectrum (Figure IV) using the NIST library (Table V).

Figure:III – GC-MS analysis and identification of components from petroleum ether extract of *Sargassum wightii*

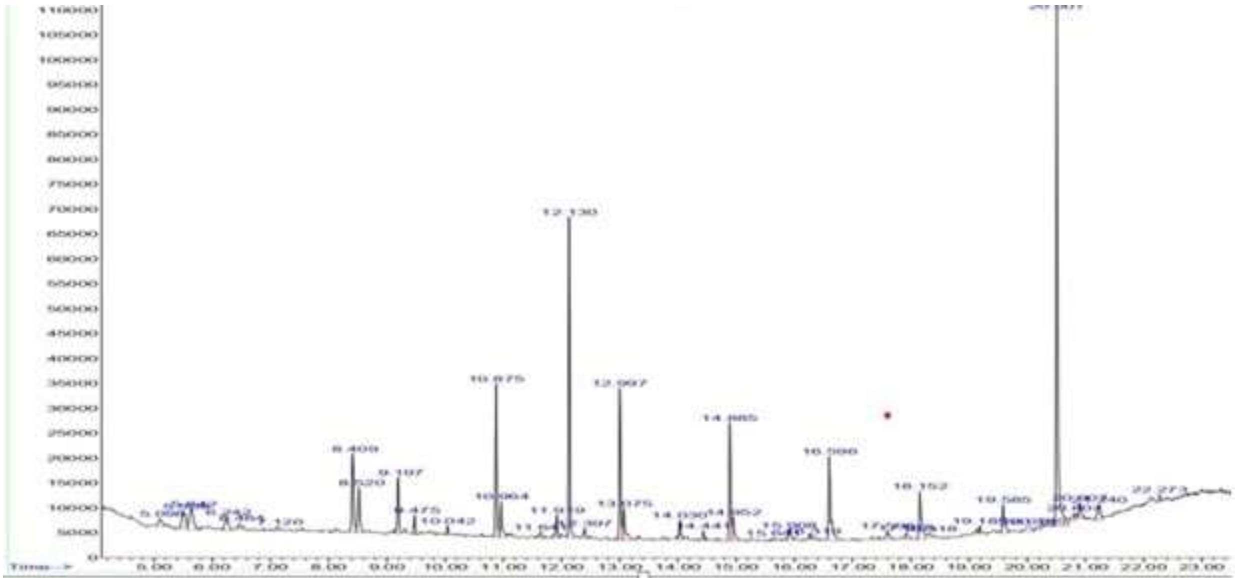

Table IV: GC-MS analysis and identification of components from petroleum ether extract of *Sargassum wightii*

S.No	Compound	RT	Area%
1.	Decane, 4-ethyl-	6.786	3.04
2.	Hexadecane, 7,9-dimethyl-	6.864	1.09
3.	Decane, 3,7-dimethyl-	7.375	1.33
4.	Heptadecane, 2,6,10,14-tetramethyl	8.808	0.91
5.	Benzene, 1,3-bis(1,1-dimethylethyl	9.297	13.93
6.	2,4-Dimethyldodecane	9.408	1.70
7.	Tridecane, 1-iodo-	9.564	3.34
8.	Cyclohexasiloxane, dodecamethyl-	9.786	2.91
9.	Dodecane	10.108	1.70
10.	Pentadecane	11.119	0.90
11.	Cycloheptasiloxane, tetradecamethyl	11.630	4.20
12.	Octane, 2,4,6-trimethyl-	11.941	4.82
13.	Heptadecane, 9-octyl-	12.008	2.05

Figure:IV – GC-MS analysis and identification of components from chloroform extract of *Sargassum wightii*

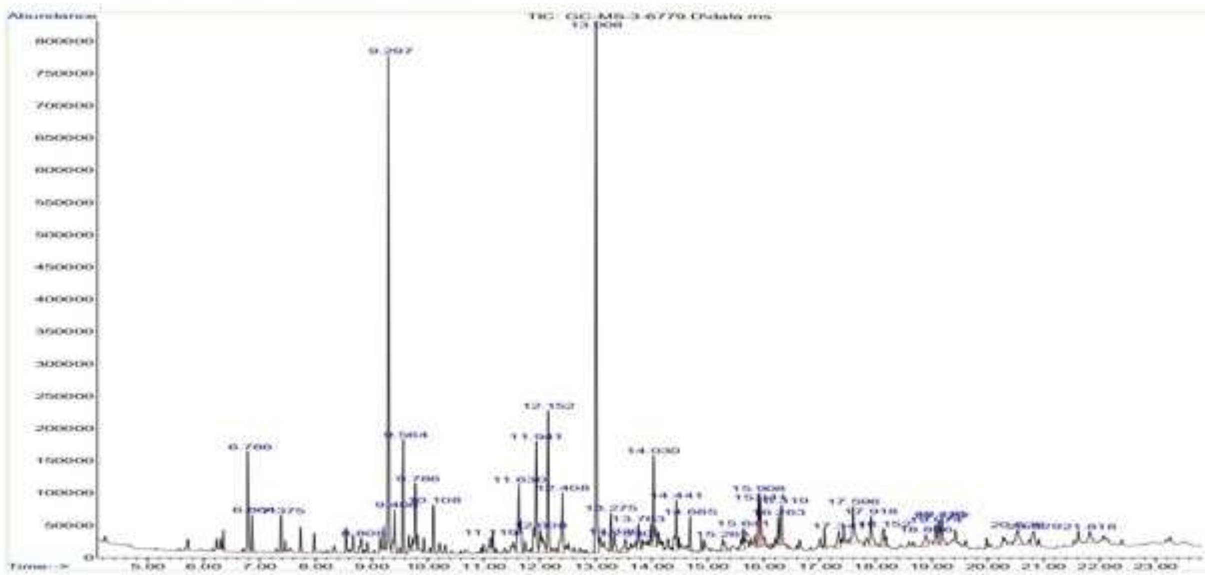

Table V: GC-MS analysis and identification of components from chloroform extract of *Sargassum wightii*

S.no	Compound	RT	Area%
1.	Trichloromethane	5.098	5.098
2.	2-Octen-4-one, 2-methyl-	5.509	1.90
3.	3-Methylbut-2-enoic acid, tetrahydropyran-2-yl ester	8.520	2.55
4.	Benzene, 1,3-bis(1,1-dimethylethyl)-	9.197	2.82
5.	Trichloroacetic acid, undecyleste	10.875	5.94
6.	1-Decanol, 2-methyl-	10.964	1.98
7.	Undecane, 4-ethyl-	11.919	1.04
8.	Phenol, 2,4-bis(1,1-dimethylethyl)	12.130	10.99
9.	Ethyl 2-(methylamino)-1-phenyl-3-c yclohexene-1-carboxylate	12.397	0.74
10.	7-Hexadecene, (Z)-	12.997	6.73

Discussion

Fourier Transform Infrared Spectroscopy

The band at 2954.40 cm^{-1} and 2848.16 cm^{-1} corresponds to C-H stretching vibration type of alkane group [28]. The band at 1730.75 cm^{-1} corresponds to C=O stretching vibration type of carbonyl group [29]. The band at 1462.40 cm^{-1} corresponds to C=C stretching vibration type of aromatic group [33]. The band at 1377.07 cm^{-1} corresponds to -C-H bending vibration type of alkane group [34]. The band at 1273.11 cm^{-1} corresponds to C-N stretching vibration type of amine group. The band at 1073.49 cm^{-1} corresponds to C-O stretching vibration type of alcohol group [30]. The band at 719.31 cm^{-1} corresponds to =C-H bending vibration type of alkene group [35]. The spectrum obtained between 800-4000 cm^{-1} can be used to analyse the structural features of chloroform extract of *Sargassum wightii* including glycosidic bonds and functional groups.

The band at 3565.11 cm^{-1} corresponds to O-H hydrogen bonded stretching vibration type of alcohol group [31]. The band at 2955.92 cm^{-1} corresponds to C-H stretching vibration type of alkane group [28]. The band at 2922.71 cm^{-1} corresponds to O-H stretching vibration type of acid group [36,37]. The band at 2161.36 cm^{-1} corresponds to -C=C- stretching vibration type of alkyne group. The band at 1599.00 cm^{-1} corresponds to C=C stretching vibration type of aromatic group [33]. The band at 1379.40 cm^{-1} and 1366.37 cm^{-1} corresponds to -C-H bending vibration type of alkane group [34]. The band at 1279.93 cm^{-1}, 1173.16 cm^{-1} and 1122.62 cm^{-1} corresponds to C-N stretching vibration type of amine group. The band at 1072.50 cm^{-1} corresponds to O-H stretching vibration type of alcohol group[34]. The band at 1040.07 cm^{-1} and 1016.84 cm^{-1} corresponds to C-O stretching vibration type of ester group [32, 34]. The band at 862.02 cm^{-1} and 771.31 cm^{-1} corresponds to =C-H bending vibration type of alkene group [35].

Gas Chromatography and Mass Spectrometry Analysis

The chromatogram of the compounds from *S.wightii* of petroleum ether fraction showed hexadecanoic acid with retention time of 6.864. Balachandran et al, 2016 reported the presence of hexadecanoic acid with retention time of 12.60 which was reported in our GC-MS NIST library. The chromatogram of the compounds from *S.wightii* of chloroform fraction showed hexadecenoic acid with retention time of 12.997. Deepak et al, 2017 reported the presence of hexadecenoic acid with retention time of 14.096 which matches with our GC-MS results and have good antibacterial, antioxidant, antifungal, nematicide, anti-inflammatory, hypocholesterolaemia, pesticide, anti-androgenic flavor, hemolytic, 5-α reductase inhibitor and mosquito larvicide [39]. The GC-MS analysis of petroleum ether and chloroform fractions indicated the presence of 40 and 40 peaks respectively (Figure I and II). The compound Benzene, 1,3-bis (1,1-dimethylethyl) with retention time 9.297 from *Sargassum wightti* of petroleum ether and chloroform fractions. Anna Masek et al, 2014 reported the presence of benzene with molecular weight of 78 in GC-MS analysis from Hops (*Humuluslupulus L.)*Benzene, 1,3-bis (1,1-dimethylethyl) was the first report from *S.wightii* in petroleum ether and chloroform fractions [40]. The compound Cyclohexasiloxane, dodecamethyl (M.wt 444) with retention time 9.786, Cycloheptasiloxane, tetradecamethyl (M.wt 518) with retention time 11.630 from *Sargassum wightti* of petroleum ether fraction. Varsha et al, 2014 reported the presence of Cyclohexasiloxane, dodecamethyl with retention time of 9.116 and Cycloheptasiloxane, tetradecamethyl with retention time of 11.862 in the spectrum of GC-MS of leaf extract *Toddaliaasiatica (L.)*[41].Cyclohexasiloxane, dodecamethyl and Cycloheptasiloxane, tetradecamethyl was found to be the first report in *S.wightii*. Eman et al, 2021 reportedCycloheptasiloxane, tetradecamethyl in *Chlorella vulgaris*[42]. Manorenjetha et al, 2013 reported Cycloheptasiloxane, tetradecamethyl have good antibacterial, antifungal, antifouling, immunomodulatory and antitumour activities. Ethyl-2-(methylamino)-1-phenyl-3-cyclohexane-1-carboxylate, Trichloroacetic acid, 2-Octen-4-1-2-methyl- are the compounds reported for the first time in *S.wightii*[43]. Venkatesan et al, 2023 reported that the Methenolone with retention time 29.26 and peak area 21.61 in *S.wightii* [44]. Vaikundamoorthy et al, 2018 reported the galactofuranose with retention time 40.914 and peak area 64.63 and Arabinose with retention time 36.426 and peak area 11.25 in *S.wightii* polysaccharides fractions [45]. Yuvaraj et al, 2014 reported that the Methylenebis (2, 4, 6-triisopropylphenylphosphine) with retention time 33.27 and peak area 09.47 and 9- Octadecenamide with retention time 32.87 and peak area 11.40 in *S.wightii* [46]. Pramitha et al, 2016 reported that the Adamantine,1-Isothiocyanato-3-methyl- with retention time 281 and peak area 207.335, Indole-2-one, 2,3-dihydro-N-hydroxy-4-methoxy3,3-dimethyl with retention time 281 and peak area 207.335 and Stigmasta-5,24(28)-dien-3-ol, (3.beta)- with retention time 281 and peak area 412.69 in *S.wightii* [47]. The above compounds was also found to have good biological activities like dopamine reuptake inhibition (NMDA antagonist), removes warts on skin, flavours for confectionary in dairy products and beverages (Table VI).

Table VI: Biological activity of compounds identified in *Sargassum wightii*

Name of compound	Biological activity
Ethyl 2-(methylamino)-1-phenyl-3-c yclohexene-1-carboxylate	NMDA antagonist (Dopamine reuptake inhibitor)
Trichloroacetic acid, undecyleste	Cosmetics (Removes warts on skin)
2-Octen-4-one, 2-methyl-	Flavours for confectionary in dairy products and beverages
Cycloheptasiloxane, tetradecamethyl	Antibacterial, antifungal, antifouling, immunomodulatory and antitumour activities

Conclusion

In the present study 13 and 10 chemical constituent identified from petroleum ether and chloroform fractions of *Sargassum wightii* respectively. Further studies have to be performed for the compounds identified by GC-MS analysis by docking analysis and interaction with NF-kB α receptor for the treatment of inflammation, pain and stomach ulceration.

This work provides evidence for the isolation, characterization, and metabolite profiling of the bioactive compounds in *Sargassum wightii*. The isolation and purification of ethanol extract of brown seaweed showed the presence of these compounds were found to be first reported in *Sargassum wightii*in petroleum ether and chloroform respectively. Several ailments, including ulcer, hypertension, diabetes, and inflammatory disorders, can be treated with the separated compounds, bringing up new opportunities for this seaweed's application in the global algae sector.

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Conflicts of Interest

The authors declare no conflict of interest.

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