

GC-MS profiling and characterization of *Sargassum prismaticum*.

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Abstract

To profile and characterize the bioactive constituents present in chloroform extract of *Sargassum prismaticum* using gas chromatography mass spectrometry (GC-MS) analysis. 10 gm sample of seaweed was extracted with chloroform by continuous shaking in dark room for 36 hrs. The concentrated seaweed extract were subjected to GC-MS analysis. The qualitative determination of the various bioactive phytochemicals from crude extract of *S. prismaticum* using gas chromatography-mass spectrometry revealed different types of high and low molecular weight chemical entities with varying quantities present in chloroform extract. These chemical compounds are considered biologically and pharmacologically important. Furthermore, the chloroform extract possess unique physicochemical characteristics which may be attributed to the compounds naturally present in significant quantities in the *S. prismaticum*. Thus, identification of different biologically active compounds in the extract of *S. prismaticum* justifies further biological and pharmacological studies.

Keywords: *S. prismaticum*, GC-MS analysis, Seaweed, Phytochemicals.

Introduction

Since ancient times, marine algae has been closely connected with human life and has been thoroughly used in several ways as a resource of food, feed, fertilizer and medicine, and chiefly used for economically important phycocolloids [1-2].

Marine macro algae are also considered as ecologically and biologically significant, contribute substantially to marine primary production, and provide a habitat for near shore benthic local communities [3-4]. They also contain protein, iodine, vitamins and substances of stimulatory and antibiotic nature. Marine algae, apart from their valuable proteins carrying essential amino acids, dietary fiber, essential fatty acids, minerals and vitamins, are also a fine source of phenolic compounds [5-6]. Marine algae are abundant and potentially renewable resources that are currently being explored for their bioactive compounds [7]. The phytochemicals from marine algae are broadly used in various industries such as food, confectionary, textile, pharmaceutical, dairy and paper, mostly as gelling, stabilizing and thickening agents. Seaweeds or marine macro algae are renewable living resources that are also used as food, feed and fertilizer in many parts of the world.

They are rich resources of structurally diverse bioactive phytochemicals with great pharmaceutical and biomedical prospective [8-9]. The bioactive compounds from the seaweeds are known to possess antibacterial, antifungal, antiviral, antimalarial, anticancer and antifouling properties [10]. Many studies have reported that seaweeds are a potential source of natural antioxidants [11]. The challenges in combating new diseases and resistant strains of microorganisms always demand the development of novel therapeutic agents. The attention in marine organisms as a prospective and shows potential source of pharmaceutical agents has increased during recent years [12].

Sargassum has been used traditionally for treating scrofula, goiter, tumor, edema, testicular pain, and swelling [13]. Algin is a carbohydrate present in *Sargassum* is used in textile, paper and pharmaceutical industries [14]. The proximate composition of *Sargassum* species are varied from species to species. Based on their environments, they contain 12-16% proteins, and 1.5-2.0% lipids [15-17].

In the present study, the selected brown seaweed, *Sargassum prismaticum* was subjected to chloroform

extractions in order to determine their phytochemicals. The GC-MS metabolite profiling of crude chloroform extract from *S. prismaticum* identifies its bioactive compounds defining its immunological properties. Hence, the present study was focused at GC-MS analysis of active compounds present in the chloroform extract of the marine brown algae, *Sargassum prismaticum*.

Methodology

Collection of seaweeds

Fresh seaweed *S. prismaticum* was collected from intertidal regions of mahim beach, dist Palghar during the month of December 2018. The collected seaweed sample was cleaned with the seawater until unwanted impurities and adhering sand particles were removed. Seaweed sample was shade dried for 7-8 days. The dried seaweed samples were powdered using mixer and it was then stored in refrigerator for further study.

Seaweed extraction

The *S. prismaticum* powdered sample (10 gm) was successively extracted with HPLC grade chloroform using cold extraction method. The sample was kept in dark room for 36 hrs with continuous shaking. The extract was collected and filtered using whattman No.1. It was evaporated to dryness by a rotavap. The final residue obtained was stored at 4 °C until further use. The volatile bioactive compounds present in chloroform extracts of the seaweeds were identified by GC-MS characterization.

GC-MS analysis

GC MS analysis of chloroform extract of *S. prismaticum* was carried out with Agilent 7890 system and gas chromatography interfaced to mass spectrometer (GC-MS) employing the following conditions: HP 5 column [(5%-Phenyl)-methylpolysiloxane] (30mm x0.25mmx 0.25 µm] thickness, Helium gas (99.999%) was used as a carrier gas at a constant flow of 1ml/min and injection volume of 1µl was employed (split ratio of 10:1) operating in electron impact mode at 70eV; injector temperature 2500C; Ion -source temperature 2800C. The

oven temperature was programmed from 800C (isothermal for 1 min), with an increase of 100C/min, to 2000C then 50C /min to 2800C ending with 9 min, isothermal at 2800C. Mass spectra were taken at 70eV; a scan interval of 0.5 seconds and fragments vary from 45-550 Da. Total GC running time was 35 minutes.

Identification of chemical constituents

The resulting peaks were analyzed with the database of National Institute Standard and technology (NIST) a library, which has more than 62,000 patterns. The spectrum of the unknown component was compared with the spectrum of the known components stored in it. The Name, Molecular weight and structure of the

components of the *S. prismaticum* sample was determined.

Results and Discussions

The volatile bioactive compounds present in Chloroform extract of *Sargassum prismaticum* were identified by GC-MS analysis. The Chloroform extracts of seaweeds indicated the presence of various types of phytocompounds (Table 1) screened under different retention time (R.T). A total of 11 peaks were observed with retention times for *S. prismaticum* respectively, as shown in Fig. 1.

Table: 1 Compounds identified in the Chloroform extract of *Sargassum prismaticum* by GC MS analysis.

Extract	RT (min)	Name of compound	Molecular formula	Molecular weight (g/mol)	Peak %
<i>Sargassum Chloroform</i>	9.3	Undecane, 3,8-dimethyl-	C ₁₃ H ₂₈	184.3614	8.697911
	9.94	Phenol, 2,4-bis(1,1-dimethylethyl)	C ₁₄ H ₂₂ O	206.3239	12.97308
	12.35	Sulfurous acid, 2-ethylhexyl hexyl ester	C ₁₄ H ₃₀ O ₃ S	278.451	13.11305
	14.15	Phytol	C ₂₀ H ₄₀ O	296.539	9.252038
	15.43	Dodecane,1-iodo-	C ₁₂ H ₂₅ I	296.236	8.790573
	19.42	L-Norvaline, N-(2-methoxyethoxycarbonyl)-, tetradecyl ester	C ₂₃ H ₄₅ NO ₅	415	15.5111
	25.12	2-Bromotetradecane	C ₁₄ H ₂₉ Br	277.29	6.574454
	25.52	Tridecane, 1-iodo-	C ₁₃ H ₂₇ I	310	4.346197
	27.23	Decane, 2,9-dimethyl-	C ₁₂ H ₂₆	170.3348	5.00967
	30.05	Octadecane, 3-ethyl-5-(2-ethylbutyl)-	C ₂₆ H ₅₄	366.7070	4.291383
	31.85	Dodecane, 1-iodo-	C ₁₂ H ₂₅ I	296.2314	11.44055

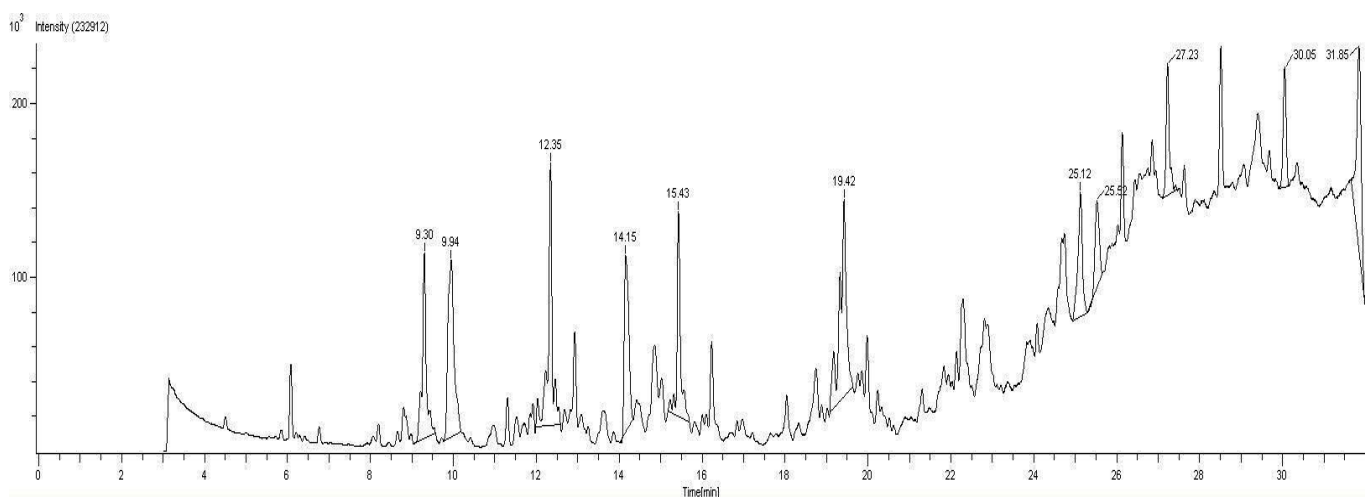


Figure 1: GC-MS chromatogram of the crude Chloroform extract of *Sargassum prismaticum*.

Chemical constituents were identified using spectral database NIST 11 software installed in the GC-MS. The compounds prediction is based on Pub Chem [18]. The compound name with RT, Molecular formula, Molecular weight and concentration % in the Chloroform extract of seaweeds *Sargassum prismaticum* are presented in (Table. 1). The major chemical constituents of crude extract of chloroform were Sulfurous acid, 2-ethylhexyl hexyl ester (RT = 12.35 min, 13.11 %) respectively. Seaweeds exhibit a high level of fatty acids compound which show potential bioactivity (Manilal *et al.*, 2010). So in the present study, biological activity *S. prismaticum* expressed the compounds like Undecane, 3,8-dimethyl-, Phenol, 2,4-bis (1,1-dimethyl-ethyl), Sulfurous acid, 2-ethylhexyl hexyl ester, Phytol, Dodecane, 1-iodo-, L-Norvaline, N-(2-methoxyethoxycarbonyl)-, tetradecyl ester, 2-Bromotetradecane, Tridecane, 1-iodo-, Decane, 2,9-dimethyl-, Dodecane, 1-iodo-, Octadecane, 3-ethyl-5-(2-ethylbutyl)- etc. Earlier, studies by kumar *et al.*, (2013) reported that GC MS analysis of *Sargassum tenerrimum* revealed the presence of different types of bioactive phytochemicals. The seaweeds have been widely recognized as producers of a wide range of bioactive compounds including polyunsaturated fatty acids, flavonoids, terpenoids, alkaloids, quinones, sterols, polyketides, phlorotannins, polysaccharide, glycerols, peptides and lipids [21] that have antimicrobial [22], anti-inflammatory [23], antiviral [24], antioxidant [25], anticancer activities [26]. Hence, the results prove that this algal species is an excellent source of bioactive compounds with a wide variety of application.

Conclusion

From the results, it could be concluded that *S. prismaticum* contains various bioactive compounds. Therefore, it is suggested as seaweed of phytopharmaceutical importance. Further studies are required to isolate individual phytoconstituents involved in the bioactivity and pharmaceuticals application. Further research is necessary to identify and purify the active compounds responsible for therapeutic activity.

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