ISSN (Print 0974-2115) (Online 2349-8552)
www.jchps.com Journal of Chemical and Pharmaceutical Sciences

Analysis of Biogenic Molecules in *Sargassum Wightii* Extract using Gas Chromatography – Mass Spectrometry

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ABSTRACT

Seaweeds are prospective renewable resource contains many biogenic compounds. In this investigation found secondary metabolite constituents in *Sargassum wightii* belong to phenolic and flavanoid compounds. Thin layer chromatography analysis report of current study showed 5 distinct phenolic and 8 distinct flavanoid compounds. The chemical constituents of seaweeds are also characterized through GC-MS and their spectrums investigation exposed the presence of 19 different compounds. Among those, hexadecanoic acid, 9,12,15 Octadecatrienoic acid, 3,7,11,15 Tetra methyl-2-hexdecen-1-ol, Heptadecanoicacid,9,12-Octadecadienoic acid,1,2-Benzenedicarboxylic acid are very significant. There are some reports insisting that the specific efficiency of some molecule presents in marine algae but in this study reference to this analytical report initiated to extend a novel formulation of medicine with multipurpose. Additional work to be expanded to find the pharmacokinetics of formulation developed from seaweeds that is useful in future to treat various diseases and develop the drugs in pharmaceutical industries.

KEY WORDS: Seaweed, GC-MS analysis, *Sargassum wightii*, TLC, Phytochemical Compounds, Flavanoids.

1. INTRODUCTION

Plants contain rich origin of biogenic compounds useful to develop safety modern medicine. Since they contain lot of life supporting compounds used by human either directly or indirectly, also produce a wide-ranging of chemically active metabolites in their surroundings in order to care for themselves from other competitive and pathogenic organisms. These active metabolites generated by marine algae having antibacterial, antialgal, antifouling and antifungal properties (Smit, 2004). Presently severe researches going on to use these clinically important biogenic compounds are as therapeutics and additives in healthcare products (Bhadury, 2004). Macroalgae seaweeds are recently using as potential renewable resources for medical and environmental application. These marine algae contain significant pharmacological and biological active components such as flavanoids, terpenoids, carotenoids, dietary fiber, protein, essential fatty acids, vitamins and minerals. Presently algae are used as a dietary food supplement to improve food quality in day today life (Ganesan, 2007). Also these food ingredients significantly improve health condition and restrict various health related problems.

Sargassum sp. contains good variety of secondary metabolites showing extensive range of biological responses such as antibacterial, antioxidant, anticancer, anticoagulant and antiviral properties (Vairappan, 2001; Athukorala, 2007). Related investigation in marine seaweed expressed the presence of valuable antioxidant compounds (Yan, 1998; Duan, 2005; Kuda, 2005). Considering all the above information current investigation is aimed to analyses the presence of these significant characteristic compounds in methanolic extract of *Sargassum wightii* with advanced phytochemical analysis method.

2. MATERIALS AND METHODS

Sample collection: Fresh edible brown seaweed *Sargassum wightii* was collected from the intertidal region of Mandapam area (09º7.4170N; 079º08.5580E) of south Tamilnadu, India. The samples used for analysis were brought to the laboratory in sterile plastic bags along with natural sea water. In laboratory thallus of collected sample was surface washed with running tap water to get rid of extraneous materials and then shade-dried to remove total water content. Dried plant material was chopped and grinding with an electric food processor and stored the powder at 4ºC until to use.

Extraction: 20 g of seaweed powder is taken in an Erlenmeyer flask and soaked in 100 ml of methanol solvent for 48 hrs in room temperature. The soluble compounds were extracted by filtering in a standard No.1 filter paper. The filtered solvent was condensed by using rotary evaporator under at 40ºC. This condensed product was stored in freezer for further use.

Identification of Phenolic and flavanoid compounds: The thin layer chromatography plate was prepared by pouring silica slurry on the microscopic slide and air dried. An aliquot of 20 µl of extracted sample was dotted on the silica gel plate and using the prepared solvent system of Chloroform: Ethanol: Acetic acid: Water (98:10:2:2 v/v) as mobile phase. Proper run plate was permitted to dried out. The separated compounds were visualized through developing spot by spraying the plates with formulated solution of 1% Potassium ferric cyanide and 1% Ferric chloride in water. Similarly flavanoid compound identified by using another formulated solvent of Toluene: Ethyl

acetate (4:5 v/v) as mobile phase. The separated flavonoid compound was identified by spraying of 1% Vanillin prepared in Sulphuric acid.

GC - MS Analysis of *Sargassum wightii:* GC-MS qualitative analysis was performed in Shimadzu 2010 plus model comprising with AOC-20i auto sampling and the generated graph of GC analyzed by tagged mass spectrophotometer. GC MS operated with standard column RTX 5Ms (Column diameter is 0.32mm, column length is 30m, column thickness 0.50µm), functioning in electron impact mode at 70eV; 99.9% Helium gas used as mobile phase with flow rate at a constant of 1.73 ml/min and sample loading volume of 0.5 µl (split ratio of 10:1); injecting temperature 270ºC; ion-source temperature 200ºC. The incubation temperature was set from 40ºC (isothermal for 2 min) to 250ºC with an increase rate of 8ºC/min and ending with a 20 min isothermal at 280ºC. Spectrums were taken with scan period of 0.5 seconds and molecule segments from 40 to 450 Da. Total time taken to prepare complete chromatograph is 51.25 min. The comparative percentage of every molecule was calculated by comparing their average peak area to the total areas. The conversion of analytical data to digital data was performed by Turbo Mass Ver-5.2.0.

Identification of components: The converted data base generated from GC MS analysis of *S.wightii* extract was interpreted with stored standard database of National Institute Standard and Technology (NIST). The separated components name, molecular weight and structure were generated accordingly.

3. RESULTS AND DISCUSSION

The developed TLC plate was examinate for the existence of phenolic compounds in *S.wightii* extract exposed the presence of 5 distinct colourful phenolic spots with different Rf values. The profile of phenolic compounds presented in Fig.1 and the calculated R_f values are plotted in Table.1. These polyphenolic compounds are having multiple clinical and physiological activities lead to developing a novel pharmacological aid. There are number of literature available to insist the characteristic features like pathogenic inhibition, destruction of microorganisms, reduction of triglycerides and active remedial agents for cardiovascular diseases, diabetes, cancer, stroke, anti-inflammation and anti-allergic effect (Ozcan, 2014).

Table.1. Rf values of Phenolic compounds

Colour of the spot	Rf value	Colour of the spot	Rf value
Light Greenish Blue	0.61	Light Greenish Blue	0.79
Light Greenish Blue	0.63	Light Pink	0.94
Greenish Blue	0.69		

Figure.1. TLC Analysis of Phenolic compounds

Similarly the presence of flavanoid compounds in *S. wightii* extract was detected after spraying with solution for flavanoid compound. There were 8 distinct flavanoid spots identified in the methanolic extract of *S.wightii* with different Rf values. The profile of developed flavanoid compounds presented in Fig.2 and their respective R_f values are in Table.2. The present qualitative analysis report indicating that the extract containing a number of characteristic flavonoids compound responsible for exhibiting effective antibacterial activities reported earlier researchers.

Table.2. Rf values of Flavanoids

Colour of the spot	Rf value	Colour of the spot	Rf value
Light Brown	0.36	Light Violet	0.85
Light Purple	0.58	Light Purple	0.90
Light Green	0.64	Light Brown	0.94
Light Green	0.74	Dark Green	0.98

Figure.2. TLC analysis of Flavanoids

GC-MS Profile of *Sargassum wightii:* GC-MS chromatogram of methanolic extract of *Sargassum wightii* presented in Fig.3. Nineteen compounds were found in methanolic extract. The segregated active compounds with their molecule retention time (RT), molecular formula, molecular weight (MW) and proportion (%) are presented in Table.3. The efficient activities of the identified compounds were listed in Table.4. N-Hexadecanoic acid showed highest peak followed by 1, 2-Benzenedicarboxylic Acid, Dibutyl in this chromatogram indicating their higher proportion compared with other components. Tetra decanoic acid or myristic acid is also reported in the seed of *Myristica fragrans* tropical tree and date fruits (Boukouada, 2014). Myristic acid is used as surfactant, cleansing, opacifying, emulsifying agent and thickening agent in cosmetic products (Chow Chng, 2008). Moreover all health care products including soaps, cleansing creams, lotions, hair conditioners, shaving products contains myristic acid. (Song, 2012). Micro algae species produces major constituents like tetra decanoic acid, hexadecanoic acid and octadecanoic acid methyl esters etc. (Musharraf, 2012). Twenty four chemical constituents in methanol extract of *Acanthophora spicifera* by GC-MS analysis and the major significant constituents reported by Flora and Rani (2013), are octanol, piperazine, benzoic acid and octadecenoic acid. Similar group of phytochemical reported in Adhatoda leaves (Srinivasan, 2013). Hexane extract of *Acantophora spicifera* have valuable phytochemical constitution with significant biological activity (Zakaria, 2011).

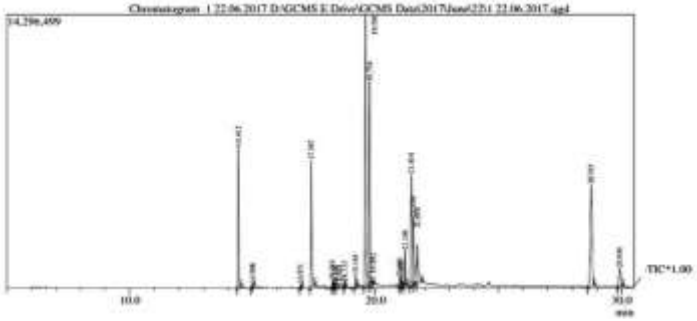
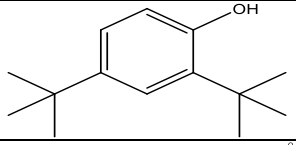
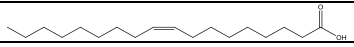
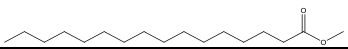
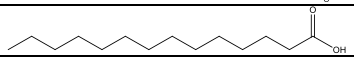
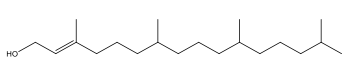
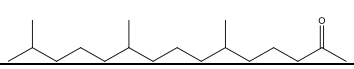
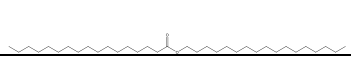


Figure.3. GC-MS Chromatogram of *Saragassumwightii*

Table.3. GC-MS analysis of Phytochemicals identified from methanolic extract of *Sargassumwightii*

S.No.	R.T	Compound Name	Compound structure	Mol. Formula	Mol. Weight	Area%
1	14.41	Phenol, 2,4-Bis(1,1-Dimethylethyl)-		$C_{14}H_{22}O$	206	8.15
2	14.99	9-Octadecenoic Acid (Z)-		$C_{18}H_{34}O_2$	282	0.35
3	6.95	Hexadecanoic Acid, Methyl Ester		$C_{17}H_{34}O_2$	270	0.21
4	17.38	Tetradecanoic Acid		$C_{14}H_{28}O_2$	228	8.90
5	18.24	3,7,11,15-Tetramethyl-2-Hexadecen-1-Ol		$C_{20}H_{40}O$	296	0.34
6	18.35	2-Pentadecanone, 6,10,14-Trimethyl-		$C_{18}H_{36}O$	268	0.15
7	18.50	Heptadecanoic Acid, Heptadecyl Ester		$C_{34}H_{68}O_2$	508	0.17

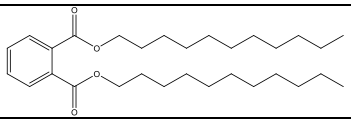
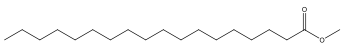
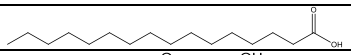
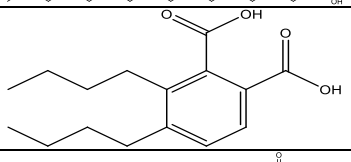
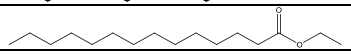
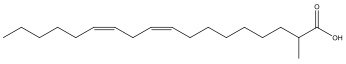
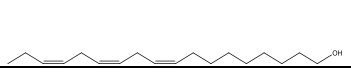
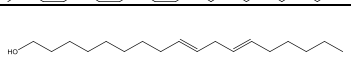
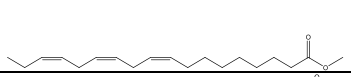
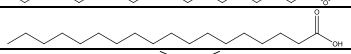
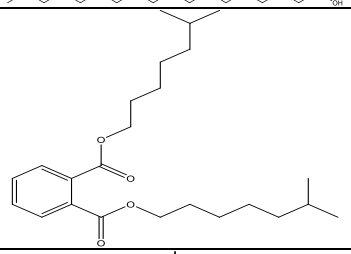
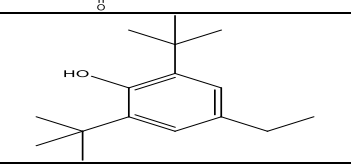
8	18.73	1,2-Benzenedicarboxylic Acid, Diundecyl Ester		$C_{30}H_{50}O_4$	474	0.32
9	19.18	Octadecanoic Acid, Methyl Ester		$C_{19}H_{38}O_2$	298	0.74
10	19.59	N-Hexadecanoic Acid		$C_{16}H_{32}O_2$	256	20.91
11	19.75	1,2-Benzenedicarboxylic Acid, Dibutyl		$C_{16}H_{22}O_4$	278	13.96
12	19.89	Tetradecanoic Acid, Ethyl Ester		$C_{16}H_{32}O_2$	256	0.20
13	20.99	9,12-Octadecadienoic Acid (Z,Z)-, Methyl		$C_{19}H_{34}O_2$	294	0.66
14	21.07	9,12,15-Octadecatrien-1-Ol, (Z,Z,Z)-		$C_{18}H_{32}O$	264	0.30
15	21.45	9,12-Octadecadien-1-Ol		$C_{18}H_{34}O$	266	13.23
16	21.53	9,12,15-Octadecatrienoic Acid, Methyl Ester, (Z,Z,Z)-		$C_{19}H_{32}O_2$	292	7.07
17	21.69	Octadecanoic Acid		$C_{18}H_{36}O_2$	284	4.52
18	28.76	Diisooctyl Phthalate		$C_{24}H_{38}O_4$	390	14.79
19	29.93	2,6-Di-Tetra-Butyl-4-Ethylphenol		$C_{16}H26O$	234	2.55

Table.4. Biological activity of Phytochemical compounds in *Sargassumwightii*

Peak	R.Time	Name of the compounds	Biological activity
1.	14.412	Phenol, 2,4-Bis(1,1-Dimethylethyl)-	Antioxidant, anti-carcinogenic, anti-inflammatory
2.	14.998	9-Octadecenoic acid (Z)-	Antihypertensive, Increase HDL and decrease LDL Cholesterol
3.	6.951	Hexadecanoicacid, Methyl ester	Antioxidant, hypocholesterolemic, Antiandrogenic, hemolytic, Alpha reductase inhibitor
4.	17.387	Tetradecanoicacid	Act as lipid anchor in biomembranes, Anxiolytic
5.	18.249	3,7,11,15-Tetramethyl-2-Hexadecen-1-ol	Antimicrobial, Anti-inflammatory, Cancer-preventive
6.	18.508	Heptadecanoicacid, Heptadecylester	Antioxidant, Pesticide, Flavor, 5-Alpha- Reductase-inhibitor, Antifibrinolytic, Hemolytic, Lubricant, Nematicide, Antialopecic
7.	19.185	Octadecanoicacid, Methyl ester	Lower LDL Cholesterol level
8.	19.591	N-Hexadecanoicacid	Antioxidant, Nematicide, 5-Alpha-Reductase-Inhibitor, Flavor, Hemolytic, Hypercholesterolemic, Antialopecic, Antiandrogenic, Antifibrinolytic
9.	19.754	1,2-Benzenedicarboxylic acid, Dibutyl	Antimicrobial, Antifouling
10.	19.892	Tetradecanoicacid, Ethyl ester	Antioxidant, Cancer preventive Nematicide, Hypocholesterolemic, Lubricant

11.	20.991	9,12-Octadecadienoic acid (Z,Z)-, Methyl	Anti-inflammatory, hypocholesterolemic cancer preventive, hepatoprotective, nematicide, insectifuge, antihistaminic, antieczemic, antiacne, 5-Alpha reductase inhibitor, antiandrogenic, antiarthritic, anticoronary, insectifuge
12.	21.536	9,12,15-Octadecatrienoic acid, Methyl ester, (Z,Z,Z)-	Hypocholesterolemic, Nematicide, Antiarthritic, Hepatoprotective, Anti androgenic, 5-Alpha reductase inhibitor, Antihistaminic, Anticoronary, Insectifuge, Antieczemic, Anticancer
13.	21.698	Octadecanoicacid	Hypocholesterolemic

The present investigated data is useful in the discovery of most beneficial aspect of seaweed *S. wightii* and in finding new product of natural medicine and other therapeutic compounds. Researches on algal species emphasis to develop a formulation have property to prevent biological oxidative damage by tracking free radicals and active oxygen molecule also controlling cancerous cell development. Though number of article available on medicinal properties of algal species, natural antioxidative property present in this seaweed attracted investigator to find an ultimate formulation for medical use. Earlier Celikler (2000), reported that seaweeds contain compounds with a relatively high pharmacological therapeutic activity useful to develop novel medicine.

4. CONCLUSION

This investigation concluded that seaweeds are the wealthy sources of structurally and biologically active biogenic molecules. The secondary metabolites created by these algae may be the potential biogenic metabolites of implication in pharmaceutical industries. The present investigation shows the existence of phenolic and flavanoid compounds with distinct Rf values of the seaweed extract for the synthesis of novel drugs. This study recommend that seaweed extract possess various phytochemical compounds they exhibit many biological activities promising a future perspective in the pharmaceutical industries. The GC-MS characterization of active compounds from *Sargassum wightii* extract exposed the presence of some major bioactive compounds such as hexadecanoic acid,9,12,15-Octadecatrienoic acid, 3,7,11,15-Tetramethyl-2-Hexadecen-1-ol etc. The marine algae might be a plentiful resource of potential corresponding and another medicine for the prevention of many degenerative diseases.

5. ACKNOWLEDGEMENT

Authors sincerely thankful to Dr.S. Velavan, Associate professor, Maruthupandiyar College of Arts & Science, Thanjavur for his kind help and support.

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