

Preliminary phytochemical screening and GC-MS analysis of *Cladophora glomerata*: green marine algae

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ABSTRACT

Background: Seaweeds since ages are excellent source of biologically active ingredients. Several Asian countries have a strong tradition of using various seaweeds in herbal medicines preparations. These plants contain various phytochemical constituents having biological activities. Seaweeds are the source of phytochemicals namely agar-agar, carrageenan and algin, which are extensively used in various industries such as food, confectionary, textiles, pharmaceuticals, dairy and paper industries mostly as gelling, stabilizing and thickening agents. They are also used for human consumption, animal feed and as manure in several countries. Several Asian countries are using various seaweeds in traditional medicines.

Methods: In the present study, the shade dried and methanolic extract of *Cladophora glomerata*, a marine green algae was subjected to preliminary phytochemical and gas chromatography-mass spectrometry analysis (GC-MS) to identify the various bioactive components.

Results: The methanolic extract of *Cladophora glomerata* revealed the presence of alkaloids, glycosides, flavonoids, saponins, diterpenes and carbohydrates. The GC-MS analysis of the methanolic extract of *Cladophora glomerata* showed the presence of 42 different compounds. The major compounds were dibutyl phthalate (27.07%), hexadecanoic acid, methyl ester (9.58%), 1,2-benzene-dicarboxylic acid (8.11%), octatriacontyl trifluoroacetate (6.81%), cholesterol (6.66%).

Conclusions: Thus, in the present study of *Cladophora glomerata*, phytochemical and GC-MS analysis provides an important novel information to support further ongoing studies to evaluate structure of bioactive compound and its pharmacological activities.

Keywords: *Cladophora glomerata*, GC-MS, Phytochemical analysis, Seaweed

INTRODUCTION

Seaweeds or macroalgae form an important constituent of marine living organisms. About 90% of marine plants are algae, which is a primary source of food for aquatic organisms as well as source of human diet in several Asian countries. People in coastal areas consume fresh and dry seaweeds.¹ Marine algae have served as a source of pharmacological active metabolites with various purpose.² Algae are reservoir of various important phytoconstituents like flavonoids, phenolic compounds, saponins, steroids,

tannins, carotenoids, pigments, enzymes, proteins.³ The active compound extracted from marine algae are used in traditional medicine. The use of active substances in curing diseases are said to have fewer side effects.⁴ Recent researches have shown that marine algae exhibit various biological activities.⁵⁻⁷ Phytochemical analysis of seaweeds lays the foundation for drug designing, drug development and drug production.

Cladophora glomerata is a filamentous green macro alga with typical branched thalli.⁸ *Cladophora* occurs both in

marine and fresh water habitat.⁸ These species contain bio-actives such as saturated and unsaturated fatty acids, sterols, typenoids and phenolic compounds.⁹⁻¹¹ The present study was performed to analyse the different phytochemical constituents present in *Cladophora glomerata* along with GC-MS finger printing.

METHODS

Collection of plant material

The fresh sample of *Cladophora glomerata* seaweed was collected from Rameswaram costal area, Tamil Nadu, India and it was authenticated by Dr. Mangaiyarkarasi, Marine Biologist, CIDRF, SBV University, Puducherry, India. The sample was thoroughly washed with seawater to remove epiphytes followed by tap water to remove salts and other extraneous materials. The seaweed was washed with water, shade dried and powdered coarsely.

Preparation of sample

Crude extract was obtained after maceration with 95% methanol at room temperature for 72 hours and repeated till exhaustion of the material. Thereafter, the methanolic extract was distilled, evaporated and dried under reduced pressure to yield methanolic extract.

Phytochemical analysis of *Cladophora glomerata*

Methanolic extract of *Cladophora glomerata* (Green algae) powder was subjected to different qualitative chemical tests for establishing profiles of the extract for its chemical composition.¹²

Phytochemical screening

Qualitative phytochemical analysis of methanolic extract of *Cladophora glomerata* was performed based on the method of Sofowara, Trease and Evans and Harborne.¹³

Detection of alkaloids

Methanolic extract of *Cladophora glomerata* was dissolved in enough of dilute hydrochloric acid and filtered.

- Mayer's test includes methanolic extract treated with Mayer's reagent (potassium mercuric iodide). Formation of a yellow coloured precipitate indicates the presence of alkaloids.
- Wagner's test includes methanolic extract treated with Wagner's reagent (iodine in potassium iodide). Formation of brown/reddish precipitate indicates the presence of alkaloids.
- Dragendroff's test includes methanolic extract treated with Dragendroff's reagent (solution of potassium bismuth iodide). Formation of red precipitate indicates the presence of alkaloids.

- Hager's test includes methanolic extract treated with Hager's reagent (saturated picric acid solution). Presence of alkaloids confirmed by the formation of yellow colored precipitate.

Detection of carbohydrates

Methanolic extract was dissolved in 5 ml distilled water and filtered. The filtrate was used to test for the presence of carbohydrates.

- Molisch's test includes methanolic extract treated with 2 drops of alcoholic α -naphthol solution in a test tube. Formation of the violet ring at the junction indicates the presence of carbohydrates.
- Benedict's test includes methanolic extract treated with Benedict's reagent and heated gently. Orange red precipitate indicates the presence of reducing sugars.
- Fehling's test includes methanolic extract hydrolyzed with dilute HCl, neutralized with alkali and heated with Fehling's A and B solutions. Formation of red precipitate indicates the presence of reducing sugars.

Detection of glycosides

Methanolic extract was hydrolysed with dilute HCl, and then subjected for glycosides test.

- Modified Borntrager's test includes methanolic extract treated with ferric chloride solution and immersed in boiling water for about 5 minutes. The mixture was cooled and extracted with equal volumes of benzene. The benzene layer was separated and treated with ammonia solution. Formation of rose-pink colour indicates the presence of anthranol glycosides.
- Legal's test includes methanolic extract treated with sodium nitroprusside in pyridine and sodium hydroxide. Formation of pink to blood red colour indicates the presence of cardiac glycosides.

Detection of saponins

- Froth test includes methanolic extract diluted with distilled water to 20 ml and this was shaken for 15 minutes. Formation of 1 cm layer of froth indicates the presence of saponins.
- Foam test includes 0.5 mg of methanolic extract shaken with 2 ml of water. If foam produced persists for ten minutes, it indicates the presence of saponins.

Detection of phytosterols

- Salkowski's test includes methanolic extract treated with chloroform and filtered. The filtrated extract was treated with few drops of concentrated sulphuric acid, shaken and allowed to stand. Appearance of golden yellow colour indicates the presence of phytosterols.

- Libermann Burchard’s test includes methanolic extract treated with chloroform and filtered. The filtrate was treated with few drops of acetic anhydride, boiled and cooled. Concentrated sulphuric acid was added. Formation of brown ring at the junction indicates the presence of phytosterols.

Detection of phenols

Ferric chloride test includes methanolic extract treated with 3-4 drops of ferric chloride solution. Formation of bluish black colour indicates the presence of phenols.

Detection of tannins

Gelatin test includes methanolic extract, 1% gelatin solution containing sodium chloride was added. Formation of white precipitate indicates the presence of tannins.

Detection of flavonoids

- Alkaline reagent test includes methanolic extract treated with few drops of sodium hydroxide solution. Formation of intense yellow colour, which becomes colourless on addition of dilute acid, indicates the presence of flavonoids.
- Lead acetate test includes methanolic extract treated with few drops of lead acetate solution. Formation of yellow colour precipitate indicates the presence of flavonoids.

Detection of proteins and amino acids

- Xanthoproteic test includes ethanolic extract treated with few drops of concentrated nitric acid. Formation of yellow colour indicates the presence of proteins.
- Ninhydrin test includes the extract, 0.25% w/v ninhydrin reagent was added and boiled for few minutes. Formation of blue colour indicates the presence of amino acid.

Detection of diterpenes

Copper acetate test includes extract dissolved in water and treated with 3-4 drops of copper acetate solution. Formation of emerald green colour indicates the presence of diterpenes.

Procedure of GC-MS analysis of Cladophora glomerata

GC-MS analysis of the active fractions of *Cladophora glomerata* seaweed was performed using GC SHIMADZU QP2010system and gas chromatograph interfaced to a mass spectrometer (GC-MS) *Cladophora glomerata* was extracted and concentrated by using rotary evaporator. The 1.5 ml upper layer of extract was taken in funnel and added 100 µl N, O-Bis (trimethylsilyl) trifluoroacetamide, trimethyl chlorosilane (BSTFA+TMCS) and 20 µl pyridine and heated at 60°C for 30 minutes. To this acetonitrile was added and filtered into a conical flask. To the filtrate added 50 µl BSTFA+TMCS and heated at 60°C in a water bath for 30 minutes. Filtered using 0.45 µm membrane filter to a vial.¹⁴

Table 1: Qualitative phytochemical analysis of *Cladophora glomerata*.

Phytochemicals	Extracts		
		Observations	Results
Alkaloids	Wagner’s test	Reddish brown solution precipitate	Present
	Mayer’s test	No yellow coloured precipitate	Absent
	Dragendroff’s test	Red coloured precipitate	Present
	Hager’s test	Yellow coloured precipitate	Present
Flavonoids	Lead acetate test	Formation of yellow colour	Present
	Alkaline reagent	Formation yellow colour precipitate	Present
Phytosterols	Salkowski’s test	No golden yellow colour precipitate	Absent
	Libermam Burchard’s test	No brown ring	
Diterpenes	Copper acetate test	Emerald green colour	Present
	Molisch’s test	Violet ring at the junction	Present
Carbohydrates	Benedict’s test	Orange red precipitate	Present
	Fehling’s test	Red coloured precipitate	Present
	Modified Borntreger’s test	No formation of rose-pink colour precipitate	Absent
Glycosides	Legal’s test	Pink to blood red colour precipitate	Present
	Froth test	Thin layer of foam	Present
Saponins	Foam test	Foam produced persists for 10 minutes	Present
	Ferric chloride test	No formation of bluish black colour precipitate	Absent
Phenols	Ferric chloride test	No formation of bluish black colour precipitate	Absent
Tannins	Gelatin test	No formation of white colour precipitate	Absent
Proteins and amino acids	Xanthoproteic test	No formation yellow colour precipitate	Absent
	Ninhydrin test	No formation of blue colour precipitate	Absent

Table 2: GC-MS activity in methanolic extract of *Cladophora glomerata*.

Retention time	Compound name	Molecular formula	Molecular weight	% peak area
6.05	Formamide, N, N-diethyl-	C ₅ H ₁₁ NO	101	0.77
6.62	Acetamide, n-ethyl-	C ₄ H ₉ NO	87	0.51
7.20	Acetamide, N, N-diethyl-	C ₆ H ₁₃ NO	115	2.94
7.38	Acetamide, n-ethyl-	C ₄ H ₉ NO	87	2.83
9.48	2-ethylhexyl acetate	C ₁₀ H ₂₀ O ₂	172	0.51
11.06	Benzene, 1,3-bis(1,1-dimethylethyl)-	C ₁₄ H ₂₂	19	0.41
14.54	Phenol, 3,5-bis(1,1-dimethylethyl)-	C ₁₄ H ₂₂ O	206	0.47
15.48	1,2-Benzenedicarboxylic acid, diethyl ester	C ₁₂ H ₁₄ O ₄	222	0.33
15.55	1-Hexadecene	C ₁₆ H ₃₂	224	1.88
16.48	8-Pentadecanone	C ₁₅ H ₃₀ O	226	0.86
16.79	Heneicosane	C ₂₁ H ₄₄	296	0.36
17.82	1-Octadecene	C ₁₈ H ₃₆	252	2.95
17.89	Heneicosane	C ₂₁ H ₄₄	296	0.38
18.54	1,2-Benzenedicarboxylic acid	C ₁₆ H ₂₂ O ₄	278	0.74
18.66	8-Octadecanone	C ₁₈ H ₃₆ O	268	1.91
19.04	Silane, trichlorooctadecyl-	C ₁₈ H ₃₇ Cl ₃ Si	386	0.80
19.17	Hexadecanoic acid, methyl ester	C ₁₇ H ₃₄ O ₂	270	9.58
19.47	Dibutyl phthalate	C ₁₆ H ₂₂ O ₄	278	0.51
19.52	Dibutyl phthalate	C ₁₆ H ₂₂ O ₄	278	27.07
19.86	1-Octadecene	C ₁₈ H ₃₆	252	3.76
19.98	Eicosyl acetate	C ₂₂ H ₄₄ O ₂	340	1.15
20.64	10-Nonadecanone	C ₁₉ H ₃₈ O	282	0.73
20.80	n-Nonadecanol-1	C ₁₉ H ₄₀ O	284	2.78
20.87	9-Octadecenoic acid, methyl ester, (E)-	C ₁₉ H ₃₆ O ₂	296	0.69
20.93	11-Octadecenoic acid, methyl ester, (Z)-	C ₁₉ H ₃₆ O ₂	296	0.54
20.98	Oxirane, hexadecyl-	C ₁₈ H ₃₆ O	268	0.67
21.04	Unknown compound	No hit compound		0.16
21.11	Methyl stearate	C ₁₉ H ₃₈ O ₂	298	0.72
21.22	14-Pentadecenoic acid	C ₁₅ H ₂₈ O ₂	240	0.53
21.43	Octadecanoic acid	C ₁₈ H ₃₆ O ₂	284	1.22
21.68	Tridecane, 3-methylene-	C ₁₄ H ₂₈	196	0.41
21.73	Behenic alcohol	C ₂₂ H ₄₆ O	326	2.90
21.84	Acetic acid n-octadecyl ester	C ₂₀ H ₄₀ O ₂	312	1.11
22.69	Eicosyl pentafluoropropionate	C ₂₃ H ₄₁ F ₅ O ₂	444	1.42
22.81	Unknown compound	No hit compound		0.42
23.39	Hexanedioic acid, bis(2-ethylhexyl) ester	C ₂₂ H ₄₂ O ₄	370	0.35
23.44	1-Heptacosanol	C ₂₇ H ₅₆ O	396	1.55
24.56	1,2-benzenedicarboxylic acid	C ₂₄ H ₃₈ O ₄	390	8.11
24.81	Oxalic acid, cyclohexyl tetradecyl ester	C ₂₂ H ₄₀ O ₄	368	0.75
25.03	Octacosanol	C ₂₈ H ₅₈ O	410	0.76
31.44	Cholesterol	C ₂₇ H ₄₆ O	386	6.66
33.42	Octatriacontyl trifluoroacetate	C ₄₀ H ₇₇ F ₃ O ₂	646	6.81

RESULT

Identification of components

Interpretation of mass spectrum GC-MS was done using the database of National Institute Standard and Technique (NIST08s), WILEY8 and FAME having more patterns.

The spectrum of the unknown component was compared with the spectrum of the known components stored in the NIST08s, WILEY8 and FAME library.

The name, molecular weight, molecular formula and structure of the component of the test material was ascertained.

Phytochemical analysis and GC-MS analysis of *Cladophora glomerata*

The phytochemical screening of methanolic extract of *Cladophora glomerata* showed the presence of alkaloids, flavonoids, carbohydrates, diterpenes, glycosides and saponins (Table 1). On contrary phytosterols, phenols, tannins, proteins and amino acids were absent.

GC- MS interpretation

The components present in the crude extract of *Cladophora glomerata* were identified by GC-MS. The various components with their retention time, molecular formula, molecular weight and percentage composition in the crude extract of the drug is shown in Table 2. Total of 42 compounds were identified in the extract. The major compounds were dibutyl phthalate (27.07%),

hexadecanoic acid, methyl ester (9.58%), 1,2-benzenedicarboxylic acid (8.11%), Octatriacontyl trifluoroacetate (6.81%), cholesterol (6.66%). All other components were less than 4% and hence found to be less significant, as their bioavailability is negligible.

DISCUSSION

Phytochemical analysis refers to extraction, screening and identification of the medicinally active substances found in plants. The bioactive substances which can be derived from plants are flavonoids, alkaloids, carotenoids, tannins, antioxidants and phenolic compounds. These bioactive compounds have great medicinal values. Seaweeds contain many bioactive compounds of medicinal value with potential pharmaceutical application. The qualitative phytochemical studies were carried out on methanolic extract of *Cladophora glomerata* for different constituents.

Table 3: Important major compounds with their pharmacological activity of *Cladophora glomerata*.

Chemical compounds	Molecular formula	Pharmacological activity
Dibutyl phthalate	C ₁₈ H ₃₇ CL	Anti-bacterial activity, antimetabolic activity ^{23,24}
Hexadecanoic acid, methyl ester	C ₁₆ H ₃₂ O ₂	Anti-bacterial, cancer preventive, antiarthritic properties ²⁵
1,2-Benzene dicarboxylic acid	C ₂₄ H ₃₈ O ₄	Neurodegenerative disorders, anti-cancer activity.
Octatriacontyl trifluoroacetate	C ₄₀ H ₇₇ F ₃ O ₂	Insecticidal ²⁶

Cladophora glomerata showed the presence of alkaloids, flavonoids, carbohydrates, diterpenes, glycosides and saponins. Flavonoids have been proved with antitumor and antioxidant properties.¹⁵

Alkaloids were found to have antimicrobial, cytotoxic and antispasmodic properties.¹⁶⁻²¹ Terpenoids possess anti-inflammatory and hypoglycaemic activities.²² In GC MS analysis, total of 42 compounds were identified in the extract. The broad range of compounds such as amines, alcohols, esters and ethers can be observed in chromatogram.

The active principles with their retention time, molecular weight and molecular formula are presented in Table 2. The major compounds are listed in Table 3.

Dibutyl phthalate is known to have anti-bacterial activity and antimetabolic activity, hexadecanoic acid, methyl ester is known to have anti-bacterial, cancer preventive and antiarthritic properties, 1,2-benzenedicarboxylic acid can be used in neuro-degenerative disorders and as anti-cancer agent, octatriacontyl trifluoroacetate an constituent found in extract can be used for insecticidal properties.²³⁻²⁶

According to previous studies fresh water *Cladophora glomerata* is a source of bioactive substances with

cosmetic importance and can be used as ingredients in cosmetic preparations.⁸

CONCLUSION

In the present study, methanolic extract of *Cladophora glomerata* contains active phytochemical compounds such as alkaloids, flavonoids, diterpenes, saponins, carbohydrates and glycosides. GC-MS analysis showed 42 different compounds. The presence of bioactive substances identified in this study need to be further investigated (in vitro and in vivo) for the potential medicinal properties of the *Cladophora glomerata* (green alga) for their pharmacological activities for their use in various clinical conditions.

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