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Characterization of Phytochemicals in Methanolic Extract of *Enteromorpha compressa* (L.) Nees Collected from Kanyakumary Coast of Tamil Nadu, India

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ABSTRACT

The present study was aimed to explore the preliminary phytochemicals analysis of *Enteromorpha compressa* (L.) Nees collected from Kanyakumari in the Southern coast of Tamil Nadu, India. The preliminary phytochemical analysis of the methanol extract was carried out using Harborne method, followed by the characterization was done using FT-IR and HPLC. The preliminary phytochemical analysis showed the presence of the following secondary metabolites such as alkaloids, emodines, tannins, flavonoids, quinones, glycosides, cardiac glycosides, terpenoids, phlobatannins, coumarins, steroids, diterpenes and triterpenoids. The crude methanol extract of *Enteromorpha compressa* (L.) Nees was passed into FTIR and it confirmed the presence of functional groups such as alcohols and phenols, aliphatic compounds, aromatic nitro compound, amides, vinyl ethers, organophosphorus compounds, amines, sulfonyl chloride, primary aliphatic amines, ketones, phosphines and aliphatic compound. HPLC fingerprint of *Enteromorpha compressa* (L.) Nees displayed three prominent peaks at the retention time of 2.050min, 2.493min and 3.427min out of six compounds separated.

Keywords: Seaweeds, Phytochemicals, Secondary metabolites, FTIR, HPLC

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INTRODUCTION

Natural products are accepted to have the benefit of having huge structural and chemical diversity, expanded protein binding characteristics and specific biological activity. Bioactive compounds from marine algae are broad over a wide span of time use in the treatment of numerous diseases and serve as compounds of attention both in their normal form and as templates for synthetic change. Marine algae have been nearly connected with human life and have been comprehensively utilized as a part of various courses as a source of nourishment, feed fertilizer and medicine and mainly used for economically important phycocolloids [1]. In India, a significant number of the rough shorelines, mudflats, estuaries, coral reefs and tidal ponds along the drift give perfect habitats to the development of seaweed growth. In all, 271 genera and 1153 species of seaweeds have been counted till date from the Indian waters [2].

Seaweeds or macroalgae belong to the lower plants that they do not have roots, stems and leaves. Instead, they are composed of a thallus. Some species have gas-filled structures to provide buoyancy. Seaweeds are categorized in to three groups namely the red (Rhodophyceae), green (Chlorophyceae) and brown (Phaeophyceae) algae [3]. Due to various biotic and abiotic pressures faced by these marine algae which influence physiological nature of the cell that leads to the production of several secondary metabolites for their defence. Several of these metabolites are constitutive, which are existing in biologically active forms. Further, there are several reports on various pharmacological activities of different solvent extracts [4, 5]. Within a decade, there were a number of dramatic advances in analytical techniques including FTIR and HPLC that were powerful tools for separation, identification and structure determination of phytochemicals [6].

MATERIALS AND METHODS

Collection of Plant sample

The plant materials used in the present study was *Enteromorpha compressa* (L.) Nees, belonging to Chlorophyceae (green algae) was made during the low tidal and subtidal regions (up to 1m depth) by hand picking. The collected materials were washed thoroughly with marine water in the field itself to remove the epiphytes and sediment particles. Then the samples were packed separately in polythene bags in wet conditions and brought to the laboratory, then thoroughly washed in tap water followed by distilled water to remove the salt on the surface of the thalli. They were stored in 5% formalin solution [7].

Preparation of extract

For the preparation of methanolic extract, the plant specimens were washed thoroughly and placed on blotting paper and spread out at room temperature in the shade condition for drying. The shade dried samples were grounded to fine powder using a tissue blender. The powdered samples were then stored in the refrigerator for further use. 30g powdered samples were packed in Soxhlet apparatus and extracted with methanol for 8h separately [8].

Preliminary phytochemical analysis

The different methanol extract of *Gracilaria textorii* (Suring.) J.Ag. was tested for the presence of alkaloids, anthocyanin, anthraquinones, cardiac glycosides, catechin, coumarins, diterpenes, emodins, flavonoids, glycosides, leucoanthocyanin, lignins, phenols, phlobatannins, quinones, saponins, steroids, tannins, terpenoids and triterpenoids. Phytochemical screening of the extract was carried out according to the standard methods [9].

Test for alkaloids

1ml of 1% HCl was added with 2ml of extract and was treated with few drops of Mayer's reagent. A creamy white precipitate indicates the presence of alkaloids.

Test for anthocyanin

1ml of 2N HCl was added to the 1ml of extract and was treated with NH_3. Pink red colour turns blue violet.

Test for anthraquinone

2ml of extract was mixed with 1ml of benzene and 1ml of 10% ammonia solution was added. The presence of red or violet color indicates the anthraquinones.

Test for cardiac glycosides

0.4ml of glacial acetic acid was added with 1ml extract and trace amount of $FeCl_3$. Blue colour indicates the presence of cardiac glycosides.

Test for catechin

1ml of plant extract was mixed with few drops of ehrlich's reagent was treated with few drops of conc. HCl. pink color indicates catechin.

Test for Coumarins

1ml of extract was added with 1ml of 10% NaOH. The formation of yellow colour indicates the presence of coumarins.

Test for diterpenes

1ml extract was added with 1ml dis. H_2O and 10 drops of copper acetate solution. Emerald green colour indicates the presence of diterpenes.

Test for emodins

1ml plant extract was added with 2ml of NH_4OH and treated with 3ml of benzene. Red color indicates emodins.

Test for flavonoids

Two drops of 1% NH_3 solution was added to 2 ml of extract in a test tube. Yellow coloration indicates the presence of flavonoids.

Test for glycosides

A few drops of 50% H_2SO_4 was added to 2ml of extract in a boiling tube. The solution was heated in boiling water bath for 5 min. 10ml of Fehling's solution was added and boiled. A red precipitate indicates the presence of glycosides.

Test for leucoanthocyanin

1ml of plant extract was mixed with 1ml of isoamyl alcohol. Upper layer appear red in color indicates leucoanthocyanin.

Test for lignins

1ml of plant extract treated with gallic acid. Formation of olive green color indicates lignins.

Test for phenols

To 1ml extract, add 2ml distilled water followed by few drops of 10% ferric chloride. The formation of blue or black colour indicates the presence of phenolic groups.

Test for phlobatannins

1ml extract was added with 1% aqueous HCl and then boiled. Red precipitate indicates the presence of phlobatannins.

Test for quinones

1ml seaweed extract added with 1ml of alcoholic KOH. Red to blue colour indicates the presence of quinones.

Test for saponins

2ml of extract was shaken vigorously with 5ml distilled water to obtain stable persistent foam. The formation of emulsion indicates the presence of saponins.

Test for steroids

1ml of extract added to 1ml $CHCl_3$ and few drops of Conc. H_2SO_4. Golden red colour or Brown colour indicates the presence of phytosteroids.

Test for tannins

To 2ml extract, 1ml of distilled water and 1-2 drops of ferric chloride solution was added and observed for brownish green or a blue black coloration indicates the presence of tannins.

Test for terpenoids

2ml extract was mixed with 2ml of $CHCl_3$ in a test tube. 3ml Conc. H_2SO_4 was added carefully along the wall of the test tube to form a layer. An interface with a reddish brown coloration confirms the presence of terpenoids.

Test for triterpenoids

1ml of plant extract was added with 1ml of $CHCl_3$ and treated with few drops of Conc. H_2SO_4. Yellow color lower layer indicates triterpenoids.

FTIR analysis

FTIR analysis of the methanol extract of *Caulerpa mexicana* Sonder ex Kuetzing was performed using Perkin Elmer

Spectrophotometer system, which was used to detect the characteristic peaks and their functional groups. The peak values of the FTIR were recorded. Each and every analysis was repeated twice and confirmed the spectrum [10].

HPLC analysis

The HPLC analysis of methanol extract of *Caulerpa mexicana* Sonder ex Kuetzing was performed on a Shimadzu LC-10AT VP HPLC system, equipped with a model LC-10AT pump, UV Visible detector SPD-10AT, a Rheodyne injector fitted with a 20µl loop and an auto injector SIL-10AT. A Hypersil® BDS C-18 column (4.6 × 250mm, 5µm size) with a C-18 guard column was used. The elution was carried out with gradient solvent systems with a flow rate of 1ml/min at ambient temperature (25-28°C). The mobile phase consisted of 0.1% v/v methanol (solvent A) and water (solvent B). The mobile phase was prepared daily, filtered through a 0.45µm and sonicated before use. Total running time was 15min. The sample injection volume was 20µl while the wavelength of the UV-Visible detector was set at 254nm [11].

Instrumentation

An isocratic HPLC (Shimadzu HPLC Class VP series) with two LC- 0 AT VP pumps (Shimadzu), a variable wave length programmable photo diode array detector SPD-M10A VP (Shimadzu), a CTO- 10AS VP column oven (Shimadzu), a SCL-10A VP system controller (Shimadzu), a reverse phase Luna 5µ C18 (2) and Phenomenex column (250 mm X 4.6mm) were used. The mobile phase components Methanol: water (45:55) were filtered through a 0.2µ membrane filter before use and were pumped from the solvent reservoir at a flow rate of 1ml/min which yielded column backup pressure of 260-270kgf/cm^2. The column temperature was maintained at 27°C. 20µl of the respective sample was injected by using a Rheodyne syringe (Model 7202, Hamilton).

RESULT AND DISCUSSION

Preliminary phytochemical analysis of *Enteromorpha compressa* (L.) Nees

In the preliminary phytochemical analysis of *Enteromorpha compressa* (L.) Nees, seventeen different types of secondary metabolites (alkaloids, anthocyanins, anthroquinones, cardiac glycosides, coumarins, diterpenes, emodins, flavanoids, glycosides, phenols, phlobatannins, quinones, saponins, steroids, tannins, terpenoids and triterpenoids) were tested in methanol extract of *Entryomorpha compressa* (L.) Nees. Among the tests for the presence or absence of the above compounds, 13 tests showed positive results and the remaining gave negative results. The 13 positive results showed the presence of alkaloids, emodines, tannins, flavonoids, quinones, glycosides, cardiac glycosides, terpenoids, phlobatannins, coumarins, steroids, diterpenes and triterpenoids (Table-1).

Table-1: Preliminary phytochemical analysis of *Enteromorpha compressa* (L.) Nees

Tests	Methanol Extract
Alkaloids	+
Anthocyanin	-
Anthraquinone	-
Cardiac Glycosides	+
Coumarins	+
Diterpenes	+
Emodins	+
Flavonoids	+
Glycosides	+
Phenols	-
Phlobatannins	+
Quinones	+
Saponins	-
Steroids	+
Tannins	+
Terpenoids	+
Triterpenoids	+

Fourier Transform Infrared (FTIR) spectrum of *Enteromorpha compressa* (L.) Nees

The FTIR spectrum of methanol extract of *Enteromorpha compressa* (L.) Nees showed different peaks at 575.71, 623.93, 720.36, 930.59, 1051.13, 1116.71, 1168.78, 1216.03, 1382.87, 1413.72, 1461.94, 1512.09, 1624.92, 1717.49, 2360.71, 2851.56, 2922.92 and 3419.56cm^{-1}. It was confirmed the presence of functional groups such as NO_2 in aromatic nitro compounds (NO_2 deformation), C-CO-C in ketones (C-CO-C bend), Ar-OH in phenols (OH out-of-plane deformation), $CH=CH_2$ in vinyl compounds (CH_2 out-of-plane wag), S=O in akyl sulfoxides (S=O stretch), $C-NH_2$ in primary aliphatic amines (C-N stretch), SO_2 in sulfones (SO_2 sym stretch), C-O-C in vinyl ethers (C-O-C antisym stretch), SO_2 in sulfonyl chlorides (SO_2 antisym stretch), C-N in primary amides (C-N stretch), CH_2 in aliphatic compounds (CH_3 antisym deformation), Benzene ring in aromatic compounds (Ring stretch), C=O in β-diketones (C=O stretch), C=O in ketones (C=O stretch), PH in phosphines (P-H stretch), CH_3 and-CH_2- in aliphatic compounds (CH antisym and sym stretching), CH_3 and – CH_2- in aliphatic compounds (CH antisym and sym stretching) and OH in alcohols and phenols (OH stretch) respectively (Figure-1 & Table-2).

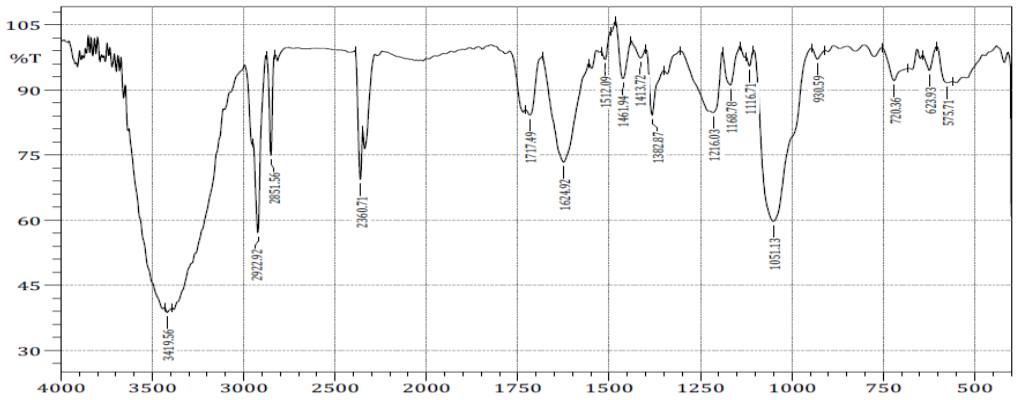

Figure-1: FTIR spectrum of methanol extract of *Enteromorpha compressa* (L.) Nees

Table-2: FTIR spectrum of methanol extract of *Enteromorpha compressa* (L.) Nees

Peak value (cm^{-1})	Functional group	Assignment
3419.56	OH in alcohols and phenols	OH stretch
2922.92	CH_3 and-CH_2 in aliphatic compounds	CH antisym and sym stretching
2851.56	CH_3 and -CH_2 in aliphatic compounds	CH antisym and sym stretching
2360.71	PH in phosphines	P-H stretch
1717.49	C=O in ketones	C=O stretch
1624.92	C=O in β-diketones	C=O stretch
1512.09	Benzene ring in aromatic compounds	Ring stretch
1461.94	CH_2 in aliphatic compounds	CH_3 antisym deformation
1413.72	C-N in primary amides	C-N stretch
1382.87	SO_2 in sulfonyl chlorides	SO_2 antisym stretch
1216.03	C-O-C in vinyl ethers	C-O-C antisym stretch
1168.78	SO_2 in sulfones	SO_2 sym stretch
1116.71	C-NH_2 in primary aliphatic amines	C-N stretch
1051.13	S=O in akyl sulfoxides	S=O stretch
930.59	CH=CH_2 in vinyl compounds	CH_2 out - of-plane wag
720.36	Ar-OH in phenols	OH out - of-plane deformation
623.93	C-CO-C in ketones	C-CO-C bend
575.71	NO_2 in aromatic nitro compounds	NO_2 deformation

High Performance Liquid Chromatographic (HPLC) spectrum of *Enteromorpha compressa* (L.) Nees

Six compounds were separated from the methanol extract of *Enteromorpha compressa* (L.) Nees at different retention time of 0.253min, 0.913min, 1.417min, 2.050min, 2.493min and 3.003min. The profile displayed three prominent peaks at the retention time of 2.050min, 2.493min and 3.003min, followed by three moderate peaks were observed at the retention time of 0.253min, 0.913min and 1.417min respectively (Figure-2 & Table-3).

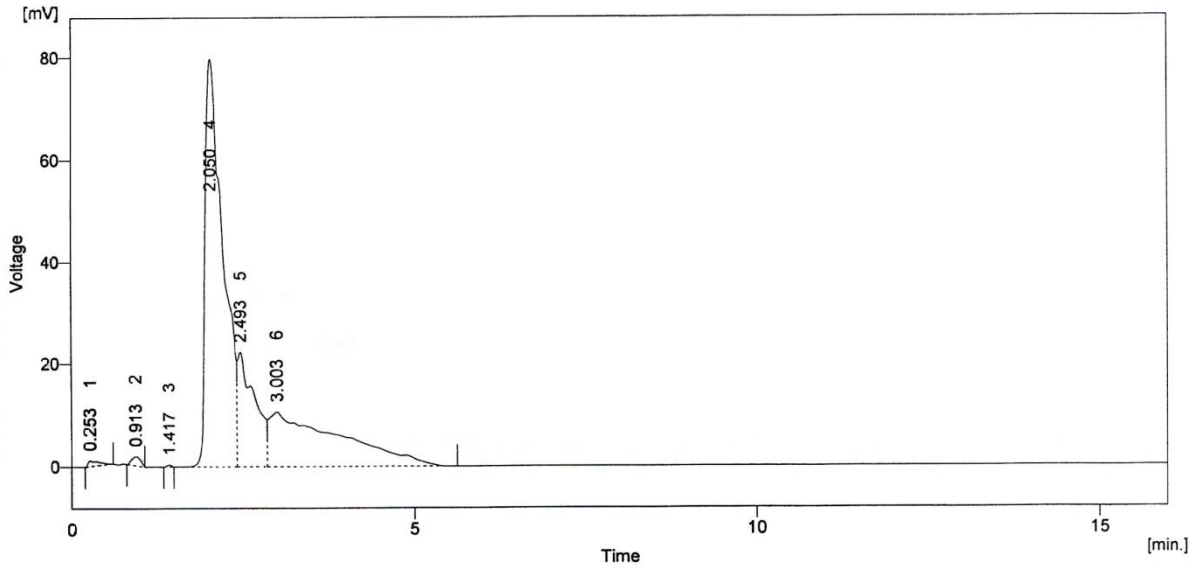

Figure-2: HPLC profile of methanol extract of *Enteromorpha compressa* (L.) Nees

Table-3: HPLC profile of methanol extract of *Enteromorpha compressa* (L.) Nees

	Reten. Time (min)	Area(Mv.s)	Height(mV)	Area (%)	Height (%)	W05(min)
1	0.253	12.338	1.135	0.5	1.0	0.28
2	0.913	16.351	1.798	0.6	1.5	0.16
3	1.417	1.844	0.400	0.1	0.3	0.08
4	2.050	1467.396	79.813	55.7	68.7	0.28
5	2.493	386.370	22.322	14.7	19.2	0.32
6	3.003	748.831	10.665	28.4	9.2	1.24
	Total	2633.130	116.133	100.0	100.0	

CONCLUSION

The present study revealed that the green seaweed *Enteromorpha compressa* (L.) Nees is a good candidate of possessing the phytochemicals. The preliminary phytochemicals were presence or absence in secondary metabolites present in the methanol extract and characterized using FTIR and HPLC profile which showed the presence of secondary metabolites. More studies are necessary to isolate the bioactive compounds and predict the bioactivities further for the knowledge and promote the exploitation of the marine green algae. The results of the present research offer a platform of using methanol extract of *Enteromorpha compresa* (L.) Nees as alternative for various diseases.

REFERENCES

1. Chapman ARO, Craigie JS, Seasonal growth in *Laminaria longicuris*: relations with dissolved inorganic nutrients and internal reserves of nitrogen. Mar. Biol., 1972; 40:197-205.
2. Rao PVS, Mantri VA, Indian seaweed resources and sustainable utilization: Scenario at the dawn of a new century. Current Science. 2006; 91(2):164-174.
3. Manilal A, Sujith S, Kiran G, Selvin S, Shakir J, Gandhimathi C, Antimicrobial potential and seasonality of red algae collected from the southwest coast of India tested against shrimp, human and phytopathogens. Ann. Microbiol. 1998; 2:207-219.
4. Luning K, Pang SJ, Mass cultivation of seaweeds: current aspects and approaches. Journal of Applied Phycology. 2003; 15:115-19.
5. Marinho SE, Fonseca PC, Carneiro MAA, Moreira WSC, Seasonal variation in the chemical composition of two tropical seaweeds. Bio-resource Technology. 2006; 97:2402-2406.
6. Roberts JKM, Xia JH, High-resolution NMR methods for study of higher plants. Methods Cell Biol, 1995; 49:245-258.
7. Iniya Udhaya C, John Peter Paul J, Screening of preliminary phytochemicals of *Gracilaria cylindrica* Boergesen in Koothankuzhi, Tirunelveli district, Tamil Nadu, India, Indo American Journal of Pharmaceutical Sciences, 2017; 4(12):4590-4594.
8. John Peter Paul J, Iniya Udhaya C, Screening of preliminary phytochemical profile of Gracilaria textorii (Suring.) J.Ag. collected from Kanyakumari coast, Tamil Nadu, India. Asian Journal of Research in Chemistry and Pharmaceutical Sciences, 2018; 6(4):183-187.
9. Harborn JB, Phytochemical Methods: A guide to modern techniques of plants analysis, Chapman & Hall. London, Ltd, 1998, Pp.49-188.
10. John Peter Paul J, Screening of phytochemicals in methanolic extract of Dictyota dichotoma (Huds.) Lamouroux from Koothankuzhi in the south east coast of Tamil Nadu, India. International Journal of Pharmacy and Biological Sciences, 2019; 9(1): 207-212.
11. John Peter Paul J, Shri Devi SDK, Phytochemical studies on Padina pavonica (L.) Thivy ex Taylor. Indo American Journal of Pharmaceutical Research, 2013; 3(6):4452-4459.