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Phytochemical Analysis of Chloroform Extract of *Nitophyllum Marginale* (Kutzing) J.Ag. using FTIR and HPLC

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Abstract

Background: Marine algae are a source of bioactive substances and they produce a great variety of secondary metabolites with a diverse range of biological activities. Phytochemical types include sterols, isopyrenoids, amino acids, terpenoids, phlorotannins, steroids, phenolic compounds, halogenated ketones and alkanes, cyclic polysulphides, fatty acids and acrylic acid have been isolated from marine algae. Hence, pharmacologists, physiologists and chemists have been paying increasing attention to the marine organisms particularly on marine macro algae for screening bioactive substances. The present study was designed to discover the phytochemical analysis of chloroform extract of *Nitophyllum marginale* (Kutzing) J.Ag., collected from Hare Island, Thoothukudi district, Tamil Nadu, India. **Methodology:** The phytochemical screening of chloroform extract was carried out by using the standard procedure of FTIR spectroscopy and HPLC. **Results:** The FTIR spectrum of the chloroform extract of *Nitophyllum marginale* (Kutzing) J.Ag. was revealed the presence of functional groups such as Alkyl halides, Alkynes, Carboxylic acid, Nitro compounds, Isocyanates, Amine hydrohalides, Alkenes, Alkanes and Alcohols. The qualitative HPLC fingerprint profile displayed thirteen compounds at different retention times. The profile displayed four prominent peaks at the retention times of 1.997min, 2.750min and 6.487min. **Conclusions:** The phytochemicals were characterized which showed the presence of secondary metabolites. The results of this study provide a base work for utilizing chloroform extract of *Nitophyllum marginale* (Kutzing) J.Ag., as a treatment for a variety of disorders.

Key words: *Nitophyllum marginale* , Macro algae, FTIR, HPLC.

Introduction

The Marine Ecosystem occupies one-third of the Earth's atmosphere which covers approximately 71 percent of the surface of the earth. In aquatic biodiversity Algae are categorised into microalgae and macro algae. A unique group of micro

algae are blue-green algae, also called Cyanobacteria and the macro algae are classified as Chlorophyceae (green algae), Phaeophyceae (brown algae) and Rhodophyceae (red algae). It is classified based on the specific pigments. Marine algae are a source of bioactive substances, and they contain a huge variety of secondary metabolites with a wide range of biological activities. Over the past several decades seaweeds have been used by humans as medicine and food as a fresh source. Seaweeds are reservoirs of carotenoids, pigments, diverse functional polysaccharides. Seaweeds are excellent source of vitamin A, B_1, B_{12}, C, D and E. (1) Seaweeds have generated an enormous amount of interest in the pharmaceutical industry as a source of bioactive compounds with immense medicinal potential. (2) The secondary metabolites synthesized by seaweeds demonstrate a broad spectrum of bioactivity. (3,4) Due to the biological interest, the present study was undertaken to investigated the phytochemical analysis of chloroform extract of *Nitophyllum marginale* (Kutzing) J.Ag., collected from Hare Island, Thoothukudi district, Tamil Nadu, India.

Materials and Methods

Collection of samples

Nitophyllum marginale (Kutzing) J.Ag., the red algal species were collected from Hare island coastal region (Latitude: 8° 77' 44.00 N, Longitutde: 78° 19' 78.00 E), Thoothukudi district, Tamil Nadu, India during the month of August 2021. The collected algal sample was authenticated by Dr. J. John Peter Paul, Assistant Professor of Botany & Director, Centre for Advanced Research in Plant Sciences (CARPS), St. Xavier's College (Autonomous), Palayamkottai and accumulated in Xavier's College Herbarium Centre for Biodiversity and Biotechnology, St. Xavier's College (Autonomous), Palayamkottai – 627002, and the given voucher number for the accumulated herbarium sample was XCH-20547. The samples were collected by hand picking during low waves and washed with marine water to remove debris and epiphytes. The entire epiphytes were turfed using a soft brush. In the laboratory, the samples were once again washed with freshwater followed by a rinse with distilled water and stored in a refrigerator for further analysis. (5)

Preparation of chloroform extract

For the preparation of chloroform extract, the collected 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 sample was packed in Soxhlet apparatus and extracted with chloroform for 8h separately. The excess amount of chloroform was evaporated and fine chloroform crude powder was prepared and stored in the refrigerator for the further analysis. (6)

FTIR analysis

FTIR analysis of the choroform extract of *Nitophyllum marginale* (Kutzing) J.Ag., 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. (7)

HPLC analysis

The HPLC analysis of chloroform extract of was *Nitophyllum marginale* (Kutzing) J.Ag., 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 (John Peter Paul and Shri Devi, 2013).

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 ethanol: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/cm2. The column temperature was maintained at 27°C. 20µl of the respective sample and was injected by using a Rheodyne syringe (Model 7202, Hamilton).

Result and Discussion

FTIR analysis

The FTIR spectrum was used to identify the functional group of the active components based on the peak value in the region of infra red radiation. The chloroform extract of *Nitophyllum marginale* was passed into the FTIR and the functional groups of the components were separated based on its peak ratio. The FTIR results of chloroform extract of *Nitophyllum marginale* showed different peaks at 517.85, 618.14, 905.52, 1386.72, 1510.16, 1641.31, 2064.66, 2309.6, 2885.31 and 3445.59. the functional groups such as Alkyl halides, Alkynes, 1°, 2° amines, Carboxylic acid, Nitro compounds, Alkenes, Isocyanates, Amine hydrohalides, Alkanes and Alcohols respectively (Figure 1 & Table 1).

Figure 1: FTIR spectrum of chloroform extract of *Nitophyllum marginale* (Kutzing) J.Ag.

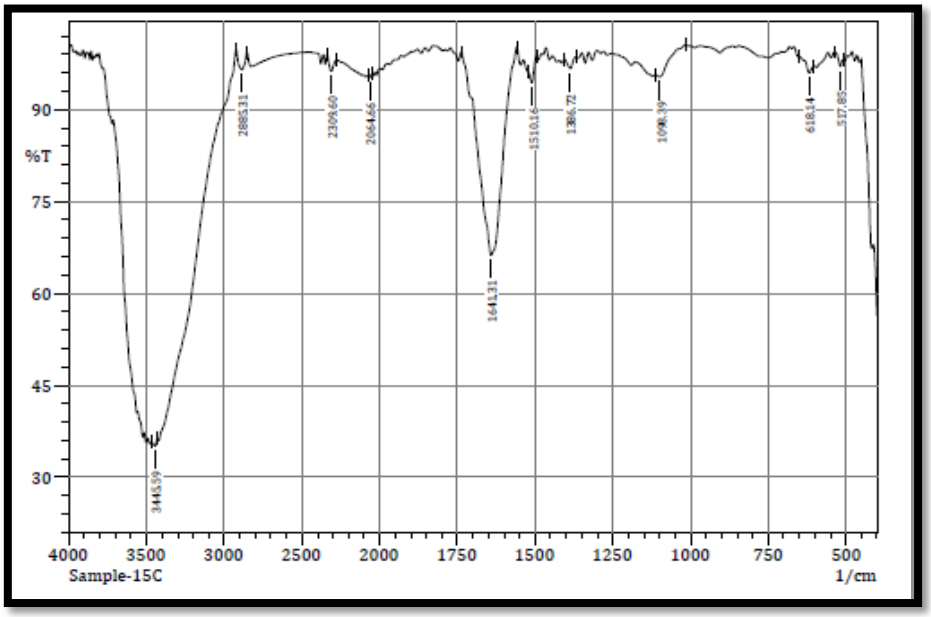

Table 1: FTIR peak values and functional groups of chloroform extract of *Nitophyllum marginale* (Kutzing) J.Ag.

S. No.	Frequency (cm^{-1})	Functional group	Possible compounds
1.	517.85	C–Br	Alkyl halides
2.	618.14	C–H	Alkynes
3.	905.52	N–H	1°, 2° amines
4.	1386.72	C–C	Carboxylic acid
5.	1510.16	N–O	Nitro compounds
6.	1641.31	–C=C–	Alkenes
7.	2064.66	N= C= O	Isocyanates
8.	2309.6	$–NH_3^+$	Amine hydrohalides
9.	2885.31	C–H	Alkanes
10.	3445.59	O–H	Alcohols

HPLC analysis

The qualitative HPLC fingerprint profile of the chloroform extract of *Nitophyllum marginale* (Kutzing) J.Ag. was prepared by hot extraction was subjected to HPLC for the separation and identification of constituents present in the *Nitophyllum marginale*. Three compounds were separated at different retention time of 1.997min, 2.750min and 6.487min respectively. The profile displayed one prominent peak at the retention time of 1.997min followed by two moderate peaks were also observed at the retention time of 2.750min and 6.487min (Figure 2 & Table 2).

Figure 2: HPLC spectrum of chloroform extract of *Nitophyllum marginale* (Kutzing) J.Ag.

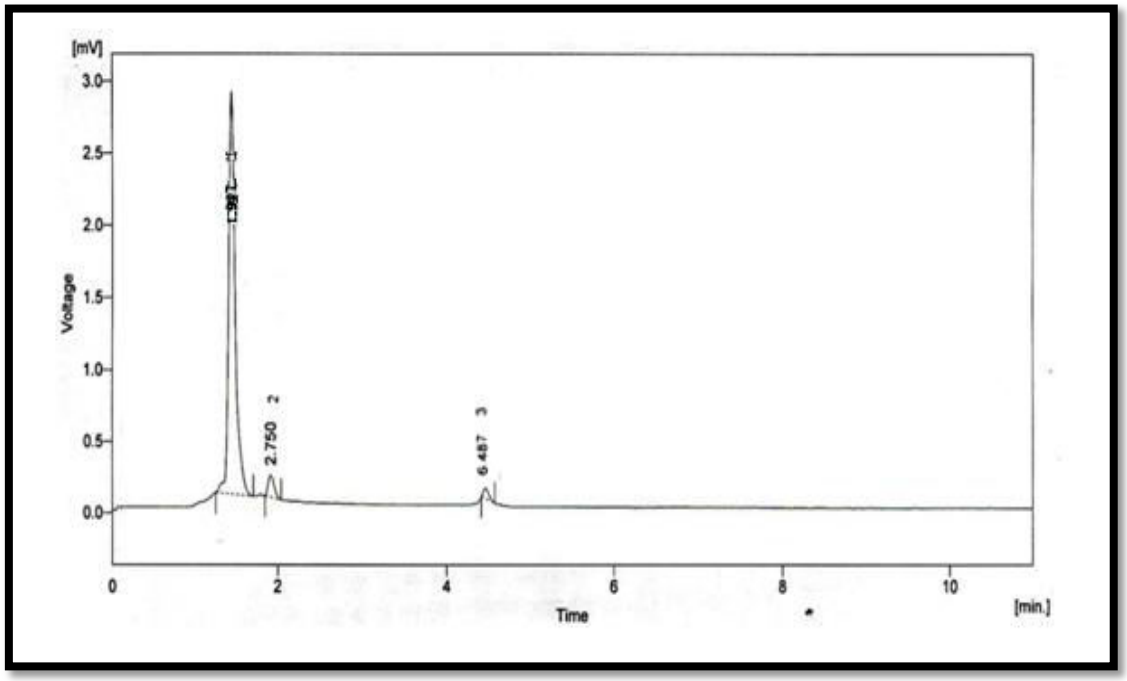

Table 2: HPLC profile of choloroform extract of *Nitophyllum marginale* (Kutzing) J.Ag.

S. No.	Retention time (min)	Area (Mv.s)	Height (mV)	Area (%)	Height (%)	W05 (min)
1.	1.997	905.664	51.834	95.0	94.2	0.30
2.	2.750	38.681	2.459	4.1	4.5	0.25
3.	6.487	8.844	0.752	0.9	1.4	0.18
	Total	953.190	55.045	100.0	100.0	

Conclusion

From the present study, it was concluded that, FTIR and HPLC analysis can be used as effective tool in identifying the phytochemicals. It also suggested that *Nitophyllum marginale* is the richest sources of phytochemicals which can be isolated and further screened for different kinds of biological activities depending on the therapeutic uses. Further work will be conducted the isolation and characterization of active principles responsible for the biopotential. The presence of various functional groups and phytocompounds in *Nitophyllum marginale* conform that it acts as a most important source of drugs against various ailments.

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