Phytoconstituent Analysis Of Methanolic Extract Of *Gracilaria Fergusonii* Collected From Hare Island, Thoothukudi District, Tamil Nadu, India.

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ABSTRACT
The main objective of this present study was to investigate the phytoconstituent present in methanol extract of *Gracilaria fergusonii* collected from Hare Island, Thoothukudi District, Tamil Nadu, India. The phytochemical analysis of methanol extract was estimated using the standard procedure for UV-Vis spectroscopic, FT-IR and HPLC. The crude extracts of the selected *Gracilaria fergusonii* J.Ag. species was scanned in a wavelength ranging from 200-900nm. Seven functional groups were identified using FTIR spectrophotometer in the range of 4000 to 400cm^{-1}. The qualitative HPLC fingerprint profile of methanol extract of *Gracilaria fergusonii* J.Ag. was chosen at a wavelength of 254nm due to sharpness of the peaks and proper baseline. There are fifteen compounds were separated from *Gracilaria fergusonii* J.Ag. The profile observed five prominent peaks at the retention time of 1.483min, 1.570min, 1.743min, 1.850min, 2.017min, followed by ten moderate peaks were predicted at the retention time of 1.273min, 2.247min, 3.520min, 3.850min, 4.220min, 4.517min, 4.703min, 5.107min, 7.037min and 12.230min.

INTRODUCTION

Phytochemicals are the chemicals that present naturally in plants. Nowadays these phytochemicals become more popular due to their countless medicinal uses. Phytochemicals play a vital role against number of diseases such as asthma, arthritis, cancer etc. unlike pharmaceutical chemicals these phytochemicals do not have any side effects. Since the phytochemicals cure diseases without causing any harm to human being these can also be considered as man-friendly medicinal. Phytochemicals are biologically active, naturally occurring chemical compounds found in plants [1]. Plants having its pharmacological benefits due to accumulation of bioactive phytochemicals in the plant tissue considered as primary and secondary metabolites. Primary metabolites are organic compounds that comprises of glucose, starch, polysaccharide, protein, lipids and nucleic acid which are helpful for growth and development of the human body. Plants produce secondary metabolites which include alkaloids, flavonoids, saponins, terpenoids etc., (2,3) they protect plants from disease and damage and contribute to the plant's color, aroma and flavor. In general, the plant chemicals that protect plant cells from environmental hazards such as pollution, stress, drought, UV exposure and pathogenic attack are called as phytochemicals [4,5]. These compounds that are not nutrients but have defined or disease-preventive qualities are called phytochemicals. These nutrients are not necessary, and plants primarily manufacture them to protect themselves. These compounds are known as secondary plant metabolites and have biological properties such as antioxidant, antibacterial, antiviral, antiallergic, antidiarrheal, and anthelmintic effects [6]. There are more than thousand known and many unknown phytochemicals. It is well-known that plants produce these chemicals to protect themselves, but recent researches demonstrate that many phytochemicals can also protect human against diseases [7]. The purpose of the present review is to provide an overview of the phytochemicals presents in the selected *Gracilaria* species.

MATERIALS AND METHODS

Collection of Plant Materials:

The plant material selected for the present study was *Gracilaria fergusonii* J.Ag. belongs to the family Gracilariaceae was collected from Hare Island (Thoothukudi district), Tamil Nadu, India. The seaweeds were identified by referring to the keys given by Umamaheswara Rao, (1970, 1987). Classification of seaweeds cited by Krishnamurthy and Joshi (1970) and Tewari (2006) were followed systematically.

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 [8]. The herbarium specimen was deposited in St. Xavier's College Herbarium (XCH), St. Xavier's College, Palayamkottai. The following Voucher number, *Gracilaria fergusonii* J.Ag. (XCH30402) was given.

Preparation of extracts

For the preparation of methanolic extract, the selected *Gracilaria fergusonii* J.Ag. was washed thoroughly and placed on blotting paper for shade dry. The shade dried samples were grounded to fine powder and stored in the refrigerator for further use. 30g powdered samples was packed in Soxhlet apparatus and extracted with methanol for 8h separately [9]. All the extracts were air dried in Petri plates and the extracts were weighed and kept in eppendorf at -4° C until used.

UV-Visible spectral analysis

The crude extract containing the bioactive compounds were analyzed by UV-Visible spectrophotometer for further confirmation of metabolites and functional groups. The crude extracts of the selected *Gracilaria fergusonii* J.Ag. species was scanned in a wavelength ranging from 200-900nm using a Shimadzu spectrophotometer and characteristic peaks were detected. The UV-Visible spectrum was repeated twice and confirmed the spectrum. The peak values of the extracts were observed and recorded.

Fourier Transform Infrared (FTIR) spectral analysis

FTIR analysis of the methanol extracts of *Gracilaria fergusonii* J.Ag. was performed using Perkin Elmer Spectrophotometer system, which was used to detect the characteristic peaks and their functional groups. About 1mg of extract was separately made in to thin discs with 10 to 100mg of potassium bromide using a mould and press under anhydrous conditions. Followed by the measurements were taken in an automatic recording FTIR spectrophotometer in the range of 4000 to 400cm^{-1}. The peak points of the FTIR were recorded and compared with the standard peak value and interpreted the functional groups.

HPLC analysis

The HPLC analysis of methanol extracts of *Gracilaria fergusonii* J.Ag. was performed on a Shimadzu LC-10AT VP HPLC system, equipped with a model LC-10AT pump, UVVisible 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

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/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

UV-Visible spectrum analysis

The UV-Visible fingerprint profile of the methanol extracts of *Gracilaria fergusonii* J.Ag. was selected at the wavelength of 200nm to 900nm due to the sharpness of the peaks and proper baseline. The profile showed the compounds separated at the nm of 979.5, 662, 606, 531.5, 498.5 and 400 with the absorption 0.046, 0.348, 0.176, 0.202, 0.236 and 1.417 respectively (Table-1).

Table-1: UV-Visible spectrum of methanol extract of *Gracilaria fergusonii* J.Ag.

Solvent	***Gracilaria fergusonii***	
	nm	**Abs**
Methanol	797.5	0.046
	662	0.348
	606	0.176
	531.5	0.202
	498.5	0.236
	400	1.417

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 methanol extract of *Gracilaria fergusonii* J.Ag. was passed into the FTIR and the functional groups of the components were separated based on its peak ratio. The results of FTIR analysis showed

different peaks at 1633.59, 2131.19, 2851.56, 2920.99, 3380.98, 3443.66 and 3523.70cm^{-1}. It was confirmed the presence of functional groups such as tertiary amides (C=O stretch), C≡C in alkynes (C≡C stretch), aliphatic (CH antisym and sym stretch), aliphatic (CH antisym and sym stretch), alcohols and phenols (OH stretch), aromatic, primary amines (NH stretch) and alcohols and phenols (OH stretch) respectively (Figure-1 and Table-2).

Figure-1: FTIR spectrum of methanol extract of *Gracilaria fergusonii* J.Ag.

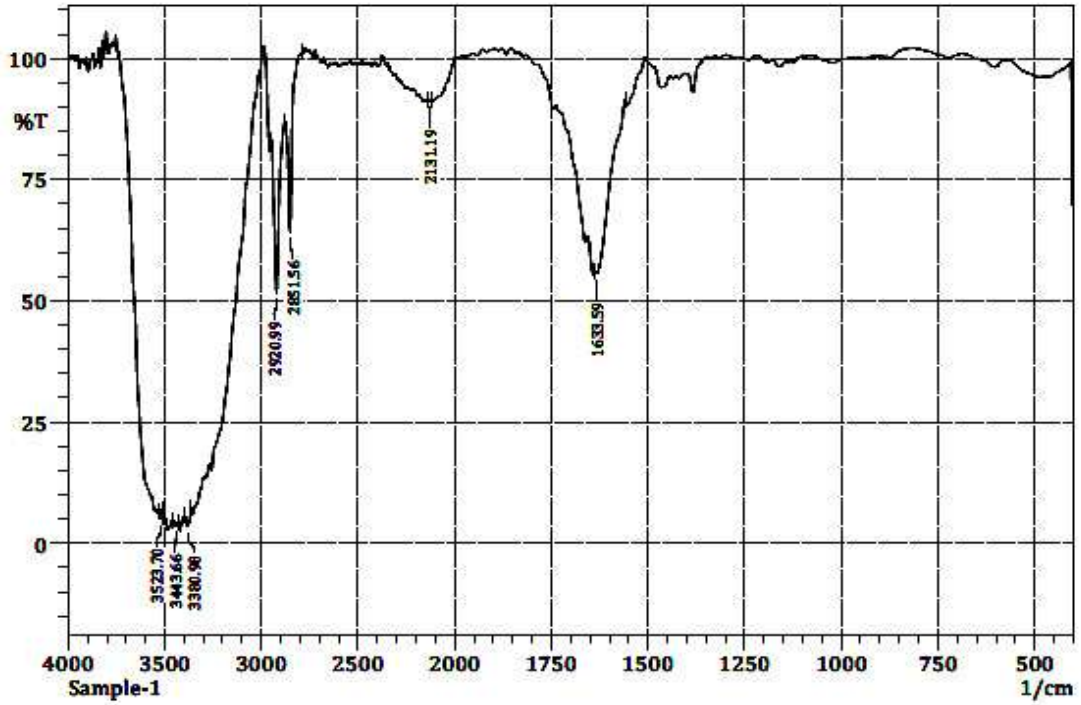

Table-2: FTIR spectrum of methanol extract of *Gracilaria fergusonii* J.Ag.

PEAK VALUE	FUNCTIONAL GROUP	ASSIGNMENT
3523.70	Alcohols and Phenols	OH stretch
3443.66	Aromatic and Primary amines	NH stretch
3380.98	Alcohols and Phenols	OH stretch
2920.99	Aliphatic	CH antisym and sym stretch
2851.56	Aliphatic	CH antisym and sym stretch
2131.19	C≡C in alkynes	C≡C stretch
1633.59	Tertiary amides	C=O stretch

HPLC analysis

The qualitative HPLC fingerprint profile of the methanol extracts of *Gracilaria fergusonii* J.Ag. was selected at a wavelength of 254nm due to the sharpness of the peaks and proper baseline. The methanol extract prepared by hot extraction was subjected to HPLC for the separation and identification of chemical constituents present in the *Gracilaria fergusonii* J.Ag. Fifteen compounds were separated from the methanol extract of *Gracilaria fergusonii* J.Ag. at different retention time of 1.273min, 1.483min, 1.570min, 1.743min, 1.850min, 2.017min, 2.247min, 3.520min, 3.850min, 4.220min, 4.517min, 4.703min, 5.107min, 7.037min and 12.230min. The profile observed five prominent peaks at the retention time of 1.483min, 1.570min, 1.743min, 1.850min, 2.017min, followed by ten moderate peaks were predicted at the retention time of 1.273min, 2.247min, 3.520min, 3.850min, 4.220min, 4.517min, 4.703min, 5.107min, 7.037min and 12.230min (Figure-2 and Table-3).

Figure-2: HPLC profile of methanol extract of *Gracilaria fergusonii* J.Ag.

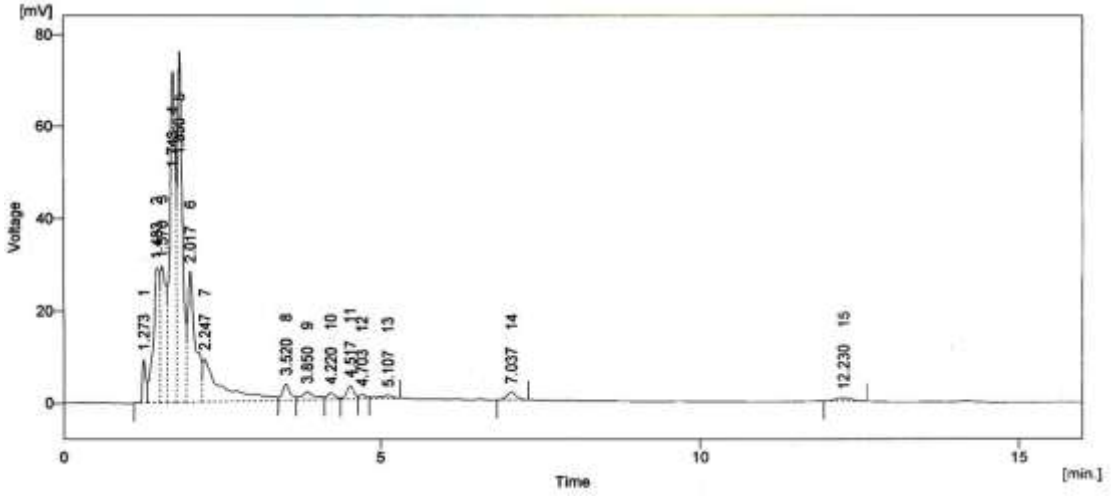

Table-3: HPLC profile of methanol extract of *Gracilaria fergusonii* J.Ag.

	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W05 [min]
1	1.273	36.591	9.524	1.9	3.5	0.07
2	1.483	207.294	29.390	11.0	10.9	0.10
3	1.570	186.540	29.693	9.9	11.1	0.11
4	1.743	457.796	71.853	24.3	26.8	0.11
5	1.850	423.827	76.220	22.5	28.4	0.09
6	2.017	245.490	28.405	13.0	10.6	0.12
7	2.247	190.766	9.287	10.1	3.5	0.17
8	3.520	29.711	3.590	1.6	1.3	0.12
9	3.850	24.422	1.738	1.3	0.6	0.18
10	4.220	12.364	1.478	0.7	0.6	0.13
11	4.517	22.666	2.901	1.2	1.1	0.12
12	4.703	6.974	0.921	0.4	0.3	0.14
13	5.107	10.059	0.647	0.5	0.2	0.24
14	7.037	16.705	1.632	0.9	0.6	0.16
15	12.230	13.078	0.739	0.7	0.3	0.28
16	76.310	3.335	0.418	0.2	0.2	0.19
	Total	1887.617	268.436	100.0	100.0	

CONCLUSION

In the present study the important red algae *Gracilaria fergusonii* J.Ag. was selected and the phytoconstituents were screened. From the results it was concluded that methanolic extract of selected *Gracilaria fergusonii* J.Ag. has more number of secondary metabolites, followed by qualitative analysis like UV, FTIR and HPLC were carried out. From this various compounds were separated at different nm, seven functional groups were separated from FTIR and fifteen compounds were identified through HPLC. The phytochemical analysis of the selected *Gracilaria* species may possess important pharmacological activities and also important for the manufacturing of new drugs. Further studies are needed to separate the bioactive components and could be useful for treating various human disorders.

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