IJNRD.ORG ISSN : 2456-4184
INTERNATIONAL JOURNAL OF NOVEL RESEARCH AND DEVELOPMENT (IJNRD) | IJNRD.ORG
An International Open Access, Peer-reviewed, Refereed Journal

HPTLC based chemical fingerprint profiling of sterols and antioxidant activity of Ulva, Sargassum and Gracilaria from Thirumullavaram, Kerala

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Abstract

The present investigation was aimed to study and compare the HPTLC fingerprint profiling of sterols and antioxidant activity in three different algae such as Ulva, Sargassum and Gracilaria belongs to Chlorophyceae, Phaeophyceae and Rhodophyceae respectively. HPTLC is a modern technique considered as a good fingerprint analytic tool having a large applicability in the field of plant material analysis and determine major active constituents present in them. The methanolic extracts of the selected algal samples obtained after Soxhlet extraction were subjected for HPTLC screening along with other phytochemical constituents at different Rf values at 366 nm. The antioxidant activity is carried out by phosphomolybdenum method. 0.3ml of sample (1mg/ml) was mixed with 3ml of phosphomolybdenum reagent. The result of HPTLC fingerprint profiling suggest that the three selected algae such as Ulva, Sargassum and Gracilaria contain sterols along with other phytochemical constituents. The result of antioxidant activity also suggests three distinct value and all the tested algae were characterized with high antioxidant activities

Keywords: HPTLC fingerprint profiling, *Ulva*, *Sargassum*, *Gracilaria*, Sterols, Chlorophyceae,Phaeophyceae, Rhodophyceae, Antioxidant activity

Introduction

Algae are known to be the largest primitive photoautotrophic and polyphyletic group of eukaryotes, which perform more than 50% of photosynthesis on this planet (Menaa F *et al*., 2020). Marine algae are one among the commercially important marine living resources. In the recent years several significant metabolites with pharmacological properties have been derived from seaweeds that have a broad range of bioactive compounds. Seaweeds create a natural source of a variety of drugs for pharmaceutical, food and cosmetic applications including carotenoids, terpenoids, steroids, amino acids, phlorotannins, phenolic compounds, halogenated ketones, alkanes and cyclic polysulphides (Taskin E *et al.*, 2007) (Guedes A *et al*, 2011). Seaweeds are rich in polyunsaturated fatty acids, steroids, terpenes, carotenoids, sulphated polysaccharides, sesquiterpene hydroquinins, mycosporins, acetogenins, phenols, amino acid derivatives, various vitamins (A, B1, B12, C, D), as main part of other biochemicals (Gressler, 2010;Safinaz and Ragaa, 2013).Many of these molecules show different biological activities such as antiviral, antibacterial, antifungal, antitumor, anticoagulant, hypotensive and antioxidant, revealing the great potential seaweeds could have in the different industries (Gressler 2010; Limberger and Gheller,2011). Phytosterols are the sterols present in plants (Piironen V *et al*., 2003).Phytosterols, also known as plant sterols, are a type of cholesterol related chemical. It has been discovered that, like higher

plants, sterols in macro algae play an important role as a bioactive molecule. Algal sterols are quite diverse, thus detecting and screening those chemicals will be important for algal identification as well. HPTLC fingerprinting has been shown to be a reliable, precise, and accurate method for herbal identification, and it can also be used to authenticate and characterise medicinally important plants (Kpoviessia DSS *et al.,* 2008). Chromatographic fingerprint is a rational option to meet the need for more effective and powerful quality assessment to the traditional system of medicine throughout the world. (Halin'ski P *et al.,* 2009). The optimised chromatographic finger print is not only an alternative analytical technique for authentication, but also a method for expressing the numerous patterns of chemical constituents distributed in herbal medications and preserving such a "database" for future multifaceted, long-term research. Algae generally has higher antioxidant activity due to a higher contents of nonenzymatic antioxidant components, such as ascorbic acid, reduced glutathione, phenols and flavonoids (Wu SC *et al*., 2010).

Materials and Methods

Study area

Kerala is situated on the South West coast of India. The coastline is dissected with sandy stretches and natural rocks. The selected site for the present study was Thirumullavaram from Kollam district, southern most part of Kerala.

Sample preparation

Fresh algal samples were handpicked and thoroughly washed first with seawater to remove all the impurities, sand particles etc., further washed with fresh water to remove salt from the surface and finally with distilled water then shade dried for 7- 10 days. The dried samples were powdered and 5 g of the powder were subjected for Soxhlet extraction in two solvents Hexane and Methanol (45:5). The extracts were made up to the required volume for HPTLC fingerprint profiling.

HPTLC screening of sterols

Methanolic extract of the selected plant was subjected to HPTLC (CAMAG, Switzerland) analysis. A Camag HPTLC instrument consisting of Linomat V automatic spotter equipped with a 100µL syringe connected to a nitrogen cylinder, Scanner-III, twin-trough developing chambers and
viewing cabinet with dual wavelength UV lamps (Camag,Muttenz, Switzerland) were used. Methanolic extracts of seaweeds were spotted on a silica gel 60F254 (Merck, Germany) TLC plate. The plate was air dried and then developed by using the solvent system Toluene: Acetone (9:1) (v/v) as mobile phase in a CAMAG- twin-trough glass chamber (20 x 10 x 4) previously saturated with mobile phase vapour for 20 minutes. After developing the plate, it was dried and scanned using Scanner 3 (CAMAG, Switzerland) at 254 and 366 nm using WinCATS software. Chromatograms were evaluated before and after spraying with Anisaldehyde- Sulphuric acid reagent. After derivatization, the plates were dried in hot air oven for 5 minutes at 105° C and viewed under UV at 254 and 366nm. Densitometric scanning was performed on Camag TLC scanner III and operated by CATS software (V 3.15, Camag).

Determination of total antioxidant activity

The total antioxidant activity was determined by phosphomolybdenum method. 0.3ml of sample (1mg/ml) was mixed with 3ml of phosphomolybdenum reagent. The blank solution was also prepared. All the tubes were incubated at 97°C for 90min. cooled and the absorbance was measured at 695nm using an UV-Vis spectrophotometer against the blank. The antioxidant capacity was expressed as ascorbic acid equivalent by using standard ascorbic acid.

Results and discussion

The phytochemical constituents in a plant material from a characteristic fingerprint, represents the quantity of active constituents. Moreover, this may help to standardize the mixtures like herbal drug formulations. Methanolic extracts of Sargassum Gracilaria and Ulva were screened for sterols using HPTLC fingerprinting with Toluene: Acetone as mobile phase. When viewed under visible light and uv at 254nm, 366 nm before and after derivatization with Anisaldehyde sulphuric acid reagent, good separation of constituents with different Rf values were observed in the chromatogram. (Fig:1a,b,c&2a,b,c). 5 peaks were found in the HPTLC chromatogram of Ulva with Rf values 0.250,0.816,0.829,0.932 and 0.952. Presence of sterols was found in the bands with Rf values 0.829 and 0.952 with peak areas of 21.8% and 78.82% respectively (Table1& Fig.2.1a) For Sargassum, 7 bands were detected with Rf values 0.240,0.634,0.647,0.816,0.832 and 0.945. Among this Sterols were detected in those bands with a Rf values 0.647,0.832 and 0.945 with peak areas 6.17%,11.31% and 82.53% respectively (Table2 &Fig.2.1b). Gracilaria showed 5 bands with maximum Rf values of 0.045,0.816,0.835,0.931 and 0.952 and sterols were found in Rf values of 0.045,0.835 and 0.952 with peak areas of 45.92%, 24.58% and 29.50% respectively (Table3 &Fig.2.1c).

Antioxidant activity of three marine algae such as Ulva, Sargassum and Gracilaria show distinct values. The algae Ulva have 12.6 µg/mL antioxidant activity(Table4). Gracilaria shows antioxidant activity of 161.3 µg/mL and Sargassum shows 53.5 µg/mL antioxidant activity.

Marine algae have been shown to constitute a rich source of bioactive compounds. Phytosterols are believed to be related to many health beneficial effects in humans and these are found to be of great relevance to the development of new drugs and functional foods. HPTLC is an important tool used for the identification, evaluation, purity and stability testing to formulate products of ayurvedic, pharmaceuticals, cosmeceuticals and nutraceuticals industry.

In the present investigation the sterols were distinguished from other components based on the Rf values. It was found that sterols are present in the selected algal samples at different Rf values. Each of the selected algae showed it's own bands for sterol in the chromatogram which will be a characteristic feature for the taxonomic identification marker among that particular genus. According to the study, Sargassum and Gracilaria show 3 different Rf values were sterol found and Ulva only contains two Rf values of sterol. Ulva and Gracilaria shares a common Rf value of 0.952.

Table 1: Peak and Rf values of the chromatogram of Ulva methanolic extract at 366nm

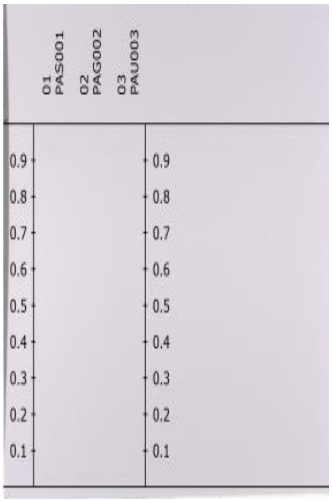
Ulva										
Peak	Start		Max			End		Area		Assigned substance
	Rf	H	Rf	H	%	Rf	H	A	%	
1	0.210	0.0046	0.250	0.0186	4.39	0.294	0.0019	0.00075	3.90	Unknown
2	0.734	0.0043	0.816	0.1205	28.44	0.852	0.0105	0.00525	27.19	Unknown
3	0.794	0.0000	0.829	0.0188	19.24	0.868	0.0006	0.00072	21.18	Sterol
4	0.853	0.0103	0.932	0.2847	67.18	0.977	0.0030	0.01331	68.90	Unknown
5	0.910	0.0000	0.952	0.0791	80.76	0.992	0.0000	0.00267	78.82	Sterol

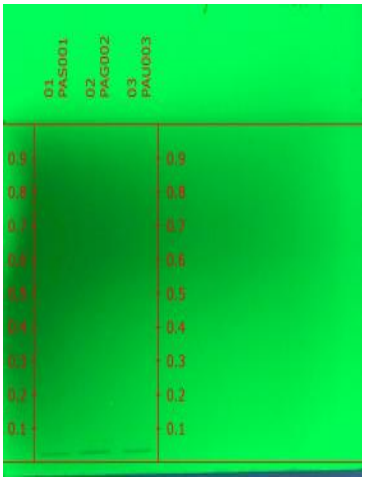
Table 2:Peak list and Rf values of the chromatogram of Sargassum methanolic extract at 366nm

Sargassum										
Peak	Start		Max			End		Area		Assigned substance
	Rf	H	Rf	H	%	Rf	H	A	%	
1	0.189	0.0032	0.240	0.0193	2.79	0.290	0.0025	0.00098	2.75	Unknown
2	0.576	0.0053	0.634	0.0678	9.79	0.684	0.0107	0.00322	9.04	Unknown
3	0.605	0.0032	0.647	0.0214	6.97	0.685	0.0000	0.00089	6.17	Sterol
4	0.760	0.0049	0.816	0.1050	15.16	0.853	0.0237	0.00466	13.07	Unknown
5	0.771	0.0000	0.832	0.0313	10.22	0.869	0.0156	0.00163	11.31	Sterol
6	0.853	0.0237	0.932	0.5003	72.25	0.987	0.0017	0.02677	75.13	Unknown
7	0.882	0.0177	0.945	0.2536	82.81	1.000	0.0000	0.01186	82.53	Sterol

Table 3: Peak list and Rf values of the chromatogram of Gracilaria methanolic extract at 366nm

Gracilaria										
peak	Start		Max			End		Area		Assigned substance
	Rf	H	Rf	H	%	Rf	H	A	%	
1	0.003	0.0000	0.045	0.0383	49.03	0.058	0.0236	0.00122	45.92	Sterol
2	0.745	0.0013	0.816	0.0696	36.18	0.853	0.0044	0.00304	36.54	Unknown
3	0.789	0.0000	0.835	0.0172	22.07	0.865	0.0030	0.00065	24.58	Sterol
4	0.890	0.0450	0.931	0.1229	63.82	0.974	0.0010	0.00528	63.46	Unknown
5	0.913	0.0000	0.952	0.0226	28.90	0.987	0.0000	0.00078	29.50	Sterol





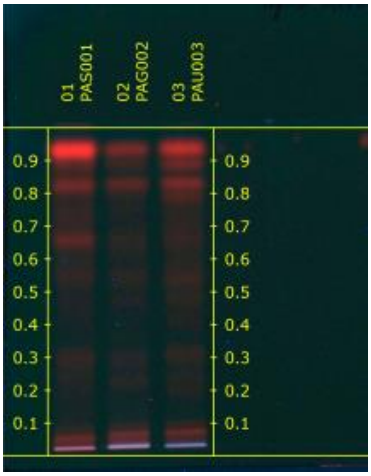


Fig. 1:a **Fig. 1:b** **Fig. 1:c**

Fig.1 :a – HPTLC plate under visible light before derivatization

Fig.1:b – HPTLC plate under UV light before derivatization at 254nm

Fig.1:c – HPTLC plate under UV light before derivatization at 366nm

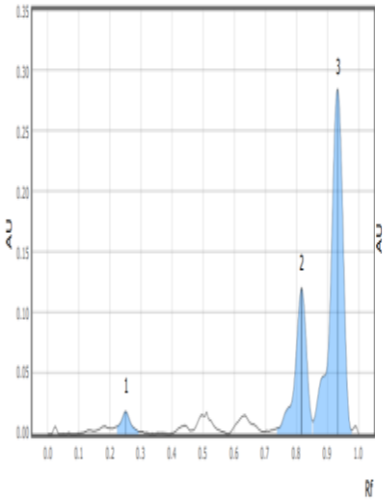


Fig.1.1:a

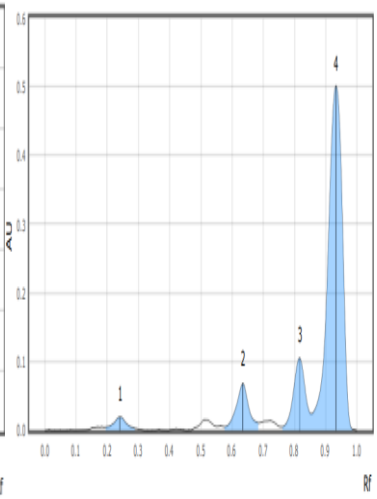


Fig.1.1:b

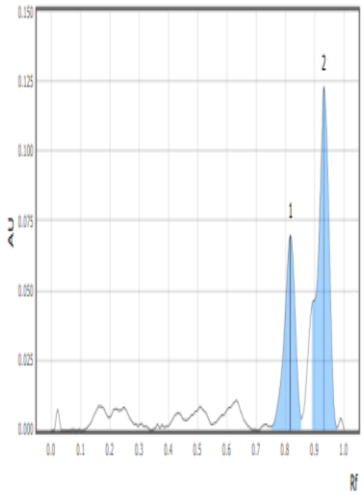


Fig.1.1:c

Fig.1.1:a – HPTLC chromatogram of Ulva before derivatization at 366nm

Fig.1.1:b – HPTLC chromatogram of Sargassum before derivatization at 366nm

Fig.1.1:c – HPTLC chromatogram of Gracilaria before derivatization at 366nm

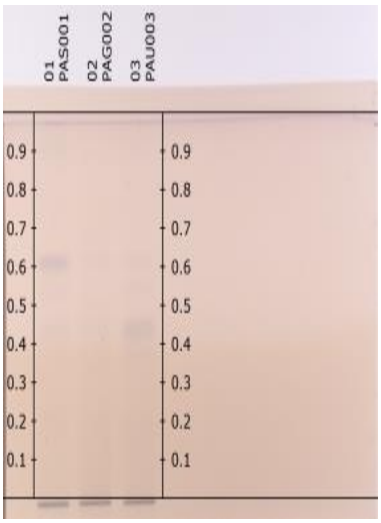


Fig.2:b

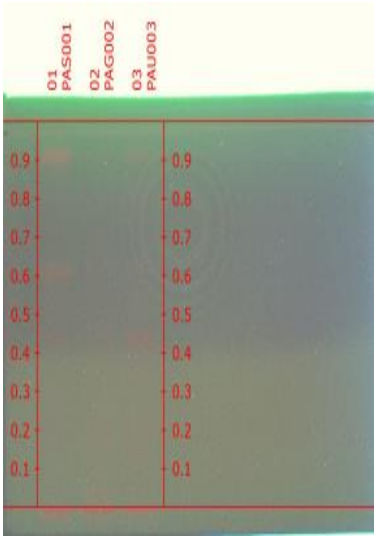


Fig.2:c

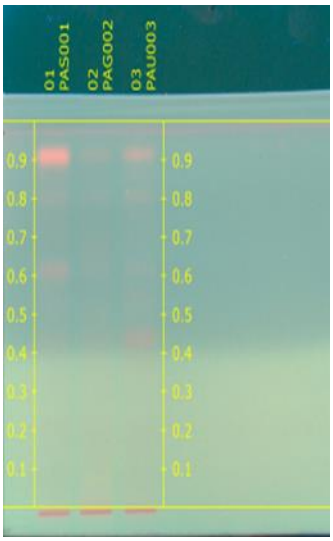


Fig.2:a

Fig.2:a – HPTLC plate under visible light after derivatization

Fig.2:b – HPTLC plate under UV light after derivatization at 254nm

Fig.2:c – HPTLC plate under UV light after derivatization at 366nm

PAU003 – Methanolic extract of Ulva

PAS001 – Methanolic extract of Sargassum

PAG002 – Methanolic extract of Gracilaria

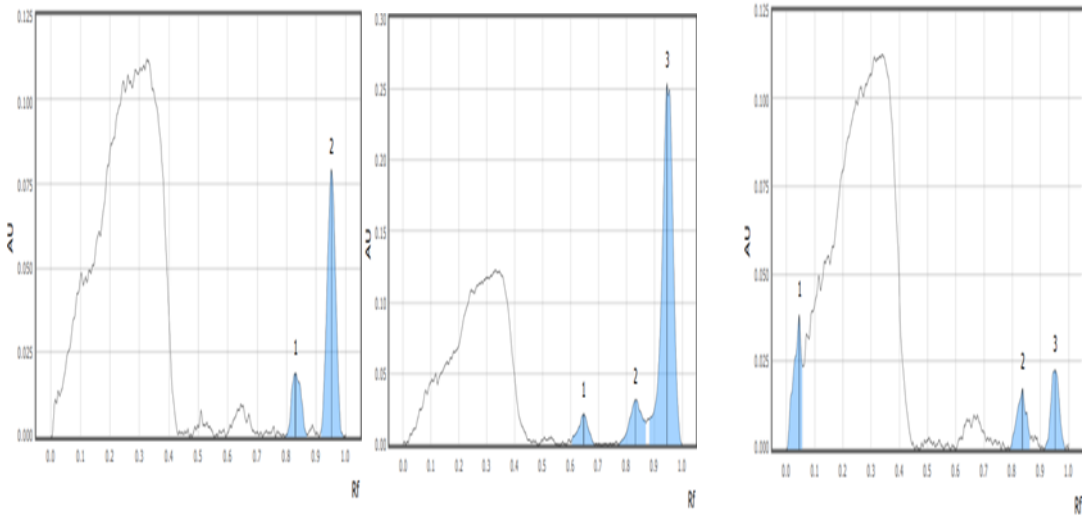


Fig.2.1:a **Fig.2.1:b** **Fig.2.1:c**

Fig.2.1:a – HPTLC chromatogram of Ulva after derivatization at 366nm

Fig.2.1:b – HPTLC chromatogram of Sargassum after derivatization at 366nm

Fig.2.1:c – HPTLC chromatogram of Gracilaria after derivatization at 366nm

Table 4: Antioxidant activity of three different algae such as Ulva, Sargassum and Gracilaria

Sl. No.	Samples	Antioxidant activity (µg/mL)
1.	Ulva	12.6
2.	Sargassum	53.5
3.	Gracilaria	161.3

Conclusion

The current study on the HPTLC fingerprint profiling of methanolic extracts of Sargassum, Gracilaria and Ulva revealed the presence of sterols along with other phytochemical constituents in different Rf values in a particular mobile phase. The presence of sterols and other phytochemical constituents in these selected algae useful for the preparation and development of novel functional ingredients for pharmaceuticals and functional foods. The results from the antioxidant activity clearly indicated that all the tested seaweeds in this investigation possess antioxidant activity.

Acknowledgement

The authors are thankful to the staffs at Central Laboratory for Instrumentation and Facilitation, Kerala university Kariavattom for the instrumentation support to carry out the work.

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