Journal of Pharmacognosy and Phytochemistry

Available online at www.phytojournal.com

J P P Journal of Pharmacognosy and Phytochemistry

E-ISSN: 2278-4136
P-ISSN: 2349-8234
JPP 2017; 6(4): 951-955
Received: 17-05-2017
Accepted: 18-06-2017

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HPTLC based chemical fingerprint profiling of sterols of selected brown algae from Kerala

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Abstract
The objective of the present investigation was to study the HPTLC fingerprint profiling of sterols in three different species of Phaeophyceae such as *Sargassum wightii*, *Sargassum cristaefolium* and *Padina tetrastromatica* from Kerala coast. Methanolic extracts of the selected algal samples were subjected for HPTLC screening with mobile phase as Toluene: Acetone. The fingerprint showed the bands of sterols along with other phytochemical constituents. HPTLC is a modern standardized technique having a large applicability in the field of plant material analysis and stability tests of extracts, finished products and can be used as a tool for chemotaxonomic studies.

Keywords: HPTLC profiling, Fingerprint, Chaemotaxonomic studies, Sterols, Phaeophyceae, *Sargassum wightii*, *Sargassum cristaefolium*, *Padina tetrastromatica*

Introduction
Marine algae are one of the commercially important marine living resources attached to the bottom of shallow and deep waters of the sea, estuaries and backwaters. Most of the marine algae are used as staple diet by human beings from different countries. Certain edible seaweeds contain significant quantities of protein, lipids, minerals and vitamins [1]. In the recent years several significant metabolites with pharmacological properties have been derived from seaweeds that have a broad range of biological activities. Although thousands of bioactive compounds have been discovered, the need for novel therapeutic compounds is still urgent in view of the emergence of a number of new diseases and the resistant strains of microorganisms [2]. Seaweeds are one of the natural sources of many organic and inorganic substances which are of nutritive and nutraceuitical value. Secondary metabolites produced by marine organisms could be the source of bioactive substance and useful in modelling compounds for drugs [3]. Marine organisms have received great attention during recent years for natural product chemistry, a promising new area of study. Phytosterols are among the most interesting compounds, being present in all plants and in foods containing plant-based ingredients. Among marine organisms, algae represent the most significant source of these compounds, and they have thus attracted the attention of researchers in recent years [4]. Phytosterols are a family of molecules related to cholesterol, and are usually found in the cell membranes of plants, where they play important roles like cholesterol in humans. It is revealed that like the higher plants, sterols in macro algae also have a major role as bioactive compound. The most common phytosterols in human diet are campesterol, sitosterol and stigmasterol [5]. Phytosterols have received much attention in the last five years because of their cholesterol lowering properties. Algal sterols are extremely diverse and the detection and screening of these compounds will be useful for algal identification also. Although the general sterol pattern is quite stable between different algal groups, the ecological differences, geographic origins, and developmental stages of the organisms can contribute to different phytosterol profiles [6, 7].
High Performance Liquid Chromatography (HPTLC) is being used for fingerprint profiling of medicinal plant extracts since long [8, 9]. The HPTLC fingerprint profile has been proved to be an effective tool in differentiating closely related species and detecting adulteration and substitution in raw drugs of Indian systems of medicine [10]. The analytical tools that can generate a fingerprint of each extract in large collections would be useful to detect stability of the same extract over time. Preferably, the method should be based on electronic storage, retrieval and analysis of the data [11]. Present study was an attempt to compare the HPTLC fingerprints of sterols in methanolic extracts of three different species of Phaeophyceae *viz*, Sargassum wightii, Sargassum cristaefolium and Padina tetrastromatica from Kerala coast.

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 and also artificial seawalls which protrude in to the sea. The selected site for the present study was Mullur from Thiruvananthapuram district, the southernmost part of Kerala. The nature of the coast is mostly sandy. However in this area different kinds of rocks are found in patches in the sub tidal and intertidal regions. Algal samples were collected during low tides at the depth of about 1.2 m.

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. After draining off water, excess water was removed from the samples using blotting and then shade dried for 7- 10 days. The dried samples were powdered and 10 g of the powder were subjected for sauxhlet extraction in two solvents Hexane and Methanol. 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. Before analysis, HPTLC plates were cleaned by predevelopment with methanol and activated at 110°C for 5 minutes for solvent removal. Methanolic extracts of seaweeds were spotted on a silica gel $60F_{254}$ (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 366 nm. Densitometric scanning was performed on Camag TLC scanner III and operated by CATS software (V 3.15, Camag).

Results and Discussion

The phyto-chemical constituents in a plant material from a characteristic fingerprint, represents the quantity of active constituents. Moreover, this helps to standardize the mixtures like herbal drug formulations and market samples[12]. Methanolic extracts of *S. wightii*, *S. cristaefolium* and *Padina tetrastromatica* were screened for sterols using HPTLC with Toluene: Acetone as mobile phase. When viewed under UV at 254 nm, 366 nm and after derivatization with Anisaldehyde sulphuric acid reagent, good separation of constituents with different R_f values were observed in the chromatogram. (Fig: 1a – 1c). In the HPTLC chromatogram it was found that number of spots (bands) obtained in the selected samples were different with different R_f values and peak areas. Sterols were detected in all the samples along with other phytochemical constituents. For *Sargassum wightii,* 9 bands were detected with R_f values 0.02, 0.06, 0.07, 0.32, 0.36, 0.45, 0.68, 0.88 and 0.95.Among this, sterols were detected in those bands with a Rf values 0.06, 0.36 and 0.45. (Table 1 &Fig: 2). *S. cristaefolium* showed 11 bands in the chromatogram with maximum R_f values of 0.05, 0.07, 0.32, 0.45, 0.59.0.70, 0.78, 0.81, 0.82, 0.88 and.0.95. (Table 2 & Fig: 3). 10 peaks were found in the HPTLC chromatogram of *P. tetrastromatica* with R_f values 0.05, 0.26, 0.45, 0.59, 0.73, 0.77, 0.80, 0.85, 0.88 and 0.96. Presence of sterols was found in the bands with R_f values 0.05, 0.26 and 0.45. (Table 3 & Fig: 4) The peak areas were also depicted in the tables. Areas covered by the sterols obtained from the peaks in the studied samples were 29.73%, 51.6%, 31.10% for *S. wightii*, *S. cristaefolium* and *P. tetrastromatica* respectively.

Marine algae have been shown to constitute a rich source of bioactive compounds, being used as food, in textile and pharmaceutical industries [13]. 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 [14]. HPTLC is an important tool used for the identification, evaluation, purity and stability testing, dissolution value and content uniformity testing of various raw materials like herbal extracts, tinctures, essential oils, fermentation mixtures, drug and excipients and formulated products of ayurvedic, pharmaceuticals, cosmaceuticals and nutraceuticals industry [15].

In the present investigation the sterols were distinguished from other components based on the R_f values. It was found that sterols with R_f value 0.45 were in all the three samples studied, which can be isolated and characterized for structural analysis of that particular sterol and this can be used as a taxonomic identification marker among the class. Along with the common bands obtained, each of the selected algae showed its own bands for sterol which will be a characteristic for that particular genus. Macroalgae sterol composition has been used for chemotaxonomic classification [16, 17]. Besides this, phytosterols are bioactive compounds, which can be found in a great variety of plant based foods [18]. Steroids may serve as an intermediate in the biosynthesis of downstream secondary natural products and it is believed to be a biosynthetic precursor for cardenolides in plants [19]. Independent of their function, the presence of steroids in practically every organism suggests that they have a powerful role in chemosystematics [20].

Table 1

Sargassum wightii				
Peak	**Rf**	**Max. height**	**Area**	**Assigned substance**
1	0.02	13.4	100.3	Unknown
2	0.06	27.0	415.7	Sterol 1
3	0.07	25.3	345.0	Unknown
4	0.32	122.2	6155.3	Unknown
5	0.36	11.4	201.1	Sterol 2
6	0.45	133.9	3694.8	Sterol 3
7	0.68	17.9	313.3	Unknown
8	0.88	31.2	601.9	Unknown
9	0.95	51.1	1275.9	Unknown

Table 2

Sargassum cristaefolium				
Peak	**Rf**	**Max. height**	**Area**	**Assigned substance**
1	0.05	21.2	301.5	Unknown
2	0.07	24.4	476.9	Sterol 1
3	0.32	101.9	4218.8	Sterol 2
4	0.45	112.0	3187.5	Sterol 3
5	0.59	27.2	1756.5	Unknown
6	0.70	24.0	530.5	Unknown
7	0.78	45.0	1587.7	Unknown
8	0.81	38.1	508.4	Unknown
9	0.82	27.1	557.2	Unknown
10	0.88	32.4	518.1	Unknown
11	0.95	58.6	1616.6	Unknown

Table 3

Padina tetrastromatica				
Peak	**Rf**	**Max. height**	**Area**	**Assigned substance**
1	0.05	25.5	410.0	Sterol 1
2	0.26	33.6	1587.9	Sterol 2
3	0.45	39.3	1477.9	Sterol 3
4	0.59	52.1	2780.0	Unknown
5	0.73	31.4	496.8	Unknown
6	0.77	38.7	1248.6	Unknown
7	0.80	39.9	588.6	Unknown
8	0.85	25.8	401.2	Unknown
9	0.88	31.4	522.0	Unknown
10	0.96	57.8	1661.8	Unknown

Table 1 -3- HPTLC fingerprinting chart showing number of peaks, R_f values, Maximum Height, Area, and Assigned substance

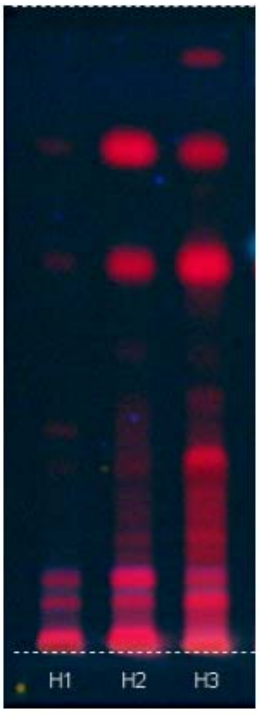

Fig 1: a

Fig 2: b

Fig 3: c

Fig 1: a- HPTLC plate under UV light before derivatization at 366nm
Fig 1: b –HPTLC Plate under Visible light after derivatization at 254 nm
Fig 1: c- HPTLC plate under UV light after derivatization at 366 nm
H1 - Methanolic extract of *Sargassum wightii*.
H2 - Methanolic extract *of Sargassum cristaefolium*
H3 - *Padina tetrastromatica.*

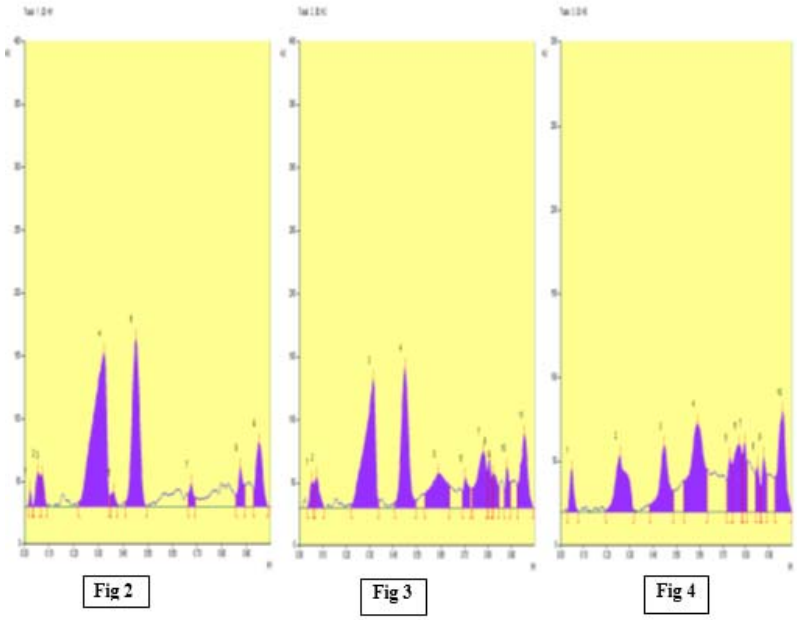

Fig 2: HPTLC fingerprint of *S.wightii*
Fig 3: HPTLC fingerprint of *S.cristaefolium*
Fig 4: HPTLC fingerprint of *P.tetrastromatica*

Conclusion

The current investigation on the HPTLC fingerprint profiling of methanolic extracts of *S.wghtii*, *S.cristaefolium* and *P. tetrastromatica* revealed the presence of sterols along with other phytochemical constituents in a particular mobile phase, which may be attributed to the bioactive properties of the selected members. This fingerprint profiling can be used as a basis for taxonomic classification, identifying the marine sources of sterols with its applications in food and pharmaceutical industries and also helps to identify active metabolites involved the plant extracts and in quality control aspects of the developed product. Further investigations are needed to be carried out to identify and characterise the structure of the sterols.

Acknowledgement

The authors are thankful to the staffs at Dalmia Centre for Research and Development, Coimbatore for giving an opportunity to carry out the analytical part work. And also wish to thank the CMFRI Cochin and Mandapam for rendering help in the identification of the seaweed samples.

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