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Original Research Article

Phytochemical screening and HPTLC fingerprint profile of marine red alga *Spyridia fusiformis* Boergesen

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Abstract: The present study was aimed to develop the high performance thin layer chromatography (HPTLC) fingerprint profile for methanol extracts of marine red alga *Spyridia. fusiformis.* A HPTLC method for the separation of the active constituents in extracts have been developed and TLC of these extracts on silica gel pre-coated aluminum plates by the automatic TLC applicator and using the solvent system n-hexane: ethyl acetate (60:40 v/v) was performed. Preliminary phytochemical screening of the extracts showed the presence of alkaloids, triterpenes, tannins, saponins, glycosides, phenolic compounds and flavonoids. HPTLC finger print scanned at 350 nm from methanol extractof *S. fusiformis* revealed 7 peaks with R_f values in the range of 0.32 to 0.92. It can be concluded that HPTLC fingerprint analysis of methanol extract of marine red alga *S.fusiformis* a phytochemical marker.

Keywords: Authentication, *Spyridia fusiformis*, Fingerprint, HPTLC, Profile, Standardization.

INTRODUCTION

Products of primary metabolism, such as amino acids, carbohydrates and proteins are playing vital role in the maintenance of life processes, while others like alkaloids, phenolics, steroids, terpenoids are products of secondary metabolism and have toxicological, pharmacological and ecological importance [1].

The phytochemical evaluations of plants, which have a suitable history of use in folklore, have often resulted in the isolation of principle compounds with remarkable bioactivities [2]. It is a widely accepted fact, which used as the quality control protocol, thus any change in the quality of extract directly affects the bioactivities.

Seaweeds are considered as a source of bioactive compounds as they are able to produce a great variety of secondary metabolites characterized by their biological activities. The environment in which seaweeds grow is harsh as they are exposed to a combination of light and high oxygen concentrations. These factors can lead to the formation of free radicals and other strong oxidizing agents, but seaweeds seldom suffer any serious photodynamic damage during metabolism. This fact implies that seaweed cells have some protective mechanisms played by the bioactive compounds [3].

In the past years, considerable progress made in the development of bio-based agrochemicals, among which products derived from algae gaining importance. Ever since the first algal extracts have been introduced in the market, a permanent rise in the development of advanced products has been observed. Standardization through quality control of natural products is very complicated because of the presence of a wide range of phyto constituents present in the extract. Often there is the variability within the same plant material or between the different parts of the same plant. The inconsistency may be from grower to grower, crop to crop and also depends on the harvest and post harvest handling. On the other hand herbal drugs have multiple phytoconstituents including active, inactive and unknown compounds, which are nutritional rather than therapeutic [4].

Hence, methodologies that can generate a finger print of each extract in large collections would be useful to detect permanence of the same extract over time. Preferably, the fingerprint method should be based on electronic storage, retrieval and analysis of the data [5]. High-performance thin layer chromatography (HPTLC) based method could be considered as a good fingerprint analytic tool, as they are being explored as an important device for routine drug analysis. HPTLC

also facilitates repeated detection of chromatogram with same or different parameters [6-9].

Spyridia fusiformis (Boergesen) is a marine red alga belongs to the class Rhodophyceae. It has been reported to possess hepatoprotective, antioxidant, antitumor, anitdiabetic, antifungal and antibacterial properties [10-11]. The present research deals with the phytochemical screening and development of HPTLC fingerprints of the methanol extracts of *S. fusiformis* can be used for identification, authentication and characterization.

EXPERIMENTAL

Algal materials

The marine red alga *Spyridia fusiformis* Boergesen was collected from intertidal regions of Mandapam, Ramanathapuram District, the South East Coast of Tamilnadu, India.

Sample Preparation

The experimental alga was washed with water and then shade dried. The crude extract was obtained after maceration with 95% methanol-water mixture at room temperature for 72 hrs and repeated till exhaustion of the material. Thereafter, the methanol crude extract was distilled, evaporated and dried under reduced pressure to yield the methanol extract of *S. fusiformis* in powder form (yield 8%). A stock solution was prepared at a concentration of 25 mg/mL and used for the HPTLC analysis.

Quantitative Phytochemical Analysis

The extracts were tested for the presence of bioactive compounds by using standard methods [12-17].

HPTLC Profile (High Performance Thin Layer Chromatography)

HPTLC studies were carried out following the method of Harborne [18] and Wagner and Baldt[19].

Sample Preparation

Methanol extracts residue obtained was re-dissolved in 1mL of chromatographic grade methanol, which was used for sample application on pre-coated silica gel 60F254 aluminum sheets (E Merck).

Developing Solvent System

A number of solvent systems were tried, for extracts, but the satisfactory resolution was obtained in the eluent containing n-hexane: ethyl acetate (60:40 v/v).

Sample Application

Application of bands of the extract was carried out (4mm in length and 1 µL in concentration to extract) using spray technique. The sample was applied in duplicate on pre-coated silica gel 60F254 Aluminum sheets (5 x 10 cm) with the help of Linomat 5 applicator attached to CAMAG HPTLC system, which was programmed through WIN CATS software.

Development of Chromatogram

After the application of sample, the chromatogram was developed in Twin trough glass chamber 10x 10 cm saturated with solvent n-hexane: ethyl acetate (60:40 v/v) extract for 15 minutes. The air-dried plates were viewed in ultraviolet radiation in midday light. The chromatograms were scanned by densitometer at 200 nm for the methanol extract after spraying with anisaldehyde/sulphuric acid. The R_f values and finger print data were recorded by WIN CATS software.

RESULTS

Qualitative and quantitative analysis of phytochemicals

The presence of various secondary metabolites in the seaweed extract is a clear indication of their pharmaceutical potential. In the preliminary phytochemical analysis of crude extracts of *S. Fusiformis* contains phenols, alkaloids, triterpenoids, steroids, tannins, saponins, flavonoids, anthraquinones and glycosides. Interestingly, the *S. fusiformis* extract showed higher content of alkaloids, triterpenoids, steroids, saponins and glycosidessas summarized in Table.1.

HPTLC Fingerprinting of phytoconstituents

The methanol extracts of *S. fusiformis* was subjected to HPTLC analysis by specific solvent system n-hexane: ethyl acetate (60:40) and detected in UV-Vis at 350 nm. The HPTLC images shown in Figs.1 and 2 indicate that all the phyto-constituents were clearly separated without any tailing and diffuseness. It is observed from the Table.2 that the developed chromatogram of methanol extract of *S. fusiformis* scanned at 350 nm there are 7 spots with varying *Rf* values 0.32, 0.44, 0.55, 0.68, 0.81, 0.86 and 0.92 indicating the occurrence of at least 7 different phytochemicals. The developed TLC chromatogram showed in Figs.3a and 3b that 7 components appeared as pale bands at 254 nm whereas appeared as bright bands at 366 nm were found to more predominant in the percentage area is more with 52.70, 18.75, 12.10, 6.50, 5.54 and 3.32% (Fig.3a and 3b; Table.2) with respect to the Rf values. And the remaining components were found to be very less in quantity as the percentage of the spots was less than 1.08%.

Table-1: Quantitative phytochemical analysis of*S. fusiformis (mg/g dry weight)*.

S.No	Phytochemicals	*S. fusiformis*
1	Total carbon	120.55 ± 0.01^{m}
2	Nitrogen	31.04 ± 0.01^{j}
3	Total protein	148.30 ± 0.10^{n}
4	Total carbohydrates	41.05 ± 0.01^{l}
5	Total lipids	9.07 ± 0.00^{g}
6	Phenols	1.16 ± 0.01^{d}
7	Alkaloids	5.25 ± 0.15^{f}
8	Triterpenoids	1.08 ± 0.01^{c}
9	Steroids	1.32 ± 0.00^{e}
10	Tannins	0.08 ± 0.00^{b}
11	Saponins	0.02 ± 0.00^{ab}
12	Flavonoids	0.01 ± 0.10^{a}
13	Antheraquinones	0.07 ± 0.01^{b}
14	Proteins	39.70 ± 0.01^{k}
15	Carbohydrates	13.15 ± 0.11^{i}
16	Glycosides	9.57 ± 0.10^{h}
F-Value		4537000.0
P-Value		0.000

Values are expressed as Mean ± SEM, n=3 as Anova test p<0.05% level.

Table-2: Peak list andR_f value of the chromatogram ofthe methanol extract of *S. fusiformis*

Peak	Start R_f	Start height	Max R_f	Max height	Max Height%	End R_f	End height	Area	Area %	Assigned substance
1	0.29	2.3	0.32	7.8	1.94	0.33	1.6	130.1	1.08	*Unknown
2	0.40	4.2	0.44	20.6	5.13	0.48	1.7	667.3	5.54	*Unknown
3	0.53	3.4	0.55	10.6	2.65	0.61	4.5	399.8	3.32	*Unknown
4	0.63	3.7	0.68	42.0	10.47	0.73	8.5	1456.6	12.10	*Unknown
5	0.73	8.3	0.81	232.5	57.94	0.84	25.6	6343.3	52.70	*Unknown
6	0.84	25.6	0.86	34.4	8.57	0.87	30.6	782.7	6.50	*Unknown
7	0.86	30.8	0.92	53.4	13.30	1.01	0.0	2257.4	18.75	*Unknown

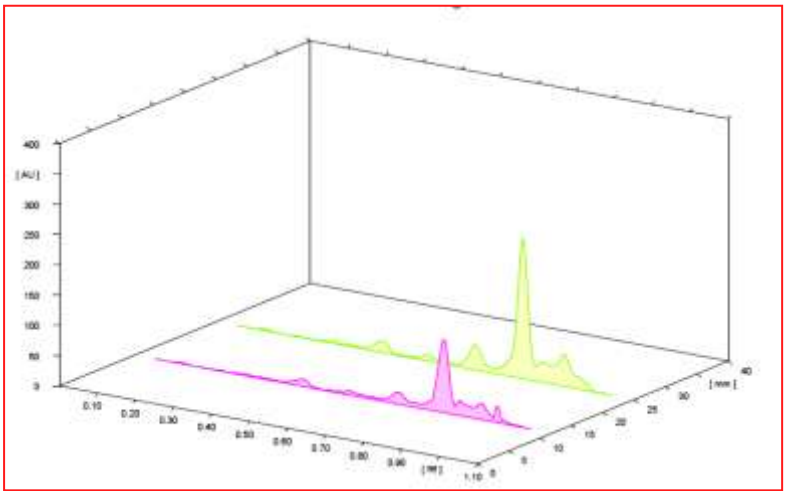


Fig-1: 3Dimensional fingerprint of methanol extract of *S. fusiformis* showing different peaks of phytoconstituents

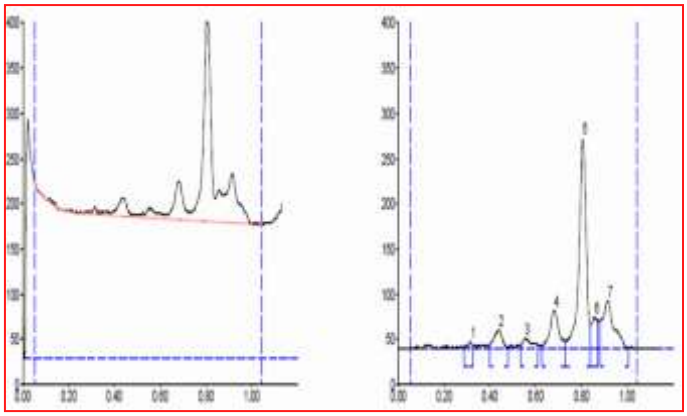
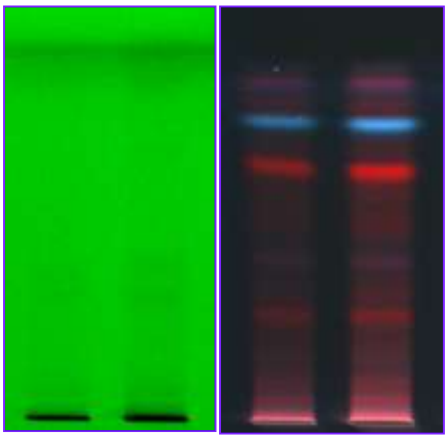


Fig-2: HPTLC Fingerprint of methanol extract of *S. fusiformis* scanned at 350 nm



(3a) **(3b)**

Fig-3: (a) HPTLC image image after derivatization observed at 254 nm. (b) HPTLC fluorescence after derivatization observed at 366 nm.

DISCUSSION

Seaweeds are rich in secondary metabolites, which include alkaloids, phenols, flavonoids, saponins, steroids and related active metabolites are of great medicinal value and have been extensively used in the drug and pharmaceutical industry. It has been reported that the presence of phyto-constituents such as flavonoids, tannin and polyphenols helps in preventing a number of diseases through free radical scavenging activity [20]. Antitumour and antioxidant properties have been attributed to the flavonoids as reported *in vitro* and *in vivo* studies [21]. Phytochemical screening of the experimental algae revealed the presence of alkaloids, saponins [22], and glycosides in methanolic extract. Alkaloids are commonly found to have antimicrobial properties [23] against both Gram-positive and Gram-negative bacteria [24]. Alkaloids are secondary metabolites known to be produced by plants and are of considerable pharmaceutical importance since they are used as drugs for the treatment of several diseases [25]. In the present study, alkaloid presence was confirmed in methanol extracts by the quantitative analysis.

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 [26]. Independent of their function, the presence of steroids in practically every organism suggests that they have a powerful role in chemosystematics [27].

Tannins are defined as naturally occurring polyphenolic compounds and are widespread among terrestrial and marine plants, and are subdivided into condensed and hydrolysable tannins [28].In contrast to terrestrial tannins, phlorotannins are tannin compounds, which have been found only in marine algae [29] and reported to possess strong antioxidant activity, which may be associated with their unique molecular skeleton [30]. The present study results confirm the presence of tannin in and *S. fusiformis.*

Saponin possesses specific physical, chemical and biological activities that make them useful as drugs. Saponin is used as mild detergents and it is utilized as hypercholesterolemia, hyperglycemia, antioxidant, anti-inflammatory and anti-fungal agent. Saponin inhibits Na^{+} and Ca^{2+}antipoter producing elevated cytosolic Ca^{2+}, which strengthens the contractions of heart muscle and thereby reduce congestive heart failure and

possess anti-carcinogenic, immunomodulatory and cholesterol lowering activity [31].

Flavonoids are important in plant defense mechanisms against invading bacteria and other types of environmental stress, such as wounding and excessive light or ultraviolet (UV) radiation [32-33]. Several researchers have made attempts to identify the plants producing bioactive substances [34-36]. In the present study also, flavonoids were observed in the methanol extracts of *S. fusiformis.* To supplement the previous observations, the present study confirms the presence of phyto-constituents in *S. fusiformis.*

The secondary metabolites, phenolics are found to play a greater role in maintaining the human body [37]. These biochemical characteristics make the seaweeds nutraceutical in nature and are important as food supplements. Hence, a more detailed pharmacognostic study of these seaweeds is necessary.

Phenolic compounds are commonly found in plants, including seaweeds, and have been reported to have a wide range of biological activities including antioxidant properties [38]. Reports have revealed that a phenolic component is one of the most effective antioxidants in brown algae [39]. In the present study, the presence of phenol was confirmed by the quantitative analysis in the crude extracts of *S.fusiformis.*

It was also suggested that *S. fusiformis* were rich sources of phytochemicals, which can be isolated and further screened for different kinds of biological activities depending on their reported therapeutic uses. Further work will emphasize the isolation and characterization of active principles responsible for bio-efficacy and bioactivity.

The HPTLC fingerprinting profile is very important parameter of herbal drug standardization for the proper identification of medicinal plants. These methods were also employed to analyze commercial samples to illustrate their application in qualitative (fingerprint) and quantitative determination, demonstrating their feasibility in the quality control of phytoconstituents from herbal drugs and formulations. This will help for the standardization of the herbal products, and alternative herbal medicine therapy. HPTLC chromatogram of methanol extract results showed that there are many compounds in *S. fusiformis* From the HPTLC studies, it has been found that the methanol extract contains a mixture of compounds and so it is established that the pharmacological activity shown by them are due to the cumulative effect of all the compounds in composite.

CONCLUSION

The present study thorough preliminary phytochemical screening and the comparative HPTLC fingerprint profiles of methanol extract of *Spyridia fusiformis.* HPTLC chromatogram of methanol extract of showed significant difference and thus the present study emphasizes and supports the use of methanol extract of *S. fusiformis* as per ethanobotanical reference for its consumption as a potent nutraceutic.

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