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Phytochemical Screening and HPTLC study of *Padina tetrastromatica* (Hauck)

Research Article

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Abstract

The marine ecology is diverse with innumerable types of natural substances, both of plant and animal origin. *Padina tetrastromatica* (PT) (Hauck) is a brown algae belonging to the order Dictyotales, found in coastal region. The objective of present investigation was to evaluate phytochemical profile of extracts of PT. The air dried plant material was defatted and extracted successively with solvents of increasing polarity. Incumbent study was performed with standard qualitative phytochemical tests and HPTLC fingerprint analysis using CAMAG HPTLC system. The results showed the presence of phytoconstituents like sterols, terpenoids, flavonoids, glycosides, alkaloids and carbohydrates. Furthermore, components present in extracts were resolved in best possible solvent system by HPTLC. The chloroform extract of PT displayed eight peaks, in which those with R_f values 0.28 and 0.72 were more predominant. Whereas ethanol extract of PT exhibited nine peaks, in which maximum R_f value was found to be 0.82. In conclusion, the data of this study provide useful guide and suitability for investigation of biological activity of the plant according to the phytochemical groups observed. However, further work is needed to standardize the above chemical constituents in comparison with biomarker and this result can also be measured along with the other data for setting up the standards to this plant.

Key Words: *Padina tetrastromatica, Brown seaweed, Marine microalgae, Phytochemical Screening, Phytoconstituents, HPTLC fingerprint.*

Introduction

Since decades, herbs or plants are used as an imperative resource of medicines due to the presence of bioactive components having therapeutic and pharmaceutical significance (1). Standardization of these plant materials is essential now days, which can be achieved by use of current methods available for describing the identification and quantification of active constituents (2). Several sophisticated analytical and extraction methods like spectrophotometric, chromatographic, electrophoresis are presently used for standardization of herbal extracts and plant based drugs. High performance thin layer chromatography (HPTLC) finger print analysis can serve as potent tool for identification, authentication and quality control of herbal medicine. This technique could be considered as a good alternative, because of its simplicity and reliability (3).

Marine macroalgae or seaweeds are found in coastal region and considered as a good source of bioactive elements as they are able to produce variety of secondary metabolites characterized by their biological activities (4). *Padina tetrastromatica* (Hauck) (PT) is a brown algae belonging to the order Dictyotales of Phaeophyta. The algae is brown to yellowish brown in colour, thallus is fan shaped and divided into several small lobes, foliaceous 5- 55cm long and1-3cm wide, irregularly branched into dichotomously fan shaped segments and apical involute (5). PT is reported for the presence of alginic acid, carbohydrates, sulphated polysaccharide (Fucoidan), fatty acids, sterols and terpenoids (6, 7). Various extracts and isolated elements of PT has been studied for its therapeutic potential. It showed spasmogenic, hypotensive, cytotoxic, antimicrobial, antifungal, antifertility, antioxidant, anti-inflammatory, antihyperglycemic and hypolipidemic activity (8-14). Further, the anxiolytic and anticonvulsant activity of PT has been studied earlier (15, 16).

The current research work was designed to carry out preliminary qualitative phytochemical screening and HPTLC fingerprinting of chloroform and ethanol extract of *Padina tetrastromatica*

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Material and Methods

Collection, authentication and extraction

The brown seaweed, PT was collected from Bhatkarwada; Ratnagiri coast and taxonomic authentication was done by Professor B. B. Chaughule,

Former Head, Botany Department, University of Pune. The collected sample was washed with sea water followed by fresh water to remove salts, suspended materials, microorganisms and dried at room temperature. Dried material were milled into a coarse powder and defatted with petroleum ether. Defatted material was then extracted successively with chloroform and ethanol using Soxhlet apparatus. The extracts were evaporated under reduced pressure using rotary vacuum evaporator until all the solvent had been removed to yield chloroform extract (CPT) and ethanol extract (EPT) respectively. Phytochemical evaluation and HPTLC profile of CPT and EPT was undertaken.

Preliminary Phytochemical study of extracts

Qualitative phytochemical investigation reveals the spectrum of chemical constituents in the chosen brown seaweed. The CPT and EPT were subjected to phytochemical evaluation for the identification of several important secondary metabolites (17, 18). Chemicals and solvents used were of analytical grade.

High Performance Thin Layer Chromatography (HPTLC) study of extracts

Chromatography is a technique used for the separation, identification and quantification of various components of extract. Many chromatographic methods are presently available, out of these HPTLC is commonly used for the analysis of herbal raw materials and formulations. Following parameters and instruments were used for fingerprint analysis of extract using HPTLC:

- Make of Instrument : CAMAG TLC scanner (Switzerland)
- Sample Applicator : Nanomat/Capillary dispenser system
- Development Chamber : Twin-trough chamber 20 X 10cm
- Stationary phase : Pre coated silica gel 60 F254 Aluminium plates (Merck, Germany)
- Plate size : 5 x 10 cm
- Syringe size : 100 µl syringe
- Application rate : 10 µl /s
- Volume applied : 2 – 5 µl
- Detection : CAMAG TLC Scanner "Scanner_211424"
- Detection wavelength : At daylight, 254 nm and 356 nm

Methodology:

Briefly, one gram of each extract added in 25 ml of ethanol. The solution was filtered and 2 to 5 microlitre of sample was applied on precoated silica gel aluminium plates. The plate was developed in Toluene: Methanol (98:2) for CPT and n-Hexane: Ethyl acetate: Methanol (78:12:10) for EPT respectively. The plate was air dried and observed under visible as well as UV light. The developed plates were scanned at different wavelength (254 nm and 356 nm) using Deuterium lamp in Camag HPTLC instrument provided with WINCATS software. Rf value of each spot was calculated (19, 20).

Results and Discussion

Extraction

The air dried plant material (400 g) was first defatted with petroleum ether and then extracted successively with solvents of increasing polarity viz. chloroform and ethanol to obtain the nonpolar, semipolar and polar constituents of the plants. The yield of the extract was found to be 12.45 % w/w and 10.21 % w/w for CPT and EPT respectively.

Preliminary phytochemical study of extracts

The preliminary phytochemical study of extracts of PT was performed to reveal the presence of different secondary metabolites. This study showed presence of sterols, terpenoids, flavonoids, alkaloids and carbohydrates in chloroform extract whereas ethanol extract of PT contain steroids, alkaloids, glycosides and carbohydrates (table1). The therapeutic values of medicinal herbs or plants are due to the presence of various active substances or secondary metabolites such as flavonoids, alkaloids, phenols, glycosides, saponin, steroids, etc (21).

Table 1: Phytochemical screening of *Padina tetrastromatica* extracts (+ Positive; - negative)

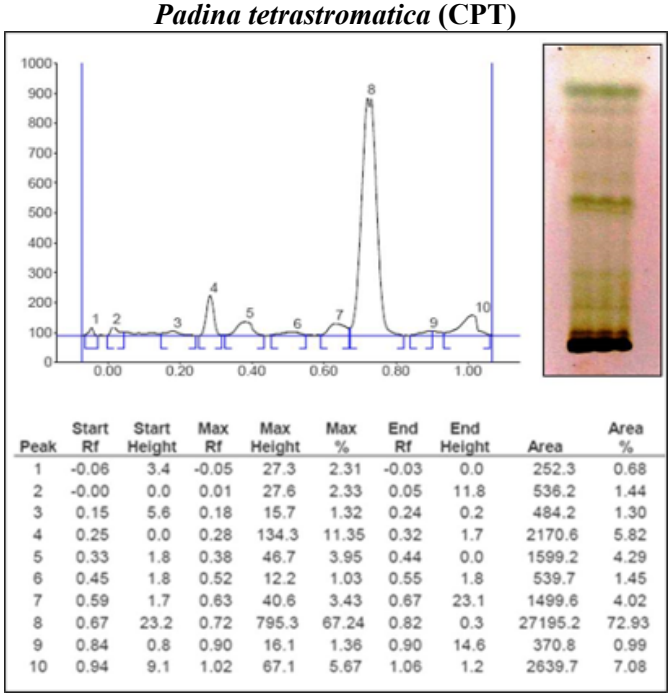
Chemical tests	*Padina tetrastromatica macroalgae extracts*	
	CPT	**EPT**
Tannins	-	-
Steroids	+	+
Terpenoids	+	-
Flavonoids	+	-
Alkaloids	+	+
Glycosides	-	+
Carbohydrates	+	+
Saponin	-	-

HPTLC study of extracts

The HPTLC profile for the CPT extract displayed eight peaks (not considering the peaks of sample application and solvent front) with R_f values ranges from 0.01 to 0.90 signifying the presence of at least eight different phytoconstituents in the extract. Amongst them, those constituents with R_f values 0.28 and 0.72 were more predominant with area percent 5.82 % and 72.93 %, respectively. The other components are very less in quantity as their area percent ranges from 0.68 % to 5.82 %. Whereas in EPT extract exhibited nine peaks indicating the presence of different compounds and corresponding rising order Rf values range from 0.01 to 0.82 in which maximum amount of the constituents was found to be 72.45 % and its corresponding R_f value was 0.82 respectively. The total number of constituents (no. of peaks) in the extract, their R_f value and chromatographic profile are shown in figure 1 and 2. The spots on the HPTLC plate after scanning were visualized by spraying 5 % methanolic sulphuric acid spraying reagent and the plates were heated at 100^0 C for 5 min in an oven. HPTLC is a valuable quality measurement tool for the assessment of plant materials. It allows for the investigation of a large number of compounds both cost effectively and

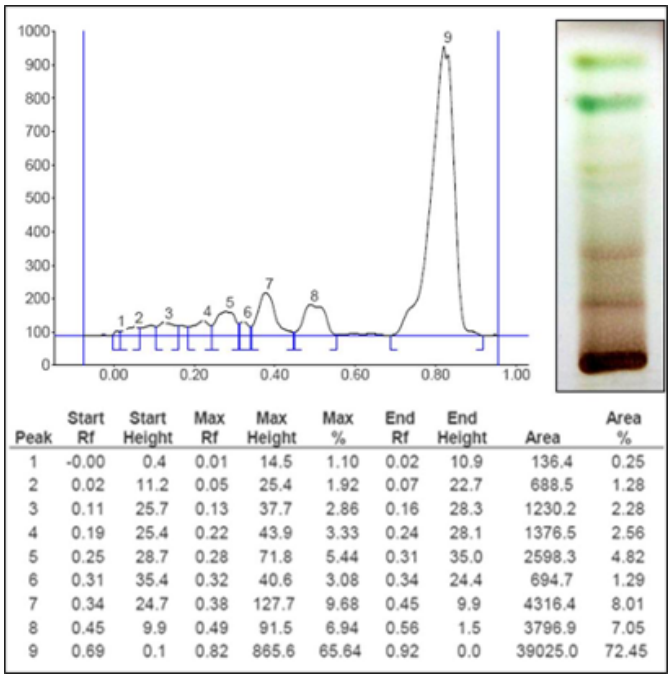
efficiently. From the HPTLC results it is evident that both the extracts contain several compounds of varied polarities. However, further work is needed to standardize the above chemical constituents in comparison with biomarker and this result can also be measured along with the other data for setting up the standards to this plant.

Figure 1: HPTLC studies of chloroform extract of *Padina tetrastromatica* (CPT)



Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %
1	-0.06	3.4	-0.05	27.3	2.31	-0.03	0.0	252.3	0.68
2	-0.00	0.0	0.01	27.6	2.33	0.05	11.8	536.2	1.44
3	0.15	5.6	0.18	15.7	1.32	0.24	0.2	484.2	1.30
4	0.25	0.0	0.28	134.3	11.35	0.32	1.7	2170.6	5.82
5	0.33	1.8	0.38	46.7	3.95	0.44	0.0	1599.2	4.29
6	0.45	1.8	0.52	12.2	1.03	0.55	1.8	539.7	1.45
7	0.59	1.7	0.63	40.6	3.43	0.67	23.1	1499.6	4.02
8	0.67	23.2	0.72	795.3	67.24	0.82	0.3	27195.2	72.93
9	0.84	0.8	0.90	16.1	1.36	0.90	14.6	370.8	0.99
10	0.94	9.1	1.02	67.1	5.67	1.06	1.2	2639.7	7.08

Figure 2: HPTLC studies of ethanol extract of *Padina tetrastromatica* (EPT)



Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %
1	-0.00	0.4	0.01	14.5	1.10	0.02	10.9	136.4	0.25
2	0.02	11.2	0.05	25.4	1.92	0.07	22.7	688.5	1.28
3	0.11	25.7	0.13	37.7	2.86	0.16	28.3	1230.2	2.28
4	0.19	25.4	0.22	43.9	3.33	0.24	28.1	1376.5	2.56
5	0.25	28.7	0.28	71.8	5.44	0.31	35.0	2598.3	4.82
6	0.31	35.4	0.32	40.6	3.08	0.34	24.4	694.7	1.29
7	0.34	24.7	0.38	127.7	9.68	0.45	9.9	4316.4	8.01
8	0.45	9.9	0.49	91.5	6.94	0.56	1.5	3796.9	7.05
9	0.69	0.1	0.82	865.6	65.64	0.92	0.0	39025.0	72.45

Conclusion

In conclusion, phytochemical screening of extracts of *Padina tetrastromatica* indicated the presence of many chemical constituents which are attributable for the varied pharmacological and traditional properties of the plant. These results provide useful guide for further investigation of biological activity of the plants according to the presence of phytochemical group. Developments of an HPTLC chromatogram are quite significant for standardization of *Padina tetrastromatica* plant and ascertaining the accurate botanical identification of the drug in a solitary form and polyherbal formulation. In this study, we present the HPTLC fingerprints of the chloroform and ethanolic extract of *Padina tetrastromatica* for the first time**.** Despite its treatment of a variety of diseases there are limited studies on the exact chemical structures of the constituents of *Padina tetrastromatica*. Therefore phytochemical screening along with these HPTLC profiles is thus needed in setting up the standard of this plant.

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