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Phytochemical Screening of *Padina Tetrastromatica* Hauck

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ABSTRACT

The present report deals with the study of phytochemicals present in *Padina tetrastromatica* Hauck collected from the south east coast of Tamil Nadu, India. The phytochemicals of different extracts was analyzed by the standard procedure for UV-Vis spectroscopic and HPLC. The UV-Vis phytochemical profile of different extracts of *Padina tetrastromatica* Hauck was predicted. The qualitative HPLC fingerprint profile of methanol extract of *Padina tetrastromatica* Hauck was chosen at a wavelength of 660 nm due to sharpness of the peaks and suitable baseline. The profile showed one prominent peak at a retention time of 1.500 min and seven moderate peaks were also noted at the retention times of 2.220, 2.400, 2.500, 2.800, 3.000, 3.300 and 6.300 min respectively. The present study on *Padina tetrastromatica* Hauck fashioned a novel phytochemical marker in standardization of the quality of the drug used for medicine.

Keywords: Phytochemistry, Seaweeds, *Padina tetrastromatica*, UV-Vis, HPLC.

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INTRODUCTION

Marine organisms are the rich source of structurally novel and biologically active metabolites. Secondary or primary metabolites produced by marine organisms may be potential bioactive compounds of interest in field of pharmaceutical research. From the literature, it is observed that the edible seaweeds contain a significant amount of the carbohydrates, proteins, vitamins and minerals essential for the human nutrition [1]. Many natural unique chemical compounds of marine origin with various biological actions have been predicted and some of the substances are under investigation and are being used to develop new pharmaceuticals [2].

Marine macro algae or seaweeds are an important resource in marine environment and also it is useful to human in health care. Seaweeds also supply oxygen to the biosphere, are a source of food for fishes, cattle, used as medicine, industrial productions and fertilizers for human. Commercially available varieties of seaweeds can be classified as red algae (Rhodophyceae), brown algae (Phaeophyceae) or green algae (Chlorophyceae) depending on the mode of nutrient and chemical composition. Fresh and dry seaweeds are extensively consumed by people especially living in the coastal areas [3].

Many metabolites isolated from seaweeds have been shown to possess bioactive efforts [4,5]. Marine algae serve as important resources for bioactive natural products which include brominated phenols, oxygen heterocyclics, nitrogen heterocyclics, sulphur nitrogen heterocyclics, sterols, terpenoids, polysaccharides and peptides [6,7]. There are several studies have been undertaken to reveal the medicinal value of seaweeds in different part of the world. These compounds are providing valuable ideas for the development of new drugs against various diseases such as cancer, microbial infections and inflammations [8].

They have some of the valuable medicinal value components such as antibiotics, antioxidant [9,10], anticoagulants, anti-ulcer products and suspending agents in radiological preparations. Most of the compounds of marine algae show anti-bacterial activities [11,12]. Oxidative stress is an important factor in the genesis of pathology, from cancer to cardiovascular and degenerative disease [13,14]. The south east coast of Tamil Nadu are abound in various ecologically and economically important seaweeds but literature pertaining to the biochemical composition of seaweeds from the south east coast of Tamil Nadu is meager. With a view to fulfill this gap, an attempt has been made in the present study to screen the phytochemicals present in *Padina tetrastromatica* Hauck collected from the south east coast of Tamil Nadu, India.

Figure 1 Natural Habit of *Padina tetrastromatica* Hauck

MATERIALS AND METHODS

Collection of samples

The samples of *Padina tetrastromatica* Hauck (Figure 1) were collected from Hare Island located in Thoothukudi, Tamil Nadu, India. The collections were made during the low tidal and subtidal regions (up to 1m depth) by hand picking. 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. For drying, washed specimens were placed on blotting paper and spread out at room temperature in the shade. The shade dried samples were grounded to fine powder using a tissue blender. The powdered samples were then stored in the refrigerator for further use.

Preparation of extracts

10 g of air dried powder of *Padina tetrastromatica* Hauck was extracted with 60 mL of solvents viz., methanol, chloroform and petroleum ether. The sample was kept in dark for 72 h with intermittent shaking. After incubation, the solution was filtered through filter paper and the filtrate was collected (crude extracts).

UV-Vis spectroscopic analysis

The different extracts of *Padina tetrastromatica* Hauck were centrifuged at 3000 rpm for 10 min and then filtered through Whatmann No.1 filter paper using high pressure vacuum pump. The sample is diluted to 1:10 with the same solvents. The crude extracts containing the bioactive compound was analyzed spectroscopically for further confirmation. They were scanned in the wavelength ranging from 200-900 nm using Shimazdu spectrophotometer and characteristic

peaks were detected [15].

HPLC analysis

HPLC method was performed on a Shimadzu LC-10AT VP HPLC system, equipped with a model LC-10AT pump, UV-Vis detector SPD- 10AT, Rheodyne injector fitted with a 20 µL loop and auto injector SIL-10AT. A Hypersil BDS C-18 column (4.6 × 250 mm, 5 µm size) with a C-18 guard column was used. The elution was carried out with gradient solvent systems with a flow rate of 1 mL min-1 at ambient temperature (25-28°C). The mobile phase was consisted of 0.1% v/v methanol (Solvent A) and water (Solvent B). The mobile phase was prepared daily, filtered through a 0.45 µm and sonicated before use. Total running time was 15 min. The sample injection volume was 20 µL while the wavelength of the UV-Vis detector was set at 254 nm [16,17].

RESULTS AND DISCUSSION

UV-VIS Spectroscopic analysis

The qualitative UV-VIS fingerprint profile of the methanol extract of *Padina tetrastromatica* Hauck was selected at the wavelength of 200nm to 800nm due to the sharpness of the peaks and proper baseline. The profile showed the compounds separated at 298nm, 418nm, 617nm and 663nm with the absorption 1.734, 1.417, 0.172 and 0.630 respectively (Table-1 and Figure-2A).

The UV-VIS fingerprint profile of the chloroform extract of *Padina tetrastromatica* Hauck was selected at a wavelength of 200nm to 800nm due to sharpness of the peaks and proper baseline. The profile showed the compounds separated at the nm of 295, 416, 536, 572, 614 and 667 with the absorption 1.868, 1.275, 0.916, 0.161, 0.129 and 0.065 respectively (Table-1 and Figure-2B).

Table 1 UV-VIS peak values of different extracts of *Padina tetrastromatica* Hauck

Solvents	Methanol		Chloroform		Petroleum ether	
Peak No.	nm	Abs	nm	Abs	nm	Abs
1	200	4.000	200	0.499	200	4.000
2	250	4.000	250	2.224	250	4.000
3	300	1.692	300	1.127	300	0.924
4	350	0.784	350	0.569	350	0.149
5	400	1.203	400	1.474	400	0.269
6	450	0.795	450	0.577	450	0.053
7	500	0.201	500	0.373	500	0.029
8	550	0.064	550	0.051	550	0.001
9	600	0.137	600	0.096	600	0.006
10	650	0.366	650	0.234	650	0.021
11	700	0.033	700	0.035	700	0.004
12	750	0.001	750	0.006	750	0.003
13	800	0.004	800	0.002	800	0.001

The UV-VIS fingerprint profile of the petroleum ether extract of *Padina tetrastromatica* Hauck

was selected at a wavelength of 200nm to 800nm due to sharpness of the peaks and proper baseline. The profile showed the compounds separated at 292nm, 408nm, 477nm, 533nm, 561nm, 610nm, 668nm and 746nm with the absorption of 1.081, 0.289, 0.122, 0.053, 0.014 and 0.001 respectively (Table-1 and Figure-2C).

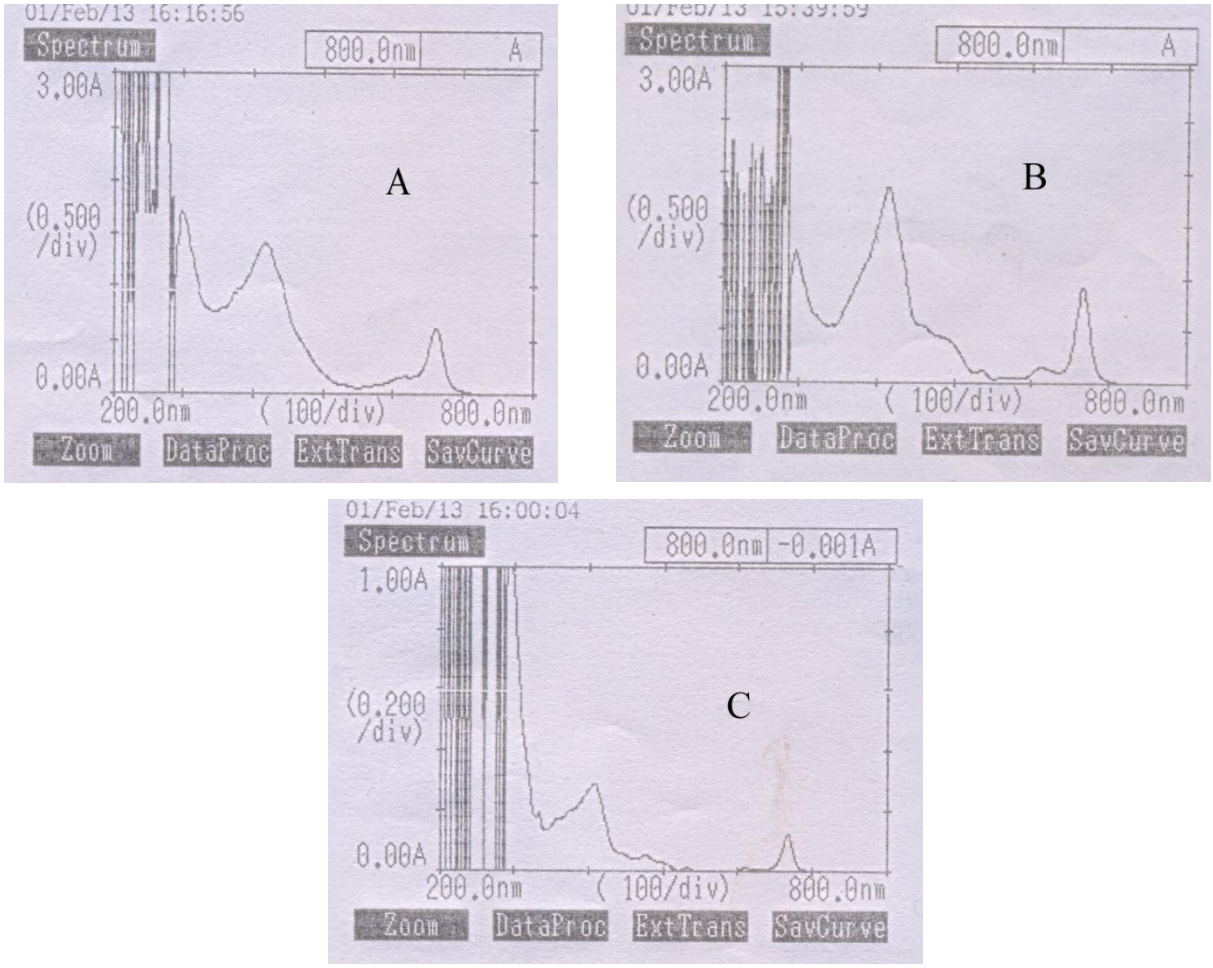

Figure 2 UV-VIS Peak Values of Different Extracts of *Padina tetrastromatica* Hauck

A. Methanol cold extract; B. Chloroform cold extract; C. Petroleum Ether cold extract

HPLC Analysis

The qualitative HPLC fingerprint profile of the methanol extract of *Padina tetrastromatica* Hauck was selected at a wavelength of 660nm due to the sharpness of the peaks and proper baseline. The methanol extract prepared by cold extraction was subjected to HPLC for the separation and identification of constituents present in the *Padina tetrastromatica* Hauck. Eight compounds were separated at different retention times of 1.500min, 2.200min, 2.400min, 2.500min, 2.800min, 3.000min, 3.300min and 6.300 min respectively. The profile displayed one

prominent peak at the retention time of 1.500, 2.113min and 2.573min and seven moderate peaks were also observed at the retention times of 2.200min, 2.400min, 2.500min, 2.800min, 3.000min, 3.300min and 6.300min (Figure-3).

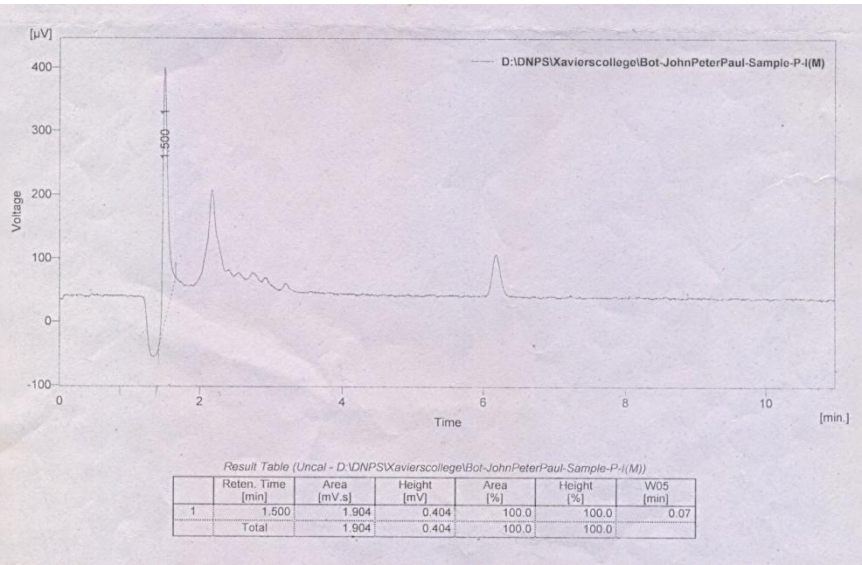

Figure 3 HPLC Profile of *Padina tetrastromatica* Hauck – Methanolic cold extract

In recent years, using traditional medicinal knowledge in drug discovery seems so promising that even large pharmaceutical companies have begun to show interest in this field [18,19]. The medicinal effects of plant materials typically result from the secondary metabolites present in the plant, although, it is usually not attributed to a single compound but a combination of the metabolites. The medicinal properties of plants are distinctive to a particular plant species or group reliable with the concept that the combination of secondary metabolites in a particular plant is taxonomically distinct [20]. Seaweeds have the immense potential for the synthesis of secondary novel bioactive compounds which are not found in terrestrial environment [21,22]. Seaweeds are considered as the richest source of bioactive compounds as they are able to produce a great variety of secondary metabolites characterized by a broad spectrum of biological activities.

Natural remedies from seaweeds are found to be safe and effective. Many seaweed species have been used in medicine to treat various ailments. Even today compounds from seaweeds continue to play a major role in primary health care as therapeutic remedies in many coastal developing countries [23]. Standardization of plant materials is the need of the day. Several pharmacopoeia containing monographs of the plant materials describe only the physicochemical parameters. Hence the modern methods describing the identification and quantification of active constituents in the plant material may be useful for proper standardization of herbals and its formulations.

Also the WHO has emphasized the need to ensure the quality of medicinal plant products using modern controlled technique and applying suitable standards [24]. High performance Layer Chromatography (HPLC) is a valuable tool for reliable identification. It can provide chromatographic fingerprints that can be visualized and stored as electronic images which can be used several times without any errors and change [25].

The chemical analysis of extracts of *Padina tetrastromatica* Hauck showed the presence of various phytoconstituents. The results of the present study also supplement the usage of the studied plant which possesses several known and unknown bioactive compounds with bio-activity. The isolation and identification of these bioactive compounds can be used to formulate new drugs to treat various diseases and disorders. In recent times during this molecule era in addition to morphological characters in plant taxonomy biochemical markers are also being used to classify the plants. HPLC finger printing profile is useful as phytochemical marker and also a good estimation of genetic variability in plant populations.

The qualitative LC method developed and employed to evaluate the *M. ilicifolia* samples, presented here, is simple and allows classifying the similarities and the differences of *M. ilicifolia.* Also, a quantitative and selective method was developed and used to identify and quantify epicatechin in raw materials of *M. ilicifolia*. Thus, these methods can be used for the quality control of raw material and herbal medicines made with this plant by pharmaceutical laboratories.

REFERENCES

1. Bhakuni DS, Rawat DS. Bioactive Marine Natural Products. New Delhi: Anamaya Publishers; 2005.
2. Athukorala Y, Ki-Wan Lee, Se-Kwon K, You-Jin J. Anticoagulant activity of marine green and brown algae collected from Jeju Island in Korea. Bioresour. Technol 2006; 98(9): 1711-1716.
3. Fayaz K, Namitha KN, Chidambara MM, Mahadeva SR, Sarada SK, Ravishankar S. Chemical composition Iron bioavailability and antioxidant activity of *Kappaphycus alvarezi* (Doty). Agric. Food Chem 2005; 53: 792-797.
4. Venkateswarlu G, Panchagnula K, Aditya L, Gottumukkala VS. Synthesis, structural revision and biological activities of 4-chloroaurone, a metabolite of marine brown alga *Spatoglossum variabile*. Tetrahedron, 2007; 63: 6909-6914.
5. Yang L, Peng, LS, Zhou S. Lactones from a brown alga endophytic fungus (No. ZZF36)

from the South China Sea and their antimicrobial activities. Bioorganic and Med. Chem. Lett 2006; 16: 4205-4208.

6. Iliopoulere D, Agias C, Harvala C, Roussis V. C15 acetogenins from the red alga *Laurencia obtusa*. Phytochemistry 2002; 59: 111-116.
7. Metzger P, Roger MN, Largean C. Botryolins A and B, two tetramethyl sequalene triethers from the green microalga *Botryoccus braunic*. Phytochemistry 2002; 59: 839-843.
8. Oh, Kim, Lee. Antimicrobial activities of the bromophenols from the red alga *Odonthalia corymbifera* and some synthetic derivatives. Bioorganic and Medicinal Chem. Lett 2008; 18: 104-108.
9. Lekameera R, Vijayabaskar P, Somasundaram ST. Potential antioxidant activity of brown alga *Loboohora variegate*. Seaweed Res. Utiln 2007; 29 (1&2): 55-61.
10. Lekameera R, Vijayabaskar P, Somasundaram ST. Evaluating antioxidant property of brown alga *Colpomenia sinuosa* (Derb. Et sol). African J. Food Sci 2008; 2: 126-130.
11. Thirumaran G, Vijayabaskar, P, Anantharaman P. Antibacterial and antifungal activities of brown Marine macro alga (*Dictyota dichotoma*) from the Gulf of mannar biosphere reserve. Environ. Ecol 2006; 24S(1): 37-40.
12. Vairappan CS, Daitoh M, Suzuki M, Abe, T, Masuda M. Antibacterial halogenated metabolites from the Malaysian *Laurencia* species. Phytochemistry 2001; 58: 291-297.
13. Parthasarathy S, Khan-Merchant N, Penunretcha, M, Santanam N. Oxidative stress in Cardio-vascular disease. J. Neuclear Cardiol 2001; 8: 379-389.
14. Croke MS, Evans MD, Dizdarognen, M, Lunec J. Oxidative DNA damage: Mechanism, Mutation and Disease. J. Federation of American Societies for Experimental Biol 2003; 17: 1195-1214.
15. John Peter Paul J. Phytochemical studies on *Turbinaria ornata* (Turner) J.Ag. American Journal of Pharm-Tech Research 2012; 2(6):582-589.
16. Sharanabasappa GK, Santosh MK, Shaila, Seetharam YN, Sanjeevarao I. Phytochemical Studies on *Bauhinia racemosa* Lam., *Bauhinia purpurea* Linn. and *Hardwickia binata* Roxb. E-Journal of Chemistry 2007; 4(1): 21-31.
17. Mallikharjuna PB, Rajanna LN, Seetharam YN, Sharanabasappa GK. Phytochemical studies of *Strychnos potatorum* L. – A Medicinal Plant. E-Journal of Chemistry 2007; 4(4): 510-518.
18. Do QT, Bernard P. Pharmacognosy and reverse pharmacognosy: a new concept for accelerating natural drug discovery. I Drugs 2004; 7(11): 1017-1027.

19. Vuorelaa P, Leinonenb M, Saikkuc P, Tammelaa P, Rauhad JP, Wennberge T, Vuorela H. Natural products in the process of finding new drug candidates. Curr Med Chem 2004; 11(11); 1375-1389.
20. Parekh J, Jadeja D, Chanda S. Efficacy of aqueous and methanol extracts of some medicinal plants for potential antibacterial activity. Turk J Biol 2005; 29: 203-210.
21. Fayaz KKM, Namitha, Chidambara MKN, Mahadeva Swamy M, Sarada R, Salma Khanam, Subbarao PV, Ravishankar GA. Chemical composition, Iron bioavailability and antioxidant activity of *Kappsphycus alvarezi* (Doty). J. Agric. Food Chem 2005; 53: 792-797.
22. Faulkner DJ. Marine natural products. Nat. Products Rep 2002; 19: 1-48.
23. Bobbarala V, Bramhachari PV, Ravichand J, Reddy YHK, Kotresha D, Chaitanya KV. Evaluation of hydroxyl radical scavenging activity and HPTLC fingerprint profiling of *Aegle marmelos* (L.) Correa extracts. J Pharm Res 2011; 4(1): 252-255.
24. Sharma P, Kaushik S, Jain A, Sikarwar SM. Preliminary phytochemical screening and HPTLC fingerprinting of *Nicotiana tabacum* leaf. J Pharm Res 2010; 3(5): 1144-1145.
25. Subramanian Sampathkumar1, Ramakrishnan N. Chromatographic finger print analysis of *Naringi crenulata* by HPTLC technique. Asian Pacific Journal of Tropical Biomedicine 2011; S195-198.