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Analysis of bioactive compounds in *Padina pavonica* using HPLC, UV-VIS and FTIR techniques

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Abstract
Identification of the chemical nature of bioactive compounds present in the seaweed, *Padina pavonica* has been evaluated using HPLC, UV VIS and FTIR. HPLC profiles and major functional groups were reported to contained five phenolic compounds namely Kaempferol, Ellagic acid, Delphinidin -3-O-glucoside, Naringenin and Ferulic Acid. The UV- VIS profile showed (ranging 226.7 nm to 664.5 nm) reveals the presence of phenolic and alkaloids derivatives. FTIR analysis confirmed the presence of phenol, alkanes, alcohol and aromatic compounds. The results of this study offer a platform of using *Padina pavonica* as an alternative for various diseases.

Keywords: HPLC, UV-VIS, FTIR, *Padina pavonica*

Introduction
Seaweeds grow in the intertidal as well as in the subtidal area up to a certain depth where 0.1% photosynthetic light is available; they are one of the ecologically and economically important living resources of the world ocean. They are able to biosynthesize secondary metabolites that can mediate a broad range of intra and inter specific ecological interactions between marine organisms, including chemical defenses against herbivores (Kavitha and Palani, 2016; Hay and Steinberg, 1992) [10, 8]. Marine algae are rich sources of structurally new biologically active metabolites (Paul, 1992) [14]. In recent years, there have been many reports of macroalgae derived compounds that have a broad range of biological activities, such as antibiotic, antiviral, antioxidant, antifouling, anti-inflammatory, cytotoxic and antimitotic activities (Ely, 2004) [6].
The active compounds produced by marine organisms are used in traditional and complementary medicine. Active compounds which can cure diseases were reported to be present in many varieties of marine algae. Use of remedies of natural origin for curing diseases are preferred by majority of the population as they are said to cause fewer side effects (Tyagi and Bohra, 2002) [17]. Many pharmacological studies on algae have reported that the chemical compounds produced by marine algae have different biological activities such as anti-inflammatory, anticancer, anti-HIV, antimutagenic and scavenging free radicals (Cornish and Garbary, 2010; Bechelli *et al*., 2011) [4, 3].
Seaweeds have been reported to contain secondary metabolites which include alkaloids, glycosides, flavonoids, saponins, tannins, steroids, and related active metabolites, and have been extensively used in the drug and pharmaceutical industry (Eluvakkal *et al*., 2010) [5]. Recently, researches have proved that compounds originating from marine algae exhibit various biological activities (Wijesekara and Kim, 2010; Wijesekara *et al*., 2010; Wijesekara *et al*., 2011) [20-22]. Therefore, there is a new trend to isolation and identification of bioactive compounds and constituents from edible seaweeds. The aim of this study is to determine the bioactive compounds present in the *Padina pavonica* extract with the aid of HPLC, UV-VIS and FTIR Techniques.

Materials and Methods
Sample collection and preparation of extract
The *Padina pavonica* were collected in August 2014 from Andaman Island, India. The collected *Padina pavonica* were dried at room temperature and coarsely powdered. The powder was extracted with methanol for 48 hours. A semi solid extract was obtained after complete elimination of alcohol under reduced pressure. The *Padina pavonica* extract was stored in refrigerator until used.

UV and FTIR Spectroscopic analysis

The extracts were examined under visible and UV light for proximate analysis. For UV and FTIR spectrophotometer analysis, the extracts were centrifuged at 3000 rpm for 10 min and filtered through Whatmann No. 1 filter paper by using high pressure vacuum pump. The sample is diluted to 1:10 with the same solvent. The extracts were scanned in the wavelength ranging from 200-600nm using Perkin Elmer Spectrophotometer and the characteristic peaks were detected. FTIR analysis was performed using Perkin Elmer Spectrophotometer system, which was used to detect the characteristic peaks in ranging from 400-4000 cm^{-1} and their functional groups. The peak values of the UV and FTIR were recorded. Each and every analysis was repeated twice for the spectrum confirmation.

HPLC analysis

Sample preparation: The sample was prepared according to the procedure. The extraction was carried out using 2 ml of fermented broth with 50 mL of 95% ethanol under 80 KHz, 45°C in ultrasonic extraction device for 30 min, repeated twice. The extract was collected and filtered; the filtrate was dried at 50°C under reduced pressure in a rotary evaporator. The dried crude extract was dissolved in the 100 ml mobile phase. After filtering through a filter paper and a 0.45 mm membrane filter (Millipore), the extract was injected into HPLC.

HPLC conditions: Flavonoids were analysed using a RP-HPLC method (Samee and vorarats 2007) [15], Shimadzu Corp., Kyoto, consisting of a LC-10ATVp pump, SCL 10A system controller and a variable Shimadzu SPD- 10ATVp UV VIS detector and a loop injector with a loop size of 20 µl. The peak area was calculated with a CLASSVP software. Reverse-phase chromatographic analysis was carried out in isocratic conditions using a C-18 reverse phase column (250×4.6 mm i.d., particle size 5 µm, Luna 5µ C-18; phenomenex, Torrance, CA, USA) at 25°C. The gradient elution of solvent A [water-acetic acid (25:1 v/v)] and solvent B (methanol) had a significant effect on the resolution of compounds. As a result, solvent gradients were formed, using dual pumping system, by varying the proportion of solvent A [water-acetic acid (25:1, v/v)] to solvent B (methanol). Solvent B was increased to 50% in 4 min and subsequently increased to 80% in 10 min at a flow rate of 1.0 mL/min. Detection wavelength was 280 nm.

Results and Discussion

Secondary metabolites consist of the phenolic compounds, alkaloids, tannins, saponins, carbohydrates, glycosides, flavonoids, steroids, etc. Most phenolic compounds such as flavonoids, glycosides, triperinoids, flavonons, carbohydrates and anthraquinones are distributed throughout the algae (Cornish and Garbary, 2010) [4]. Similarly, the polyphenolic compounds most commonly found in seaweed extracts are the phenolic acids, flavonoids and tannins (Kim *et al*., 2005) [11]. These compounds together with other phenolic structures of origin have been reported as scavengers of Reactive Oxygen Species (ROS) and are seen as promising therapeutic drugs for free radical mediated pathologies including diabetic, cardiovascular diseases (Velavan, 2011) [19]. Most flavonoidic compounds exhibit antipyretic, analgesic, anti-inflammatory, anti-arthritic, antioxidant and immuno-modulatory properties (Balasundram *et al*., 2006; Gill *et al*., 2011) [2, 7]. These activities of flavonoidic compounds may be due to the presence of gallic acid, ellagic acid, quercitin, tannin acid, vanillin, resorcinol, catechin, etc.

Previous studies on marine microalgae have investigated and contributed to the isolation and chemical determination of over 15,000 compounds, including fatty acids, sterols, phenolic compounds, terpenes, enzymes, polysaccharides, alkaloids, and flavonoids. It was also recently reported that marine algae are a source of antioxidant compounds with free radical scavenging activity (Viswanathan *et al*., 2014) [18]. Many industrial products such as agar, algin and carrageenan use seaweeds as the raw material and also they are consumed as food in Asian countries (Kavitha and Palani, 2016) [10].

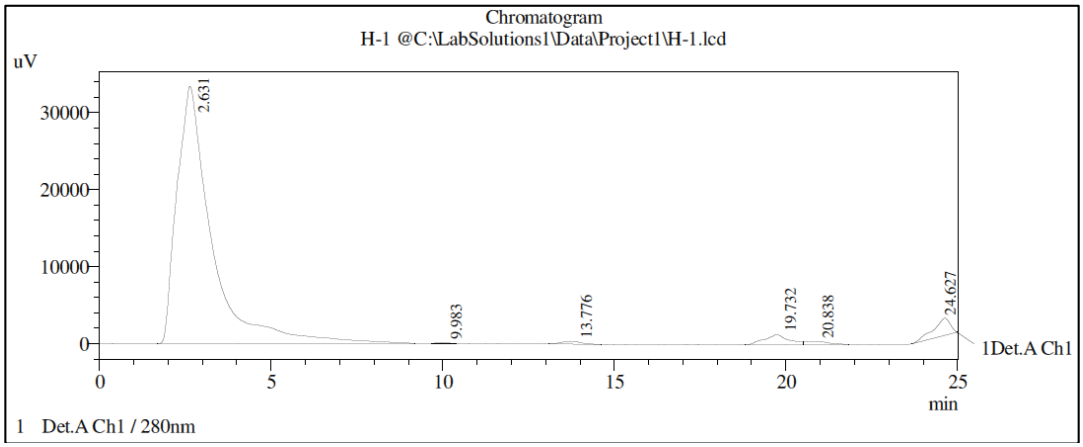

Fig 1: HPLC analysis of *Padina pavonica* extract

Table 1: HPLC analysis of *Padina pavonica* extract

Peak	Area %	Retention Time (Seaweed)	Literature (RT)	Name of the compounds
1.	93.110	2.631	2.66	Kaempferol
2.	0.052	9.983	9.59	Ellagic acid
3.	0.557	13.776	13.30	Delphinidin -3-O-glucoside
4.	2.510	19.732	20.18	Naringenin
5.	0.701	20.838		
6.	3.070	24.627	24.17	Ferulic Acid

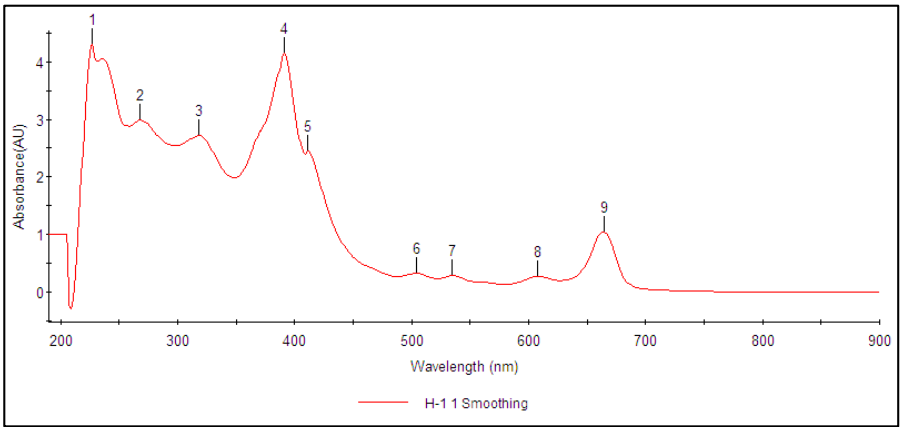

Fig 2: UV-Vis Spectral analysis of *Padina pavonica* extract

Table 2: UV-VIS peak values of extract of *Padina pavonica*

Peak	Wave length (nm)	Absorption Peak (O.D.)
1	226.7	4.304
2	267.5	2.999
3	318.3	2.722
4	391.3	4.153
5	411.3	2.467
6	504.3	0.327
7	535.5	0.287
8	607.5	0.275
9	664.5	1.047

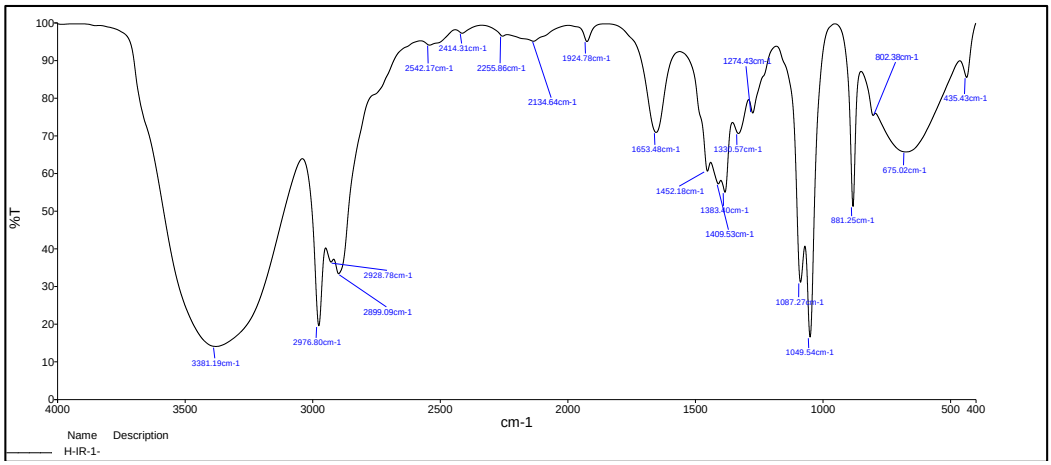

Fig 3: FTIR analysis of *Padina pavonica* extract

Table 3: FTIR peak values of extract of *Padina pavonica* extract

Peak Value	Bond	Functional group
2976.80	C-H stretch	Alkenes
2899.09	O-H stretch	Carboxylic acids
2255.86, 2134.64	-C=C- stretch	Alkynes
1452.18, 1409.53	C-C stretch (in-ring)	Aromatics
1330.57, 1274.43	C-N stretch	Aromatic amines
1049.54, 1087.27	C-O stretch	Alcohols, Phenols, Carboxylic acids, esters, ethers
881.25	C-H "loop"	Aromatics

HPLC profile of *Padina pavonica*

HPLC profiles of *Padina pavonica* were analysed and five phenolic compounds namely Kaempferol, Tannic acid, Epigallocatechin, Quercetin and Caffeic acid having different elution times could be obtained (Fig 1 and Table 1) when each compound was analyzed individually using the mobile gradient phase consisting of methanol and 1% acetic acid in water during 30 minutes run time. The profile displayed a prominent peak at the retention times of 2.631min. HPLC profiles of *Padina pavonica* reported to contained five phenolic compounds namely Kaempferol, Ellagic acid, Delphinidin -3-O-glucoside, Naringenin and Ferulic Acid. Earlier review of literature (Mradu *et al*., 2012; Alam *et al*., 2011 and Paranthaman *et al*., 2012) [12, 1, 13] supported the findings of these compounds.

Spectrophotometric analysis

The UV-VIS profile of *Padina pavonica* extract was taken at the 200 to 800nm wavelength due to the sharpness of the peaks and proper baseline. The UV-visible spectra were

performed to identify the compounds containing σ- bonds, π-bonds, and lone pair of electrons, chromophores and aromatic rings. The profile showed the peaks from 226.7nm to 664.5nm with the absorption (Fig 2 and Table 2). Occurrence of peaks at 234-676 nm reveals the presence of phenolic and alkaloids in the *Padina pavonica*. Our results agreement with earlier reports as the peak values ranges from 200 nm to 680 nm in *Padina pavonica* (John Peter Paul. and Shri Devi, 2013) [9].

Functional groups identification

The FTIR spectrum was used to identify the functional groups of the active components present in extract based on the peaks values in the region of IR radiation. When the extract was passed into the FTIR, the functional groups of the components were separated based on its peaks ratio. The results of FTIR analysis confirmed the presence of phenol, alkanes, aldehyde, alcohol, aliphatic amines, aromatic and nitro compounds (Fig 3 and Table 3). FTIR analysis of *Aerva lanata* confirmed the presence of alcohols, phenols, alkanes, carboxylic acids, alkenes, aromatics, esters and ethers compounds which shows major peaks at 2976.80, 2899.09, 2255.86, 2134.64, 1452.18, 1409.53, 1330.57, 1274.43, 1049.54, 1087.27 and 881.25 cm^{-1} respectively (Yamunadevi *et al*., 2012) [23].

Conclusion

HPLC profiles of *Padina pavonica* reported to contained five phenolic compounds namely Kaempferol, Ellagic acid, Delphinidin-3-O-glucoside, Naringenin and Ferulic Acid. The UV- VIS profile showed the peaks from 226.7 nm to 664.5 nm reveals the presence of phenolic and alkaloids derivatives. The results of FTIR analysis confirmed the presence of phenol, alkanes, alcohol and aromatic compounds. The results of this study offer a platform of using *Padina pavonica* as alternative for various diseases including diabetic, cardiovascular etc.

References

1. Alam N, Hossain M, Khalil MI, Moniruzzaman M, Sulaiman SA, Gan SH. High catechin concentrations detected in *Withania somnifera* (Ashwagandha) by high performance liquid chromatography analysis. BMC Complementary and Alternative Medicine. 2011; 11:65.
2. Balasundram N, Sundram K, Samman S. Analytical, Nutritional and Clinical Phenolic compounds in plants and agri-industrial by-products: Antioxidant activity, occurrence, and potential uses. Food Chemistry. 2006; 99(1):191-203.
3. Bechelli J, Coppage M, Rosell K, Liesveld J. Cytotoxicity of algae extracts on normal and malignant cells. Leuk Res Treat, 2011, 1-7.
4. Cornish ML, Garbary DJ. Antioxidants from macroalgae: Potential applications in human health and nutrition. Algae. 2010; 25:155-171.
5. Eluvakkal T, Sivakumar SR, Arunkumar. Fucoidan in some Indian brown seaweeds found along the coast gulf of manner. Int. J Bot. 2010; 6:176-181.
6. Ely R, Supriya T, Naik CG. Antimicrobial activity of marine organisms collected off the coast of South East India. J Exp Mar Biol Ecol. 2004; 309:121-127.
7. Gill NS, Arora R, Kumar SR. Evaluation of antioxidant, anti-inflammatory and analgesic potential of the *Luffa acutangula Roxb Var amara*. Res J Phytochem. 2011; 5:201-208.
8. Hay ME, Steinberg PD. The chemical ecology of plant herbivore interactions in marine versus terrestrial communities, In: Rosentha, GA, Berenbaum MR, (Ed.). Herbivores – their interactions with secondary plant metabolites. New York: Academic Press, 1992, 371-413.
9. John Peter Paul J, Shri Devi. Phytochemical studies on *Padina pavonica* (L.) *Thivy ex Taylor*. Indo Am J Pharm Res. 2013: 3(6).
10. Kavitha J, Palani S. Phytochemical screening GC-MS analysis and antioxidant activity of marine algae *Chlorococcum humicola.* World J Pharm and Pharmaceutical Sci. 2016: 5(6):1154-1175.
11. Kim SJ, Woo S, Yun H, Yum S, Choi E, Do JR. Total phenolic contents and biological activities of Korean seaweed extracts. Food Sci Biotechnol. 2005; 14:798-802.
12. Mradu G, Saumyakanti S, Sohini M, Arup M. HPLC profiles of standard phenolic compounds present in medicinal plants. Int J Pharmacognosy and Phytochem Res. 2012; 4(3):162-167.
13. Paranthaman R, Praveen kumar P, Kumaravel S. GC-MS analysis of phytochemicals and simultaneous determination of flavonoids in *Amaranthus caudatus* (Sirukeerai) by RP-HPLC. J Anal Bioanal Techniques. 2012; 3:5.
14. Paul VJ. Ecological roles of marine natural products. Ithaca, New York: Comstock Publishing Associates. (Cornell University Press), Ithica, USA, 1992, 119-163.
15. Samee W, Vorarat S. Simultaneous determination of gallic acid, catechin, rutin, ellagic acid and quercetin in flower extracts of *Michelia alba, Caesalpinia pulcherrima* and *Nelumbo nucifera* by HPLC. Thai Pharm Health Sci J. 2007; 2:131-137.
16. Sofowora A. Medicinal Plants and Traditional Medicine in Africa. John Wiley and Son Ltd. 1993, 150-153.
17. Tyagi N, Bohra A. Screening of phytochemicals of fruit plant and antibacterial potential against *Pseudomonas aeruginosa*. Biochem Cell Arch. 2002; 2:21-24.
18. Viswanathan S, Ebciba C, Santhiya R, Nallamuthu T. Phytochemical screening and *in vitro* antibacterial, antioxidant and anticancer activity of *Amphiroa fragilissima* (Linneaus) Lamoroux JV. Int J Innov Res Sci Eng Technol. 2014; 3(5).
19. Velavan S. Free radicals in health and diseases- A mini review Pharmacologyonline. 2011; 1:1062-1077.
20. Wijesekara I, Kim SK. Angiotensin-I-Converting Enzyme (ACE) inhibitors from marine resources. Mar Drugs, 2010; 8:1080-93.
21. Wijesekara I, Yoon NY, Kim SK. Phlorotannins from *Ecklonia cava* (Phaeophyceae): Biological activities and potential health benefits. Biofactors. 2010; 36:408-14.
22. Wijesekara I, Pangestuti R, Kim SK. Biological activities and potential health benefits of sulfated polysaccharides derived from marine algae. Carb Polymer. 2011; 84:14-21.
23. Yamunadevi M, Gnanaraj WE, Marimuthu J. FTIR spectroscopic studies on *Aerva lanata* (l.) Juss. ex Schult. Asian J Pharm Clin Res. 2012; 5(2):82-86.