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UV-VIS AND HPLC STUDIES ON *PADINA GYMNOSPORA* (KUTZING) SONDER AND *PADINA TETRASTROMATICA* HAUCK

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ABSTRACT

The present study was aimed to investigate the phytochemical constituents and physico-chemical parameters present in the seaweeds *Padina gymnospora* (Kutzing) Sonder and *Padina tetrastromatica* Hauck. Preliminary phytochemical screening was carried out by the method described by Harborne. The extracts of *P. gymnospora* and *P. tetrastromatica* were scanned in the wavelength ranging from 200-1100 nm by using Shimadzu Spectrophotometer. HPLC method was performed on a Shimadzu LC-10 AT VP HPLC system, which was 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. The qualitative phytochemical screening revealed the presence of alkaloids, steroids, phenolic groups, saponins, tannins, flavonoids, glycosides, proteins and sugars. The results of ash analysis discovered the existence of sulphur, calcium, magnesium, iron, phosphorous and chlorine in all the extracts of *P. gymnospora* and *P. tetrastromatica*. The fluorescence analysis results showed different colours both under ordinary and UV light. The UV-VIS fingerprint profile of various extracts of *P. gymnospora* and *P. tetrastromatica* produced different peaks which confirmed the presence of variety of metabolites. The HPLC profile showed the compound separated at a retention time of 2.657 was identical in both the species *P. gymnospora* and *P. tetrastomatica.* The generated data may be useful in suggesting chemotaxonomical interrelationship of two species. Further advanced studies are required for structural elucidation and identification of compounds detected in *P. gymnospora* and *P. tetrastomatica*.

INTRODUCTION

Seaweeds are marine plants constituting important renewable marine resources. They are divided into three categories based on their colours such as red (4,500 species), green (900 species) and brown (1,000 species). In India seaweeds are found more abundantly along the southeastern and northeastern parts of the coast. There are 681 known species of seaweeds in India, of which 60 are commercially important. Seaweeds have been used as food, fertilizer and for medicinal purposes. In food manufacturing, seaweeds have been developed as raw or semi-processed food products. Fresh and dry seaweeds are extensively consumed by people especially living in the coastal areas. Edible seaweeds contain significant amount of carbohydrates, proteins, vitamins and minerals essential for human nutrition. Marine organisms are rich source of structurally novel and biologically active metabolites. Secondary or primary metabolites produced by these organisms may be potential bioactive compounds of interest in the pharmaceutical industry. Seaweeds have some of the valuable medicinal uses such as antibiotics, laxatives, anticoagulants, anti-ulcer products and suspending agents in radiological preparations [1]. Historically seaweeds provide essential economic, environmental, aesthetic and cultural benefits to humanity. Marine algae are continuously exposed to many biotic and

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abiotic pressures which influence the organism's physiology, which in turn leads to the production of multifunctional natural secondary metabolites. More than 2400 seaweed secondary metabolites are described so far and many of them are natural blueprints for the development of new drugs [2, 3]. Majority of them (about 60%) are terpenes and some fatty acids are also common (20%) with nitrogenous compounds. Since then, numerous studies have been carried out to detect antimicrobial and phytochemical compounds from marine algae of all three groups *viz*. Rhodophyceae, Phaeophyceae and Chlorophyceae. Several of these compounds are constitutive, existing in biologically active forms in healthy seaweeds. The major secondary metabolites produced by seaweeds are halogenated compounds displaying anti-bacterial, anti-fungal, anti-viral, antioxidant, anti-fouling and anti-feedent properties [4, 5].

Although thousands of bioactive compounds have been discovered, the need for novel therapeutic compounds is still urgent in concern of number of new diseases and resistant strains of microorganisms. Few reports are available on the bio-potential and biochemical studies on *Padina* and other seaweeds from marine sources of Tamil Nadu, India [6, 7]. In addition, Shanmughapriya *et al* [8], Kandhasamy and Arunachalam [9], Vallinayagam *et al* [10] and Erturk and Tas [11] observed the antimicrobial activity of the crude extracts of *Padina*. With this knowledge, the present study was aimed to investigate the phytochemical constituents using UV-VIS and HPLC and the physico-chemical parameters of different extracts of seaweeds viz. *Padina gymnospora* (Kutzing) Sonder and *Padina tetrastromatica* Hauck.

MATERIALS AND METHODS

Collection of seaweeds

Padina gymnospora (Kutzing) Sonder and *Padina tetrastromatica* Hauck were collected by handpicking from the coast of Chotthavillai beach, Kanyakumari District, Tamil Nadu, India. The collected samples were cleaned well with seawater to remove all the extraneous matter such as epiphytes, sand particles, pebbles and shells and brought to the laboratory in plastic bags. The samples were then thoroughly washed with tap water followed by distilled water. For drying, washed seaweeds were blotted on the blotting paper and spread out at room temperature in shade. Shade dried samples were grounded to fine powder using tissue blender. The powdered samples were then stored in refrigerator for further use.

Preparation of extracts

To compare the hot and cold extraction, the dried and powdered materials (5 g) were extracted successively with 250 mL of petroleum ether, methanol, chloroform, benzene, isopropanol and water using Soxhlet extractor for 8 h at a temperature not exceeding the boiling point of the solvent. The aqueous extracts were filtered using Whattman filter paper (No.1) and then concentrated in vacuum at 40ºC using Rotary evaporator. The residues obtained were stored in a freezer -20ºC until further tests. For cold extraction, 2 g of air dried powder of sample was extracted with 50 ml of solvents viz., ethanol, petroleum ether, chloroform, benzene, isopropanol and water for 72 h. 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).

Phytochemical analysis

The different extracts were tested for steroids, triterpenoids, reducing sugars, phenolic compounds, saponins, xanthoproteins, tannins, flavonoids, protein, glycosides and anthroquinones. Phytochemical screening of the extracts was carried out according to the standard methods [12]. For the proximate analysis, the extracts were examined under visible and UV light. These powdered materials were also treated with various reagents such as 50% nitric acid, ethanol, 50% sulphuric acid, 1N HCl, 1N NaOH and changes in colour were recorded [13]. For UV-VIS spectrophotometer and HPLC analysis, the extract was 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 solvent. The crude extracts containing the bioactive compound was analyzed spectroscopically for further confirmation. To detect the UV-VIS spectrum profile of the crude extracts of *P. gymnospora* and *P. tetrastromatica*, the extracts were scanned in the wavelength ranging from 200-1100 nm by using Shimadzu Spectrophotometer and the characteristic peaks were detected. The qualitative UV-VIS fingerprint profile of different extracts of was selected at a wavelength of 300-700 nm due to the sharpness of the peaks and proper baseline. HPLC method was performed on a Shimadzu LC-10 AT 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. An isocratic HPLC (Shimadzu HPLC Class *VP* series) with two LC-10 AT *VP* pumps (Shimadzu), variable wave length programmable photo diode array detector SPD-M10A *VP* (Shimadzu), CTO- 10AS *VP* column oven (Shimadzu), SCL-10A *VP* system controller (Shimadzu) and reverse phase Luna 5°C18 (2) Phenomenex column (250mm X 4.6mm) was used. The mobile phase components methanol: water (45:55) were filtered through 0.2 µ membrane filter before use and were pumped from the solvent reservoir at a flow rate of 1ml/min which yielded column backup pressure of 260-270 kgf/cm^2. The column temperature was maintained at 27°C. 20µL of respective sample was injected by using Rheodyne syringe (Model 7202, Hamilton). 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 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 [14, 15].

RESULTS

The results of phytochemical screening of *Padina gymnospora* and *Padina tetrastromatica* revealed the presence of secondary metabolites with varied degree. In *P. gymnospora* cold and hot extract, sugars showed the maximum presence in all the six extracts followed by saponins and alkaloids in 5 different extracts. Phenol is present in 4 different extracts, glycosides in 3 different extracts, steroids, flavonoids and tannins in 2 different extracts. Among the six different extracts, methanolic extract showed the presence of maximum number (6) of metabolites viz., alkaloids, flavonoids, tannins, saponins, glycosides and sugar. Next to that, aqueous, isopropanol and petroleum ether extracts showed the presence of 5 metabolites viz., alkaloids, phenolics, steroids, glycosides and sugar and chloroform and benzene showed 4 metabolites alkaloids, phenolics, saponins and sugar. Similar to *P. gymnospora* extracts, the hot and cold extracts of *P. tetrastomatica* also displayed the similar profile in their presence of phytoconstituents. Sugars and saponins illustrated the maximum presence in six different extracts followed by phenols, tannins and alkaloids in 4 extracts. Glycosides are present in 3 different extracts, flavonoids and steroids in 2 different extracts. Among the six different extracts, methanolic extract showed the presence of maximum number (8) of metabolites viz., alkaloids, phenolics, flavonoids, saponins, steroids, tannins, glycosides and sugar. Next to that, aqueous, chloroform and petroleum ether extract showed the presence of 5 metabolites viz., phenolics, saponins, tannins, glycosides and sugar and isopropanol and benzene extracts displayed 4 metabolites each. The isopropanolic cold and hot extracts of *P. tetrastomatica* showed the presence of alkaloids, saponins, tannins and sugar. The alkaloids, phenolics, saponins and sugars were displayed their presence in the benzene extracts of *P. tetrastomatica*. The benzene, aqueous and petroleum ether extracts of both the *Padina* species showed the presence of same number of metabolites. The isopropanolic extract of *P. gymnospora* (5) illustrated the presence of one extra metabolite when compared to *P. tetrastromatica* (4). Similarly, the chloroform extract of *P. tetrastromatica* (5) illustrated the presence of one extra metabolite when compared to *P. gymnospora* (4).The methanolic extract of *P. gymnospora* showed the presence of 6 different metabolites whereas *P. tetrastromatica* showed the presence of 8 different metabolites.

The results of the ash analysis revealed the presence of sulphur, calcium, magnesium, iron, phosphorous and chlorine in all the extracts of *P. gymnospora* and *P. tetrastromatica*. The fluorescence analysis of different extracts and chemical reagents of *P. gymnospora* and *P. tetrastromatica* are recorded in Table 1.

The qualitative UV-VIS fingerprint profile of benzene extract of *P. gymnospora* was selected at a wavelength of 300 to 700 nm due to sharpness of the peaks and proper baseline. The profile showed the compounds separated at the nm of 670, 612, 456, 416 and 324 with the absorption 0.491, 0.095, 0.558, 1.32 and 0.458 respectively. The qualitative UV-VIS fingerprint profile of chloroform extract of *P. gymnospora* was selected at a wavelength of 300 to 700 nm due to sharpness of the peaks and proper baseline. The profile showed the compounds separated at the nm of 668, 610, 414 and 326 with the absorption 0.117, 0.002, 0.347 and 0.119 respectively. The qualitative UV-VIS fingerprint profile of isopropanol extract of *P. gymnospora* was selected at the wavelength of 400 to 700 nm due to sharpness of the peaks and proper baseline. The profile showed the compounds separated at the nm of 662, 612 and 402 with the absorption 0.256, 0.063 and 0.716 respectively. The qualitative UV-VIS fingerprint profile of aqueous extract of *P. gymnospora* was selected at the wavelength of 200 to 300 nm due to sharpness of the peaks and proper baseline. The profile showed the compound separated at 296 nm with the absorption of 1.028 (Table 2).

The qualitative UV-VIS fingerprint profile of chloroform extract of *P. tetrastromatica* was selected at a wavelength of 400 to 700 nm due to sharpness of the peaks and proper baseline. The profile showed the compounds separated at the nm of 658, 610, 564 and 426 with the absorption 0.396, 0.079, 0.039 and 0.677 respectively. The qualitative UV-VIS fingerprint profile of aqueous extract of *P. tetrastromatica* was selected at the wavelength of 300 to 400 nm due to sharpness of the peaks and proper baseline. The profile showed the compound separated at 304 nm with the absorption of 2.069 (Table 3).

Benzene and isopropanolic extract prepared by cold extraction was subjected to HPLC for the separation and identification of constituents present in *P. gymnospora.* The qualitative HPLC fingerprint profile of benzene and isopropanol extract of *P. gymnospora* was selected at a wavelength of 254 nm due to sharpness of the peaks and proper baseline. The benzene extract of *P. gymnospora* HPLC profile displayed four prominent peaks at a retention time of 1.143, 1.803, 2.933 and 3.723 min respectively (Fig.1 a). The HPLC profile of *P. gymnospora* isopropanolic extract displayed one prominent peak at a retention time of 2.657 min (Fig. 1 b). Benzene extract prepared by cold extraction was subjected to HPLC for the separation and identification of constituents present in the *P.tetrastomatica*. The HPLC profile of *P. tetrastomatica* benzene extract showed only one prominent peak at a retention time of 2.657 min and one moderate peak was observed with a retention time 4.063 min (Fig.1 c).

Figures 1a. HPLC chromatogram of *P. gymnospora* benzene extract; 1b.HPLC chromatogram of *P. gymnospora* isopropanolic extract and 1c. HPLC chromatogram of *P. tetrastomatica* benzene extract

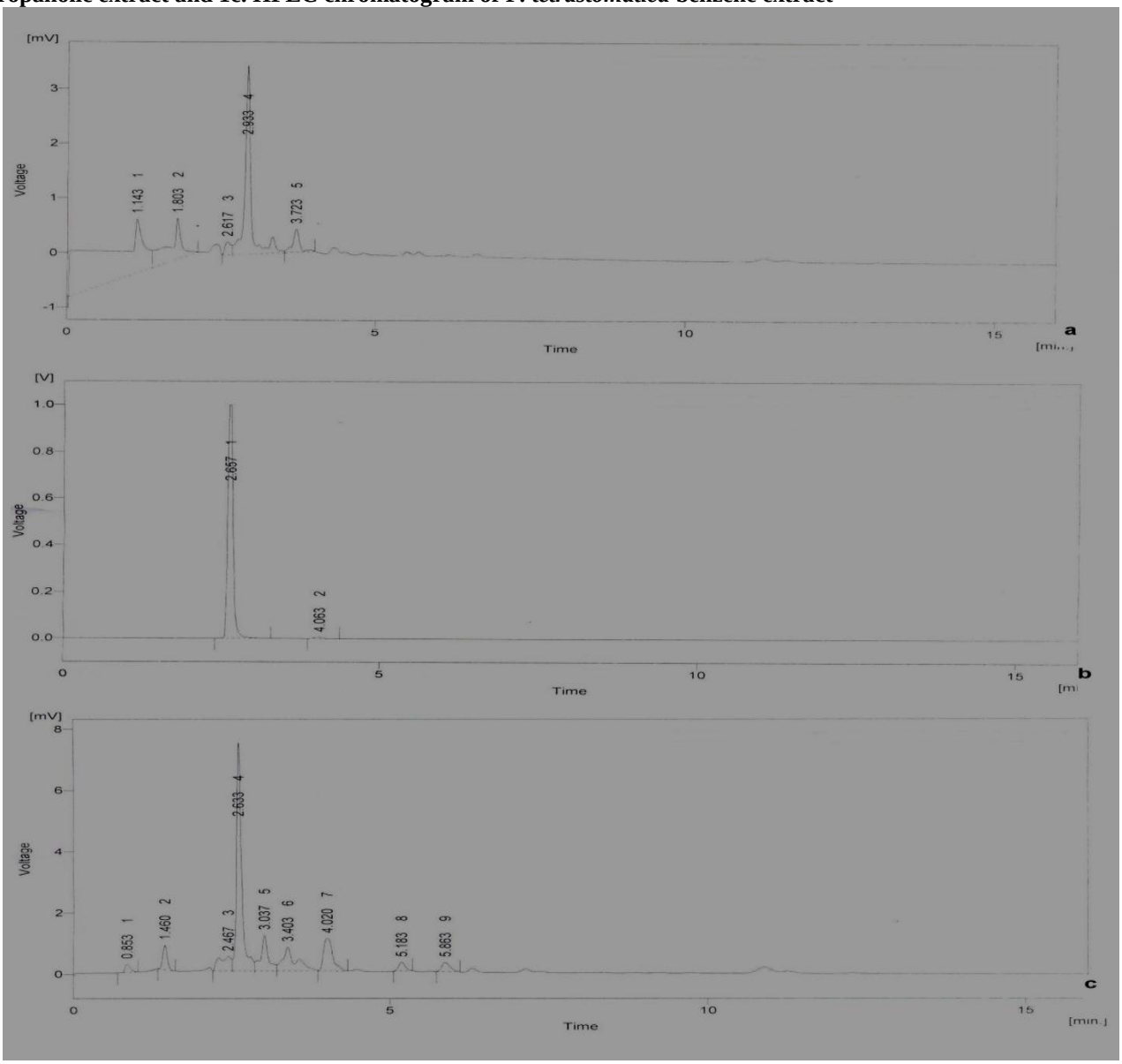

Table 1. Proximate Analysis of *P. gymnospora* and *P. tetrastomatica*

Solvents	*P. gymnospora*		*P. tetrastomatica*	
	Ordinary light	**UV light**	**Ordinary light**	**UV light**
Chloroform	Green	Brownish green	Dark green	Brownish green
Benzene	Dark green	Yellowish green	Dark green	Dark green
Methanol	Green	Yellowish green	Yellowish green	Yellowish green
Aqueous	Yellowish green	-	Greenish yellow	Light green
50% H_2SO_4	Dark green	-	Dark green	-
1N HCl	-	-	-	-
Ethanol	Greenish yellow	-	Greenish yellow	-
NaOH	-	-	Yellowish green	-
HNO_3	Greenish yellow	-	Greenish yellow	-

Table 2. UV-VIS Peak Values of Different Extracts of *Padina gymnospora*

Benzene		Chloroform		Isopropanol		Aqueous	
λ	Abs	λ	Abs	λ	Abs	λ	Abs
670	0.491	668	0.117	662	0.256	296	1.028
612	0.095	610	0.002	612	0.063		
456	0.558	414	0.347	402	0.716		
416	1.32	326	0.119				
324	0.458						

Table 3. UV-VIS Peak Values of Different Extracts of *Padina tetrastromatica*

Chloroform		Aqueous	
λ	Abs	λ	Abs
658	0.396	304	2.069
610	0.079		
564	0.039		
426	0.677		

DISCUSSION

It is a real fact that the importance of marine organisms as a source of new substances is growing. With marine species comprising approximately a half of the total global biodiversity, the seaweeds offer an enormous resource for novel compounds and it has been classified as the largest remaining reservoir of natural molecules to be evaluated for drug activity. Different kinds of substances have been obtained from marine organisms because they are living in a very exigent, competitive and aggressive surrounding diverse in many aspects from the terrestrial environment, a situation that demands the production of quite specific and potent active molecules. Many marine plants including seaweeds, often carry significantly less macro and microepibionts on their thalli compared to co-occurring biofilms on inanimate substrata [16]. Therefore it has been assumed that seaweeds defend themselves against bacterial fouling by production of secondary metabolites that prevent attachment and growth of bacterial colonizers. Recently, consumers are demanding foods which are fresh, natural and minimally processed along with the requirement for enhanced safety and quality. This perspective has put pressure on the food industry for progressive removal of chemical preservatives and has fuelled research into alternative natural antimicrobials. Plant products with antimicrobial properties have obtained emphasis for possible application in food production in order to prevent bacterial and fungal growth [17]. The plants known as medicinal are rich in secondary metabolites which include alkaloids, glycosides, flavonoids, steroids and phenolics which have been extensively used in the drug and pharmaceutical industry. Recently, a number of studies have been reported on the phytochemistry of plants across the world [18-20]. In the present investigation also, two seaweeds of same genus *Padina* have been selected from South India for phytochemical screening on the basis of their traditional uses. The present phytochemcial studies revealed the presence of phenols, alkaloids, tannins, steroids, glycosides, saponins and flavonoids in both the seaweeds with varied degree. Alkaloids are commonly found to have antimicrobial properties against both Gram-positive and Gram-negative bacteria. Presence of alkaloids in different extracts exerts a remarkable antibacterial activity against Gram-positive and Gram-negative bacteria [21]. In the present study, alkaloids were present in both the *Padina* species tested, which can be one of the key chemical exerting the antimicrobial activity and is supported by earlier presented research. Seaweed extracts are considered to be a rich source of phenolic compounds and 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 [22]. In the present study also the phenolics presence were confirmed by the qualitative and quantitative analysis in the crude extracts. Reports have revealed that phenolic compounds are one of the most effective antioxidants in brown algae [23]. The total phenolic content of *P. gymnospora* obtained in this study was higher than some reports for other brown seaweeds. Brown seaweeds had a phenolic content of 24.61 and 49.16 mg GAE/g of seaweed extract [24]. The total phenolic content in different Icelandic seaweeds ranges from 4 to 242 mg PGE/g extract [23]. The results of the present study are promising as algal polyphenolic compounds are effective antioxidants in delaying oil rancidity, therefore the seaweed extracts could have potential in food applications.

Tannins are defined as naturally occurring plant polyphenolic compounds and are widespread among terrestrial and marine plants [25]. In contrast to terrestrial tannins, phlorotannins are tannin compounds which have been found only in marine algae. Phlorotannins are formed by the polymerization of phloroglucinol (1, 3, 5-trihydroxybenzene) monomer units and synthesized in the

acetate-malonate pathway in marine algae. Phlorotannins purified from several brown algae have been reported to possess strong antioxidant activity which may be associated with their unique molecular skeleton [26]. Phlorotannins are more potent free radical scavengers than other polyphenols derived from terrestrial plants including green tea catechins which only have three to four rings. Many tannin-containing drugs are used in medicine as astringent. They are used in the treatment of burns as they precipitate the proteins of exposed tissues to form a protective covering. They are also medicinally used as healing agents in inflammation, leucorrhoea, gonorrhoea, burns, piles and as antidote. Tannins has been found to have antiviral, antibacterial, antiparasitic effects, anti-inflammatory, antiulcer and antioxidant property for possible therapeutic applications [27]. The present study results confirm the maximum (8) presence of tannins in *P. tetrastromatica* when compared to *P. gymnospora* (4). It suggests that *P. tetrastromatica* can be used as antiviral, antibacterial, antiparasitic agents and to treat the diseases like ulcer, gonnorrhoea, leucorrhoea etc.

Flavonoids, the largest groups of phenolic compounds are known to contain a broad spectrum of chemical and biological activities including antioxidant and free radical scavenging properties. Flavonoids include flavonols, flavones, catechins, proanthocyanidins, anthocyanidins and isoflavonoids [28]. Saponins are considered a key ingredient in traditional Chinese medicine and are responsible for most of the observed biological effects. Saponins are known to produce inhibitory effect on inflammation. There is tremendous, commercially driven promotion of saponins as dietary supplements and nutraceuticals. Saponins possess specific physical, chemical and biological activities that make them useful as drugs. Some of the biological properties include antimicrobial, anti-inflammatory and hemolytic effects. In the present investigation also, saponins showed its maximum presence in more extracts of seaweeds. These observations cited on phytochemical compounds support our findings on the usefulness of seaweeds in traditional medicines.

The antimicrobial and pharmacological activity of *P. gymnospora* and *P. tetrastromatica* may be due to one or more group of above phytoconstituents. As both the seaweeds belong to the same genus *Padina*, the presence of more number of phytochemicals in *P. tetrastromatica* will helpful to identify the crude powder from *P. gymnospora*. The HPLC profile showing the compound separated at a retention time of 2.657 was identical in both the species *P. gymnospora* and *P. tetrastromatica*. Hence the generated data may be useful in suggesting chemotaxonomical interrelationship of two species.

CONCLUSION

From the results, it can be concluded that the crude extracts of seaweeds are used as broad-spectrum bioactive agent after extensive investigation. Further work will emphasize the isolation and characterization of active principles responsible for bio-efficacy and bioactivity.

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