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UV- VIS Spectroscopic and HPLC Studies on *Dictyota bartayresiana* Lamour

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ABSTRACT

Objective: The present study was aimed to explore phytochemical constituents present in *Dictyota bartayresiana* Lamour and produce the UV-VIS and HPLC spectrum profile for *Dictyota bartayresiana*. **Methods:** Phytochemical screening of the extracts was carried out according to the standard methods. For the HPLC analysis, the methanol: water (45:55) was used as mobile phase. **Results:** The phytochemical results showed the presence of alkaloids, steroids, phenolic groups, saponins, tannins, glycosides and sugars. The UV- VIS profile of methanolic, petroleum ether, chloroform, isopropanol of *D. bartayresiana* extract showed various peaks with different functional groups. The HPLC profile of *D. bartayresiana* petroleum ether, chloroform and benzene extracts showed some prominent and moderate peaks with different retention time. **Conclusions:** The results of the present study showed that *Dictyota bartayresiana* Lamour may be rich sources of phytoconstituents which can be isolated and further screened for different kinds of biological activities, depending on their reported therapeutic uses.

1. Introduction

Marine algae are continuously exposed to many biotic and abiotic pressures which influence the organism's physiology which in turn leads to the production of multifunctional natural secondary metabolites. So far, more than 2400 Seaweeds Secondary metabolites (SSM) are described and many of the SSM are natural blueprints for the development of new drugs [1, 2]. Bio-stimulant properties of seaweeds are explored for use in agriculture and the antimicrobial activities for the development of novel antibiotics. Seaweeds have some valuable medicinal components such as antibiotics, laxatives, anti-coagulants, anti-ulcer products and suspending agents in radiological preparations. Seaweeds have recently received significant attention for their potential as natural antioxidants. Most of the compounds of marine algae show anti-bacterial activities. Many metabolites isolated from marine algae have bioactive efforts [3, 4]. Many of these compounds are bioactive and have been extensively studied by using bioassays and pharmacological assays [5]. Potential antitumor promoting properties of 36 edible/common marine algae from sea near Maozuru, Kyoto, Japan were examined and strong inhibitory activities were found in Undaria pinnatifida, Laminaria and Sargassum species. The production of inhibitory substances from seaweeds was noted as early as in 1917. Like other plants, seaweeds contain various inorganic and organic substances which can benefit human health [6, 7]. Seaweeds are considered as a source of bioactive compounds as they are able to produce a great variety of secondary metabolites characterized by a broad spectrum of biological activities. Compounds with antioxidant, antiviral, antifungal and antimicrobial activities have been detected in brown, red and green algae [8, 9]. 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 and compounds. Although thousands of bioactive compounds have been discovered, the need for novel therapeutic compounds is still urgent in view of the emergence of a number of new diseases and the resistant strains of microorganisms. Although a number of phytochemical and bio-efficacy studies were carried out at global level, only few reports are available on the bio-potential and biochemical studies on the seaweeds from Gulf of Mannar

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and Peninsular coast of India [10-15]. With this background, the present study was aimed to explore phytochemical constituents present in *Dictyota bartayresiana* Lamour and produce the UV-VIS and HPLC spectrum profile for *Dictyota bartayresiana*.

2. Materials and Methods

Dictyota bartayresiana Lamour. was collected by handpicking from the coast of Manapad, Tiruchendur, Tuticorin 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 a refrigerator for further use. To compare the hot and cold extraction, the dried and powered materials (5 g) were extracted successively with 250 ml of petroleum ether, methanol, chloroform, acetone, benzene, isopropanol and water by using soxhlet extractor for 8 h at a temperature not exceeding the boiling point of the solvent. The aqueous extracts were filtered by using Whattman filter paper (No.1) and then concentrated in vacuum at 40 ℃ using Rotary evaporator. The residues obtained were stored in a freezer -20 ℃until further tests. 2 g of air dried powder of sample was extracted with 50 ml of solvents such as ethanol, acetone, petroleum ether, chloroform, benzene 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). The different extracts were tested for steroids, triterpenoids, reducing sugars, phenolic compounds, saponins, xanthoproteins, tannins, flavonoids, saponin, protein, glycosides and anthroquinones. Phytochemical screening of the extracts was carried out according to the standard methods [16-21]. 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, acetone, ethanol, 50% sulphuric acid, 1N HCL and 1N NaOH and changes in colour were recorded [22]. 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 by 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 *Dictyota bartayresiana*, the extracts were scanned in the wavelength ranging from 200 - 1100 nm by using Shimazdu Spectrophotometer and the characteristic peaks were detected. 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 ℃18 (2) Phenomenex column (250mm X 4.6mm) was used. The mobile phase components methanol: water (45:55) were filtered through 0.2 μm 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 / cm2. The column temperature was maintained at 27 ℃. 20 μl 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 ℃). 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 [23, 24].

3. Results

By preliminary phytochemical screening, thirteen different chemical compounds (steroids, alkaloids, phenolic groups, saponins, tannins, flavonoids, anthraquinone, carbohydrates, carboxylic acid, coumarins, proteins, sugars and xantoproteins) were tested for their presence or absence in the extracts of *Dictyota bartayresiana*. Out of 156 (2 x 6 x 13 = 156) tests for the presence or absence of the above compounds, 50 tests gave positive results and the remaining 103 gave negative results. The 50 positive results showed the presence of alkaloids, steroids, phenolic groups, saponins, tannins, glycosides and sugars. Proteins, xantoproteins, terpenoids, flavonoids, coumarins and catechin did not show any positive result for their presence in any of the six extracts of *Dictyota bartayresiana*.

Dictyota bartayresiana Lamour - Cold extract

Steroids and glycosides showed the maximum presence in five different extracts followed by saponin, alkaloids, tannins and sugar in 3 extracts, phenol in 2 different extracts. Among the six different extracts, benzene and petroleum ether extracts showed the presence of maximum number (6) of compounds. Next to that, chloroform extracts showed the presence of 5 compounds and isopropanol, and methanol extracts showed 3 compounds each and aqueous extracts showed only one compound (Table -1).

Dictyota bartayresiana Lamour. - Soxhlet extract

Similar to cool extracts of *Dictyota bartayresiana*, steroids and glycosides showed the maximum presence in five different extracts followed by tannins and sugar in 4 extracts, alkaloids and saponin in 3 different extracts. Among the six different extracts, chloroform, benzene and petroleum ether extracts showed the presence of maximum number (6) of compounds. Next to that, isopropanol extracts showed the presence 4 compounds and methanol extracts shows 3 compounds each and aqueous extracts showed only one compound (Table -1).

The behaviour of the drug powder with different chemical

Table 1

Preliminary Phytochemical Studies Dictyota bartayresiana Lamour

Compounds	*D. bartayresiana* – Cold						*D. bartayresiana* – Soxhlet						Tot
	C	M	I	B	Aq	P	C	M	I	B	Aq	P	
Alkaloids	+			+		+	+			+		+	6
Phenols		+	+					+	+				4
Flavonoids													0
Saponins	+			+		+	+			+		+	6
Proteins													0
Terpenoids													0
Steroids	+	+	+	+		+	+	+	+	+		+	10
Tannins				+	+	+	+			+	+	+	7
Xanthoprotiens													0
Catechin													0
Glycosides	+	+	+	+		+	+	+	+	+		+	10
Coumarins													0
Sugars	+			+		+	+		+	+		+	7
Total	5	3	3	6	1	6	6	3	4	6	1	6	50

C– Chloroform; B – Benzene; Aq– Aqueous; M– Methanol; I – Isopropanol; *P*– Petroleum ether

reagent will also be helpful in analyzing the characterization of the crude drug. In the present study, fluorescence analysis of *Dictyota bartayresiana* was carried out. The result of the present study revealed the various behaviour characteristics of *Dictyota bartayresiana* crude drug. The result of the present study depicted in Table – 2.

Table 2

Fluorescence Analysis of D. bartayresiana

Solvents	*D. bartayresiana* – Sox		*D. bartayresiana* – Cold	
	Ordinary	UV	Ordinary	UV
Chloroform	YG	LG	YG	G
Benzene	G	FG	Y	LG
Methanol	DG	FG	G	FG
Isopropanol	G	FG	G	FG
Aqueous	Colourles	Colourles	Colourles	Colourles

The qualitative UV–VIS profile of *Dictyota bartayresiana* methanolic extract was selected at the wavelength of 400 nm to 900 nm due to the sharpness of the peaks and proper baseline. The profile showed the peaks at 866, 658, 606, 532, 500 and 410 nm with the absorption 0.125, 0.942, 0.369, 0.375, 393 and 3.957 respectively (Table –3). The qualitative UV–VIS profile of *Dictyota bartayresiana* petroleum ether extract was taken at wavelength from 400 nm to 600 nm due to sharpness of the peaks and proper baseline. The profile showed the peaks at 666, 608, 500 and 408 nm with the absorption of 0.161, 0.048, 0.06 and 0.462 respectively (Table –3). The qualitative UV–VIS spectrum profile of chloroform extract of *Dictyota bartayresiana* was selected at a wavelength of 200 nm to 700 nm due to sharpness of the peaks and proper baseline. The profile showed the peaks at 666, 610, 536, 504, 412 and 298 nm with the absorption 0.217, 0.015, 0.005, 0.021, 0.689 and 0.805 respectively (Fig. 1; Table –3). The qualitative UV–VIS spectrum profile of isopropanol extract of *Dictyota bartayresiana* was taken at the wavelength of 400 nm to 700 nm due to the sharpness of the peak and proper baseline. The profile showed the peaks at 600, 608, 536, 502 and 410 nm with the absorption of 0.11, 0.034, 0.023, 0.028 and 0.274 respectively (Table –3).

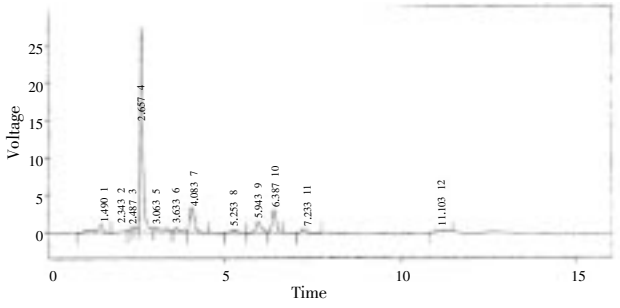

Fig. 1: HPLC profiles of *Dictyota bartayresiana* Lamour. – Petroleum ether Cold Extract

The qualitative HPLC profile of petroleum ether extract *Dictyota bartayresiana* was selected at a wavelength of 254 nm due to the sharpness of the peaks and proper baseline. The profile showed one prominent peak at a retention time of 2.657 min and some moderate peaks were also observed with the retention time 4.083 min, 6.387 min and 1.490 min respectively (Fig. 1). The qualitative HPLC fingerprint profile of chloroform extract of *Dictyota bartayresiana* was taken at a wavelength of 254 nm due to the sharpness of the peaks and proper baseline. The profile showed one prominent peak at a retention time of 2.670 min and some moderate peaks were also observed with the retention time 4.060 min, 3.077 min and 1.507 min respectively (Fig. 2). Benzene extract prepared by soxhlet apparatus was subjected to HPLC for the separation and identification of constituents present in the *Dictyota bartayresiana*. The profile illustrated one prominent peak at a retention time of 2.633 min and some moderate peaks were also observed with the retention time 4.030 min and 5.917 min (Fig. 3).

Table 3
UV-VIS Peak Values of Different Extracts of Dictyota bartayresiana Lamour.

Solvents	Isopropanol		Pet. Ether		Methanol		Chloroform	
S. No.	nm	Abs	nm	Abs	nm	Abs	nm	Abs
1	660	0.11	666	0.161	866	0.125	666	0.217
2	608	0.034	608	0.048	658	0.942	610	0.015
3	536	0.023	500	0.06	606	0.369	536	0.005
4	502	0.028	408	0.462	532	0.375	504	0.021
5	410	0.274			500	0.393	412	0.689
6					410	3.957	298	0.805

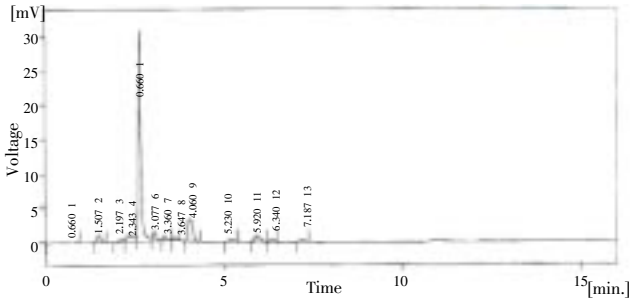

Fig. 2: HPLC profiles of *Dictyota bartayresiana* Lamour. – Chloroform Soxhlet Extract

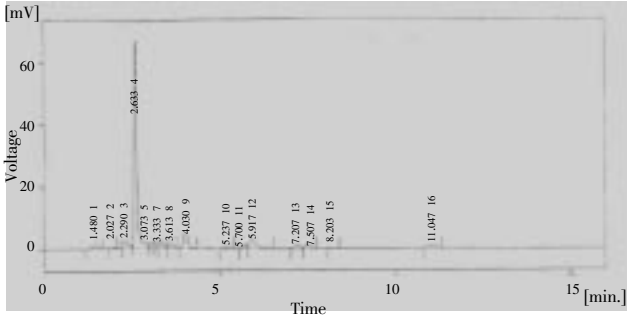

Fig. 3: HPLC profiles of *Dictyota bartayresiana* Lamour. – Benzene Soxhlet Extract

Discussion

Plant substances continue to serve as the viable source of drugs for the world population and several plant-based drugs are in extensive clinical use. For the past few decades, several plants have been widely used for the treatment of various diseases due to their antioxidant properties. The metabolic and physiological capabilities of marine organisms that allow them to survive in complex habitat types provide a great potential for production of secondary metabolites which are not found in terrestrial environments. Thus, marine algae are among the richest sources of known and novel bioactive compounds [25, 26]. The result of the phytochemical analysis of various solvent extracts revealed the presence of alkaloids, glycosides, saponins, steroids, phenols and tanins in *D. bartayresiana* (Table – 1). The seaweeds known as medicinal are rich in secondary metabolites which include alkaloids, glycosides, flavonoids, saponins, tannins, steroids, related active metabolites are of great medicinal value and have been extensively used in the drug and pharmaceutical industry [27]. Recent findings revealed that seaweeds contained antibacterial [28- 31], antiviral [32], antifungal [33, 34], cytotoxic [35] and larvicidal potentials [36]. The secondary metabolites synthesized by seaweeds demonstrate a broad spectrum of bioactivity varying from neurologically active in humans to algicidal, nematicidal, insecticidal and ichthyotoxicity in lower form of animals [35]. In the present we revealed the secondary metabolites by the qualitative tests. The results suggested that the extracts of the seaweed can be used as anti-inflammatory, hepatoprotective, analgesic, antimicrobial, antimycotic, virostatic, immunomodulatory agent.

The extractive values are useful to evaluate the chemical constituents present in the crude drug and also help in the estimation of specific constituents soluble in a particular solvent. In the present investigation the extractive values for *D. bartayresiana* were produced. In addition the behaviour of the drug powder with different chemical reagent also produced. It will be helpful in the characterization of the crude drug and identify and classify the seaweeds. The crude extract prepared by soxhlet apparatus and cool extraction was subjected to UV – VIS and HPLC for the identification of constituents present in crude extracts of *D. bartayresiana*. HPLC identification tests are required to confirm the presence of the active constituents and potential adulterant in ayurvedic drugs. In the present study, the UV – VIS and HPLC profile for the *D. bartayresiana* was evolved. Thus the present studies on *D. bartayresiana* exhibited novel markers in standardization as useful analytical tools to check not only the quality of the powder but also the presence of adulterants in ayurvedic drugs. Fluorescence, UV-VIS and HPLC analysis can be used as effective markers in identifying authentic from its adulterants. Therefore, using newer analytical techniques as markers can be generated for the researches as a chain of markers for use of the common man to evaluate the quality of herbal drug and also incorporated in pharmacopoeias. Further advanced spectroscopic studies are required for the structural elucidation and identification of compounds. The results of the present study showed that *Dictyota bartayresiana* Lamour may be rich sources of phytochemicals particularly flavonoids, terpenes, steroids, tannins, alkaloids, phenol and glycosides, which can be isolated and further screened for different kinds of biological activities, depending on their reported therapeutic uses. Quantitative analyses of these phytochemicals may also be done to guide the researchers on which particular bioactive class of compounds may be subjected to subsequent target isolation.

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