ORIGINAL RESEARCH PAPER

Botany

HPTLC FINGER PRINT PROFILE OF METHANOLIC EXTRACT OF HALYMINIYA DILATATA AND LIAGORA CERNOIDES AS THERAPEUTIC AGENTS

KEY WORDS: Seaweeds, Phytochemicals, HPTLC

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ABSTRACT

Analysis of pharmaceutical and natural compounds is commonly used in all stage of drug discovery and development process. High – performance thin layer chromatography is one of the sophisticated instrumental techniques based on the full capabilities of thin layer chromatography. The present study is focused to develop the high performance thin layer chromatography (HPTLC) fingerprint profile of methanolic extracts of marine red alga *H.dilatata and L. ceranoides*. The aim of present study is to perform the qualitative phytochemical analysis of red seaweeds *H.dilatata* and *L.ceranoides*. Among the 2 seaweeds, *H.dilatata* and *L.cernoides* showed the of active constituents in the ethanol, methanol and aqueous extracts. *H.dilatata* and *L. ceranoides* showed of compounds are present in three extracts. HPTLC profiling of the algal extract confirm about the presence of various phytochemicals. HPTLC method is used for the separation of the active constituents and TLC of these extracts on silica gel pre-coated aluminum plates of Merck by the automatic TLC applicator and using the solvent system Chloroform : Methanol (6.0 : 4.0) from *H.dilatata* and another solvent system like Ethyl acetate : Formic acid (7.3: 2.7 : 0.01) from *L.ceranoides* was performed. The HPTLC finger print scanned at 254 and 366 nm for method for extraction 12 µl was performed algal extract revealed in the seaweeds of *H.dilatata* 5 peaks and *L.ceranoides* shows the 10 peaks and with Rf value in the range of both seaweeds 68.38 to 2.10 and 45.80 to 0.53 respectively.

INTRODUCTION

The marine environment comprises of complex ecosystem with a plethora of organisms and many of these organisms are known to possess of bioactive compounds. The marine bioactive of various, are of immense ecological and great economic important seaweeds. The secondary metabolites produced by marine organisms could be the source of bioactive substance and useful in modeling compounds for drugs. Marine organisms have received great attention during recent years for natural product chemistry, a promising new area of study. Lately a large number of marine organisms have been reported to exhibit various kinds of bioactivities. Methodology that can generate a fingerprint of each extraction large collections would be useful to detect permanence of the same extract over time. Preferably, the fingerprint method should be based on electronic storage, retrieval and analysis of the data. High performance thin layer chromatography (HPTLC) based method could be considered as a good fingerprint analytic tool, as they are explored as an important device for routine drug analysis.

Recent years have seen a surge in interest in marine creatures due to the exciting new field of research known as natural product chemistry. Recently, several marine creatures have been shown to engage in a variety of bioactivities. To determine the persistence of the same extract across time, methodology that can create a fingerprint of each extraction from big datasets would be beneficial. The fingerprinting technique should ideally be based on electronic data storage, retrieval, and analysis. As they are investigated as an essential device for regular drug analysis, high performance thin layer chromatography (HPTLC) based methods might be considered as a useful fingerprint analytical tool.

The objective of this work was to developed an HPTLC method for fracination and quantification of red pigments which could complement to Hptlc and classic chemical determination in red pigments. Hence methodology that can generate a finger print of extract in large collections would be useful to detect phyto constituents of the same red algal extract, over time the fingerprint method should be based on electronic storage retrieval and analysis of the data . Hptlc based on the method could be considered as a good finger print analytical tools as they are being explored as an important device for routine drug analysis.

The present research deals with the HPTLC finger of the methanol extract of *H.dilatata* and *L.ceranoides* can be used for identification authentication and characterization. HPTLC used for determination and quantity and purity of active ingredients. HPTLC is a most versatile techniques and is known for uniformity purity profile assay value and precision and accuracy of results.The marine biota of various kinds are of immense ecological and of great socioeconomic importance.

Seaweeds are considered as a source of bioactive compounds as they are able to produce a great variety of secondary metabolites characterized by their biological activities. The environment in which seaweeds grow is 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 have some protective mechanisms due to the bioactive compounds. Even since the algal extract have been introduced in the market, a permanent rise in the development of advance products has been observed. Standardization through quality control of natural products is very complicated because of the presence of a wide range of phyto constituents present in the extract. Often there is the variability within the same algal material or different parts of the same algae. The herbal drug have multiple phyto constituents including active and unknown compounds which are nutritional therapeutic activity

MATERIALS AND METHOD

The marine red algae *H.dilatata* and *L.ceranoides* was collected from mandabam cost Rameswaram Tamil Nadu.

Sample preparation

One gram of algal sample was extracted with 10 ml of different solvents systems such as methanol, ethanol and aqueous in a beaker for 24 hours at room temperature. Then the solvent portion was centrifuge at 5000rpm for 10minutes. The supernatant was collected from the centrifuge tube and the solvent were evaporated. Finally crude extract was obtained. The extracts were collected in separate plastic vials and

stored in the refrigerator for further studies.A stock solution was prepared at a concentration of 25mg/mL and used for HPTLC analysis.

Chromatographic condition

The chromatogram was developed on 5 x 10 cm aluminium TLC plate procoated with a 0.2 mm layer of silica gel 60 F254 (E. Merck Ltd, Darmstadt, Germany) stored in a desiccators. The application was done by Hamilton micro syringe (Switzerland), mounted on a linomat V applicator. Application of bands of each extract was carried out using spray gas technique. The sample was applied in duplicate on procoated silica gel 60 f 254 aliminum sheets (5 x 10 cm) with the help of linomat 5 applicatiors attached to CAMAG TLC system. The Rf values and finger print data were recorded by WIN CAT CATS software

Developing the solvent system

H.dilatata shoes the spotting was done on the TLC plate ascending development of the plate migration distance 80 mm was performed at 20°c with chloroform methanol(6.0:4.0)as a mobile phase in a camag chamber previously saturated with solvent vapour for 30 mins. The concentration of the sample (12)uL was applied in the track as8 mm bands at a spraying rate 15s/l. After development the plate was dried at 60°c in an oven for 5 mins. *L.ceranoides* shows the spotting was done on the TLC plate ascending development of the plate migration distance 80 mm was performed at 20°c with Ethyl acetate:Formic acid (7.3 : 2.7 : 0.01) as a mobile phase in a camag chamber previously saturated with solvent vapour for 30 mins. The concentration of the sample (10) ul was applied in the track as 8 mm bands at a spraying rate 15s/l. After development the plate was dried at 60°c in an oven for 5 mins. Densitometric scanning was then performed with a camag TLC scanner with the equipped with the win CATS soft were

Development of chromatogram

The application of sample the chromatogram was developed in Twin trough glass chamber 10×10 ca saturated with solvent chloroform methanol (6.0:4.0)extract for 15 mins. After the development allow the plate to dry in air , record the finger print and densitometric chromatogram of the two batch samples of the single compound scanned at 254 and 366nm for alcohol and chloroform extract after spraying with N_2 gas. The R1 value and finger print data were recorded by WINCATS soft ware

Detection of spots

The air dried plates were viewed under ultra violet radiation in middy light. The chromatograms were scanned by the densitometric at 254nm and 366nm with Visible light after derivatised using vanillin-sulphuric acid. The Rf values and finger print data were recorded by WINCATS soft were

RESULTS

Seaweed contribution to its efficacy as neutraceutical and traditional medicine based on the presence of their chemical compounds. Some seaweeds shows climatic condition, season, species, subspecies. Harvesting and the method used for extraction of compounds will algae change the chemical composition of the algal extract. They contain different vitamins, minerals, trace elements, proteins and bioactive substance. In our present study the ethanol, methanol and aqueous extract of the seaweed showed the highest number of compounds in both seaweeds.Priliminary phytochemical screening of ten different chemical compounds like (alkaloids, terpinoids, steroids, tannins, flavonoids, phenols, coumarins, quinines and glycosides) were tested in algal extract. In the present study, the phytochemical screening was performed with thyree extracts of *H.dilatata* and *L.ceranoids.*

The preliminary phytochemical test of *H.dilatata* alkaloids, terpinoids, tannins, flavonoids, phenols, coumarins, quinines and glycosides are presented in methanol, ethanol and aqueous extract. The preliminary phytochemical test of *L.ceranoides* alkaloids, terpinoids, tannins, flavonoids, phenols, coumarins, quinines and glycosides are presented in methanol, ethanol, aqueous extract. In both two seaweeds. Saponins only absent in two seaweeds and three extracts The methanol extract of H.dilatata was subjected to HPTLC analysis by specific solvent system chloroform methanol(6.0:4.0) and detected in UV at254 and 350 nm.

The HPTLC image show in Figs 1a and 1b that 5 components appeared as bright band at 366 nm were found to more predominant in the % area is more with 65.38,14.87,12.38,5.25% (1a and 1b Table)with respect to the Rf values, And the remaining compounds were found to be very less in quality as the percentage of the spots was less then 2.10% The methanol extract of *L.ceranoides* was subjected to HPTLC analysis by specific solvent system Ethyl acetate : Formic acid (7.3 : 2.7 : 0.01)and detected in UV at254 and 350 nm.

The HPTLC IMAGE SHOW IN Figs 1a and 1b that 5 components appeared as bright band at 366 nm were found to more predominant in the % area is more with 61.85,9.20,7.62,6.27,6.24,4.33,2.25,% (1a and 1b Table)with respect to the Rf values, And the remaining compounds were found to be very less in quality as the percentage of the spots was less then 2.23%

Qualitative phytochemical analysis of H.dilatata

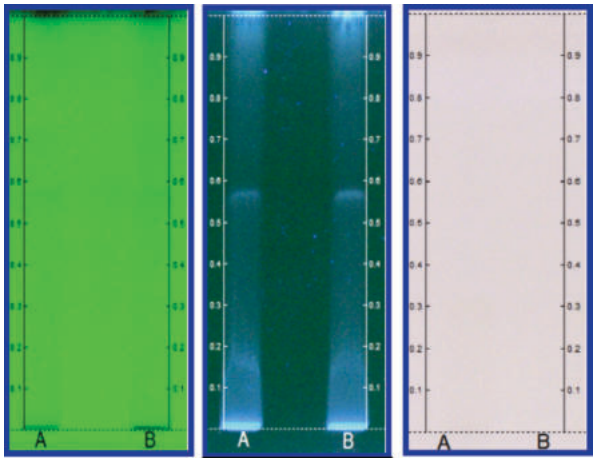
S. NO	phytochemical characters L	Name of the test	Methanol	ethanol	Aqueous
1	Flavonoide	Ferric chloride test	+	+	+
		Alkaline reagent test	+	+	+
		Lead acetate test	+	+	+
2	Test for Glycosides	Borntrager's test	+	+	+
3	Test for steroids	Libermann Burchard test	+	+	+
4	Test for phenolic compounds	Ferric chloride test	+	+	+
		Lead acetate test	+	+	+
5	Detection of Terpenoids	Salkowski's test	+	+	+
6	Test for saponins	Froth test	-	-	-
7	Detection of Tannins	Ferric chloride test	+	+	+
8	Detection of Anthraquinones	Anthraquinones	+	+	+
9	Cardiac Glycosides	Killer killianis test	+	++	++
10	Test for Quinones	Quinones	+	+	+

Qualitative phytochemical analysis of *L.ceranoides*

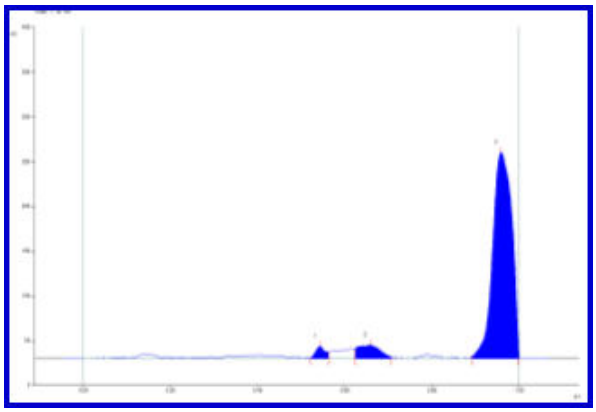
S.NO	phytochemical characters L	Name of the test	Methanol	Ethanol	Aqueous
1	Flavonoide	Ferric chloride test	+	+	+
		Alkaline reagent test	-	-	-
		Lead acetate test	-	-	-

2	Test for Glycosides	Borntrager's test	+	+	+
3	Test for steroids	Libermann Burchard test	+	+	+
4	Test for phenolic compounds	Ferric chloride test	+	+	+
		Lead acetate test	+	+	+
5	Detection of Terpenoids	Salkowski's test	+	+	+
6	Test for saponins	Froth test	-	-	-
7	Detection of Tannins	Ferric chloride test	+	+	+
8	Detection of Anthraquinones	Anthraquinones	_	_	_
9	Cardiac Glycosides	Killer killianis test	++	++	++
10	Test for Quinones	Quinones	-	+	-

This picture shows on *H.dilatata*



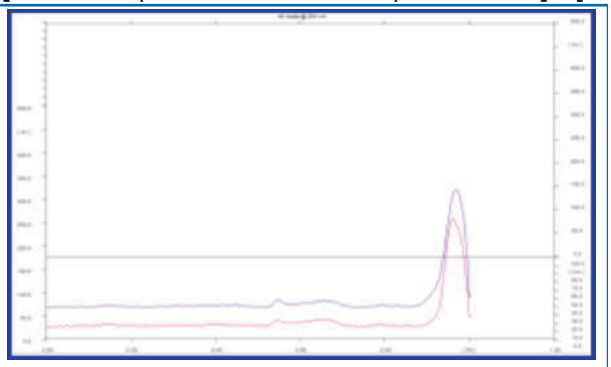
UV – 254 nm UV – 366 nm V - S Reagent
Solvent System: Chloroform : Methanol (6.0 : 4.0) 12 µl
Track A. Alcohol extract; Track B. Chloroform extract



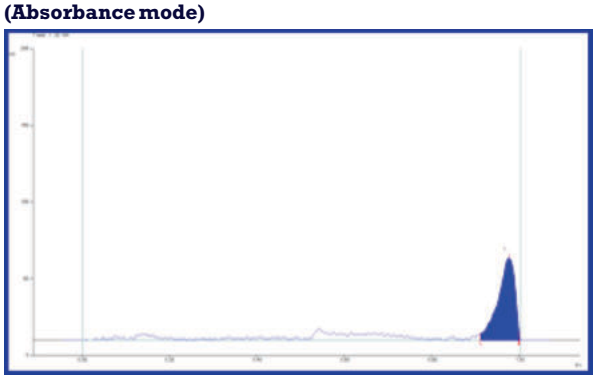
HPTLC finger print of *H.dilatata* at 254 nm (Absorbance mode)

R_f values of *H.dilatata* at 254 nm (Absorbance mode)

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.52 Rf	0.8 AU	0.55 Rf	13.9 AU	5.32 %	0.57 Rf	7.2 AU	262.0 AU	2.78 %
2	0.63 Rf	11.4 AU	0.66 Rf	15.3 AU	5.89 %	0.71 Rf	1.7 AU	659.6 AU	7.01 %
3	0.89 Rf	2.5 AU	0.96 Rf	231.2 AU	88.79 %	1.00 Rf	0.0 AU	8491.3 AU	90.21 %



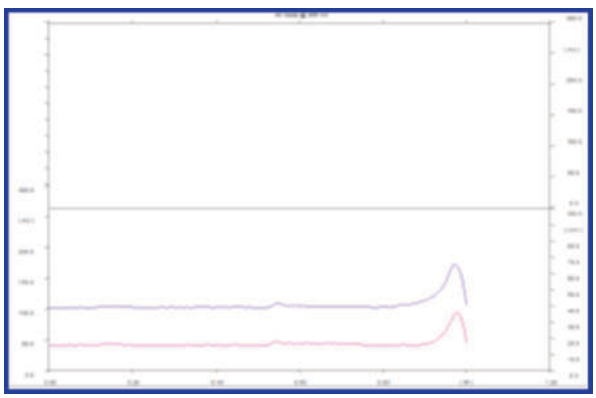
Densitometric chromatogram of *H.dilatata* at 254 nm (Absorbance mode)



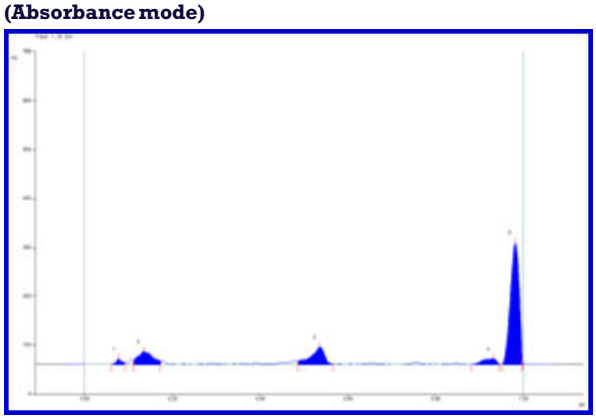
HPTLC finger print of *H.dilatata* at 366 nm (Absorbance mode)

R_f values of *H.dilatata* at 366 nm (Absorbance mode)

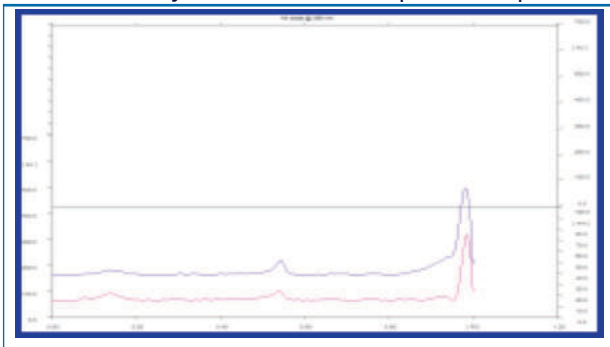
Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.91 Rf	4.3 AU	0.98 Rf	53.5 AU	100.00 %	1.00 Rf	5.0 AU	1709.8 AU	100.00 %



Densitometric chromatogram of *H.dilatata* at 366 nm (Absorbance mode)



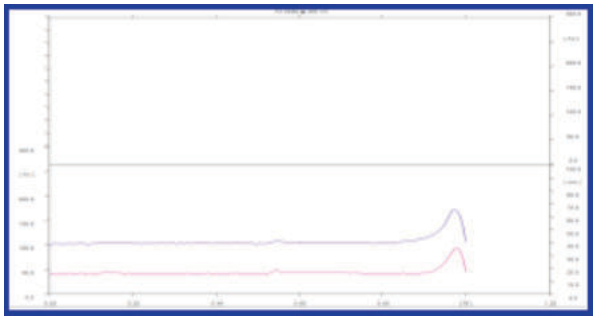
HPTLC finger print of *H.dilatata* at 366 nm (Fluorescence mode)



HPTLC finger print of *H.dilatata* at 366 nm (Fluorescence mode)

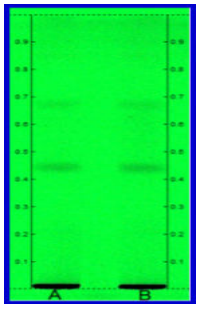
R_f values of *H.dilatata* at 366 nm (Fluorescence mode)

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.06 Rf	0.0 AU	0.08 Rf	12.2 AU	3.60 %	0.09 Rf	4.4 AU	135.1 AU	2.10 %
2	0.11 Rf	9.0 AU	0.14 Rf	27.1 AU	8.02 %	0.17 Rf	7.2 AU	795.5 AU	12.39 %
3	0.49 Rf	7.4 AU	0.54 Rf	36.2 AU	10.71 %	0.57 Rf	1.3 AU	954.8 AU	14.87 %
4	0.88 Rf	0.4 AU	0.93 Rf	12.5 AU	3.69 %	0.95 Rf	0.3 AU	336.7 AU	5.25 %
5	0.95 Rf	0.2 AU	0.98 Rf	249.9 AU	73.98 %	1.00 Rf	33.6 AU	4197.2 AU	65.38 %

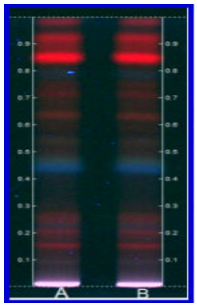


Densitometric chromatogram of *H.dilatata* at 366 nm (Fluorescence mode)

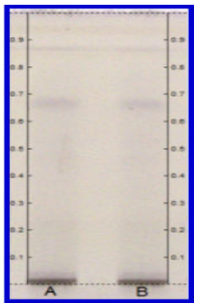
This picture shows on *L.ceranoides*



UV – 254 nm

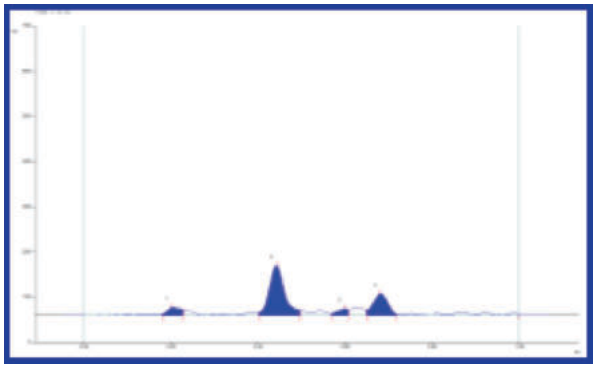


UV – 254 nm



V - S Reagent

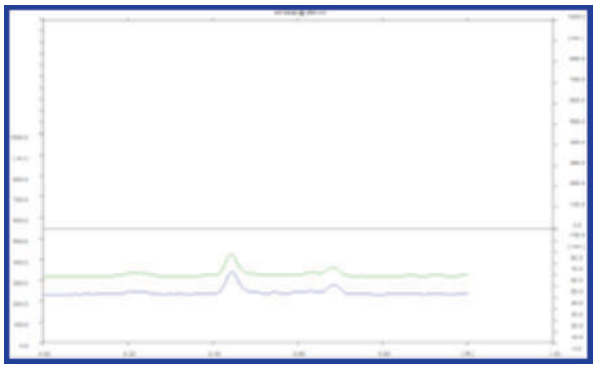
Solvent System: Toluene : Ethyl acetate : Formic acid (7.3 : 2.7 : 0.01) 10 µl Track A. Alcohol extract; Track B. Chloroform extract



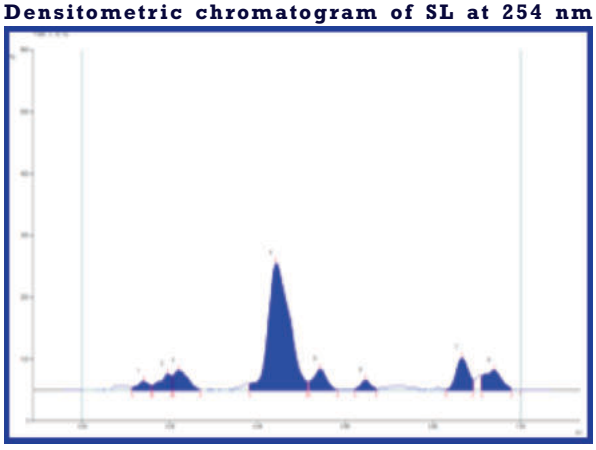
HPTLC finger print of SL at 254 nm (Absorbance mode)

R_f values of SL at 254 nm (Absorbance mode)

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.18 Rf	2.9 AU	0.20 Rf	16.7 AU	8.92 %	0.23 Rf	12.0 AU	410.3 AU	8.16 %
2	0.40 Rf	5.7 AU	0.44 Rf	109.4 AU	58.39 %	0.50 Rf	10.2 AU	3067.3 AU	61.03 %
3	0.57 Rf	2.9 AU	0.60 Rf	14.4 AU	7.68 %	0.61 Rf	10.8 AU	272.7 AU	5.43 %
4	0.65 Rf	11.6 AU	0.68 Rf	46.9 AU	25.01 %	0.72 Rf	1.9 AU	1275.2 AU	25.37 %



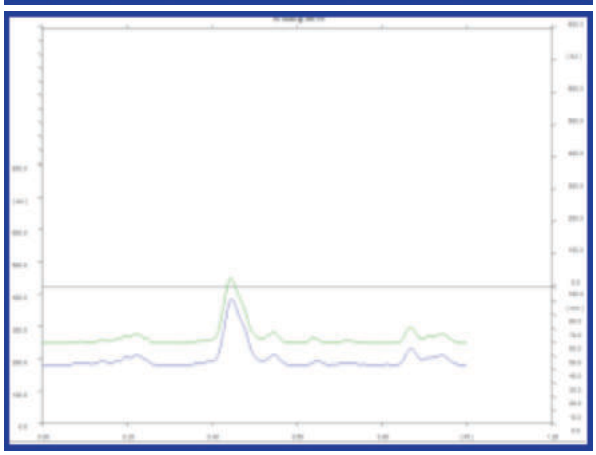
Densitometric chromatogram of SL at 254 nm



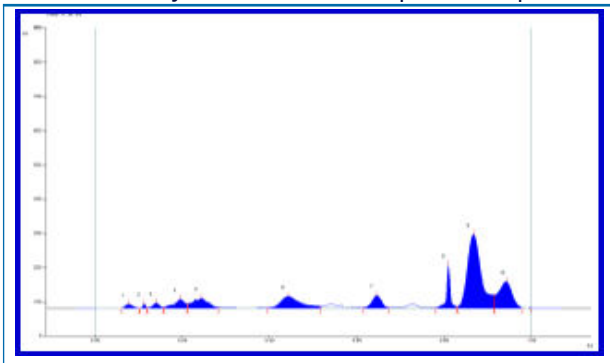
HPTLC finger print of SL at 366 nm (Absorbance mode)

R_f values of SL at 366 nm (Absorbance mode)

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.12 Rf	3.7 AU	0.14 Rf	13.8 AU	3.37 %	0.16 Rf	7.3 AU	296.8 AU	2.25 %
2	0.16 Rf	7.4 AU	0.20 Rf	25.8 AU	6.32 %	0.21 Rf	22.9 AU	572.7 AU	4.33 %
3	0.21 Rf	23.2 AU	0.22 Rf	31.4 AU	7.69 %	0.27 Rf	0.1 AU	825.3 AU	6.24 %
4	0.38 Rf	9.7 AU	0.44 Rf	205.1 AU	50.22 %	0.52 Rf	13.7 AU	8174.3 AU	61.85 %
5	0.52 Rf	13.6 AU	0.55 Rf	33.2 AU	8.13 %	0.58 Rf	0.6 AU	828.8 AU	6.27 %
6	0.62 Rf	1.4 AU	0.65 Rf	15.5 AU	3.79 %	0.68 Rf	1.4 AU	295.1 AU	2.23 %
7	0.83 Rf	1.3 AU	0.87 Rf	52.0 AU	12.73 %	0.89 Rf	16.1 AU	1216.4 AU	9.20 %
8	0.91 Rf	23.3 AU	0.94 Rf	31.6 AU	7.75 %	0.98 Rf	0.7 AU	1007.5 AU	7.62 %



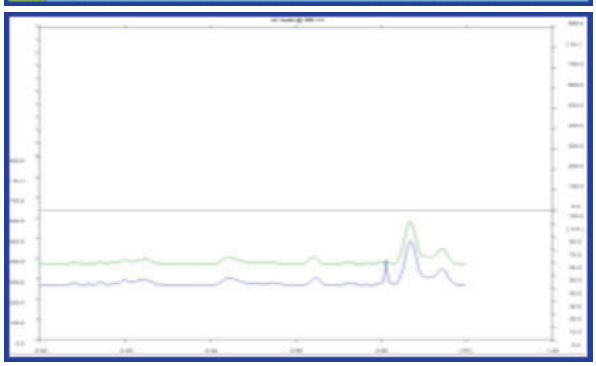
Densitometric chromatogram of SL at 366 nm (Absorbance mode)



HPTLC finger print of SL at 366 nm (Fluorescence mode)

R_f values of SL at 366 nm (Fluorescence mode)

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.06 Rf	0.1 AU	0.08 Rf	11.6 AU	1.98 %	0.10 Rf	0.3 AU	165.5 AU	1.32 %
2	0.10 Rf	0.3 AU	0.11 Rf	13.6 AU	2.32 %	0.12 Rf	0.8 AU	66.4 AU	0.53 %
3	0.12 Rf	1.0 AU	0.14 Rf	15.2 AU	2.58 %	0.16 Rf	2.8 AU	201.9 AU	1.61 %
4	0.16 Rf	2.8 AU	0.20 Rf	25.9 AU	4.40 %	0.21 Rf	13.8 AU	545.5 AU	4.35 %
5	0.21 Rf	13.9 AU	0.25 Rf	28.9 AU	4.91 %	0.28 Rf	2.5 AU	876.7 AU	6.99 %
6	0.40 Rf	1.2 AU	0.44 Rf	34.7 AU	5.91 %	0.52 Rf	5.4 AU	1335.1 AU	10.65 %
7	0.61 Rf	1.8 AU	0.65 Rf	37.1 AU	6.31 %	0.68 Rf	1.5 AU	693.9 AU	5.53 %
8	0.78 Rf	1.3 AU	0.81 Rf	126.6 AU	21.52 %	0.83 Rf	4.7 AU	910.5 AU	7.26 %
9	0.83 Rf	5.0 AU	0.87 Rf	216.4 AU	36.80 %	0.92 Rf	38.6 AU	5743.5 AU	45.80 %
10	0.92 Rf	38.8 AU	0.94 Rf	78.1 AU	13.29 %	0.98 Rf	0.2 AU	2001.4 AU	15.96 %



Densitometric chromatogram of SL at 366 nm (Fluorescence mode)

DISCUSSION

The phytochemical analysis of methanol, aqueous and ethanolic extracts of marine algae indicates the presence of the various phytochemical constituents such as alkaloids, aminoacids, flavanoids, carbohydrates, and sterols. Bioactive compounds of the isolates revealed the presence of sterols and amino acids by TLC.The HPTLC finger printing profile is very important parameter of herbal drug standardization for the proper identification of algae. These methods were also employed to analyze commercial samples to illustrate their application in medicinal properties of seaweeds. HPTLC chromatogram of chloroform and methanol extract result showed that there are many compounds in *H.dilatata* and *L.cernoides* from the HPTLC studies, it has been found that the methanol and chloroform extract contains a mixture of compounds and so it is established that the pharmacological activity. Now a days the interest in the study of natural products is growing rapidly, especially as a part of drug discovery programs. Seaweeds contain several bioactive secondary metabolites that ellucit pharmacological or toxicological effects in human beings and animals. Due to natural variability, the qualitative and quantitative composition of seaweeds. This preliminary study was carried out with HPTLC and the results showed that there are many compounds in *H.dilatata and L.cernoides*.

The present study to identification and detection of phytohormone can be used to characterize the methanol extract of experimental algae for further therapeutic uses.

The development of chromatogram will be specific with selected solvent system chloroform and methanol (6.0.4.0), Rf values are serving a better tool for standardization of the drug. HPTLC is feasible for development of chromatographic fingerprints to determine major active constituents of algae. The separation and resolution are much more better, and the results are much more reliable and reproducible then TLC. Combined with digital scanning profiling , it has the main advantage of *in situ* Quantitative measurement by scanning densitometry. Furthermore the colourful pictorial HPTLC image provides extra intuitive visible colour and fluorescence parameters for parallel assessment on the sample plate. From HPTLC studies, it has been found that the methanol extract marine red algae *H.dilatata* and *L.ceranoides* contains mixture of compounds and it will provide sufficient information about the therapeutic efficacy of the drug and also used in the identification, standardization and quality control. So it is established that the pharmacological activity shown by the algal extract are due to the cumulative effective effect of all the compounds.

CONCLUSION

The present study thorough HPTLC finger print profiles of methanolic extract of *H.dilatata and L.ceranoides* HPTLC Chromatogram of methanol extract of showed significant and therapeutic agents. Further studies is focused on the purification and analysis of sample using GC-MS and therapeutic evaluation of the purified sample

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