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Research Article

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HPTLC finger printing profile of brown alga *Lobophora variegata* (J.V. Lamouroux)

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ABSTRACT

To establish the fingerprint profile of Lobophora variegata using high performance thin layer chromatography (HPTLC) technique. HPTLC studies were carried out using CAMAG HPTLC system equipped with Linomat V applicator; TLC scanner 3, Reprostar 3 and WIN CATS-4 software were used. HPTLC finger printing of methanolic extract of Lobophora variegata revealed 9 peaks with Rf values in the range of 0.18 to 0.86. It can be concluded that HPTLC fingerprint analysis of methanolic extract of Lobophora variegata can be used as a diagnostic tool for the correct identification of the alga and it is useful as a phytochemical marker and also a good estimator of genetic variation in algal populations.

Keywords: *Lobophora variegata*, Phytochemical screening, HPTLC Fingerprinting.

INTRODUCTION

The importance of pharmacologically active natural compounds from biological sources are being re-evaluated in recent years and constitute one of the most active research in this field. These phytochemicals are often present in low concentration in the plants and are chemically sensitive. Besides, phytochemical progress has been aided enormously by the development of rapid and accurate methods of screening plants for particular chemicals. One such group of phytochemicals widely distributed in the plant kingdom is polyphenols.

The plant kingdom still holds many species of plant containing substance of medicinal value which have yet to be discovered and the large number of plants is constantly being screened for their possible pharmacological value in addition to already exploited plants. As the results of modern isolation technique and pharmacological screening procedure, new plant drugs usually find their way into modern medicines.

Standardization of plant materials is the need today. Several pharmacopoeia containing monographs of the plant materials describes only the physicochemical parameters. Hence, the modern methods describing the identification of phytoconstituents of the plant and in the present study the HPTLC fingerprinting of *Lobophora variegata* extracts has been performed which may be used as markers for quality evaluation and standardization of the drug.

EXPERIMENTAL SECTION

2.1Algal materials

The marine brown alga *Lobophora variegata* (J.V. Lamouroux) was collected from intertidal regions of Mandapam, Ramanathapuram District, the South East Coast of Tamilnadu, India.

2.2 Sample preparation

The experimental alga was washed with water and then shade dried. The crude extract was obtained after maceration with 95% methanol at room temperature for 72 hrs, and repeated till exhaustion of the material. Thereafter, the methanol crude extract was distilled, evaporated and dried under reduced pressure to yield a methanol extract of experimental alga (yield 8%). A stock solution was prepared at a concentration of 25 mg/ml and used for the analysis. The residue was redissolved in 1 ml of chromatography grade chloroform, ethyl acetate and 90% ethanol, which was used for sample application on pre-coated silica gel 60F 254 aluminium sheets.

2.3 HPTLC Profile (High Performance Thin Layer Chromatography)

HPTLC studies were carried out following the method of [2, 4].

2.3.1 Chromatographic conditions

Chromatogram was performed on 10 x 10 cm aluminum packed TLC plate coated with a 0.2 mm layer of silica gel 60F254 (E. Merck Ltd, Darmstadt, Germany) stored in a desiccator, 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 technique. Sample were applied in duplicate on pre-coated silica gel 60F254 aluminum sheets (5 x 10 cm) with the help of Linomat 5 applicator attached to CAMAG HPTLC system, which was programmed through WIN CATS software (Version 1.3.0) at λmax = 254 nm and 366 nm using Deuterium light source, the slit dimensions were 6.00 X 0.45 mm and at λmax = 620 nm using Tungsten light source. The chromatograms were recorded.

2.3.2 Developing Solvent System

Spotting was done on the TLC plate, ascending development of the plate, migration distance 80 mm (distance to the lower edge was 10 mm) was performed at 20°C with n-Hexane: ethyl acetate (60:40 v/v) as a mobile phase in a camag chamber previously saturated for 30 mins. The concentration of the sample (2.5 µl) was applied in the track as 8 mm bands at a spraying rate of 15s/L. After development the plate was dried at 60ºC in an oven for 5 mins. Densitometric scanning was then performed with a Camag TLC Scanner 3 equipped with the win CATS Software.

2.3.3 Development of Chromatogram

After the application of the sample, the chromatogram was developed in Twin trough glass chamber 10 x 10 cm saturated with solvent n-Hexane: ethyl acetate (60:40) for 15 mins.

2.4.4 Detection of Spots

The air-dried plates were viewed in ultraviolet radiation to mid-day light. The chromatograms were scanned by the densitometer at 254 nm and 366 nm with or without staining with Permanganate, Potassium dichromate, Phosphomolybdic acid, Anisaldehyde-sulphuric acid stains, and Iodine vapour. The Rf values and finger print data were recorded by WIN CATS software. Documentation of chromatograms was carried out with digital camera SNR & Lens. DXA252: 223971607.Computer, 12 mm, 14.0.

RESULTS AND DISCUSSION

Nowadays, the interest in the study of natural products is growing rapidly, especially as a part of drug discovery program. 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 may vary considerably.

Table.1 Peak list and Rf value of the chromatogram of the methanolic extract of *L.variegata*

Peak	Start Rf	Start height	Max Rf	Max height	Max Height%	End Rf	End height	Area	Area %	Assigned substance
1	0.10	0.50	0.18	8.0	3.83	0.22	4.7	386.6	6.70	*Unknown
2	0.31	6.20	0.34	22.4	10.67	0.36	10.7	518.2	8.99	*Unknown
3	0.41	7.90	0.43	46.4	22.14	0.48	5.3	1201.0	20.82	*Unknown
4	0.51	8.30	0.53	31.4	14.97	0.56	7.7	752.1	13.04	*Unknown
5	0.56	7.80	0.59	20.7	9.89	0.63	8.8	731.7	12.69	*Unknown
6	0.67	5.90	0.68	15.5	7.42	0.70	6.7	215.4	3.73	*Unknown
7	0.71	4.90	0.72	32.3	15.41	0.73	5.5	387.8	6.71	*Unknown
8	0.74	8.30	0.78	14.0	6.98	0.80	10.3	522.0	9.05	*Unknown
9	0.80	10.4	0.86	18.2	8.69	0.93	1.8	1053.3	18.26	*Unknown

The chromatogram shown in Figs.1-2 indicates that all samples constitute were clearly separated without any tailed and diffuseness. It is evident from Fig.1, that there are 9 spots were visualized from the developed chromatogram of *L.variegata* scanned at 490 nm. Table.1 indicates the occurrence of at least 9 different components in the methanol extract. With the R_f values of 0.18, 0.34, 0.43, 0.53, 0.59, 0.68, 0.72, 0.78 and 0.86. As the percentage area was more with 6.70%, 8.99%, 20.82%, 13.04%, 12.69%, 3.73%, 6.71%, 9.65% and 18.26% (Table.1), these compounds were found to be more prominent. The remaining components are less in quantity as the percentage area of all spots was 7.00%. Thus, the developed chromatogram will be specific with selected solvent system hexane: ethyl acetate (60:40), R_f *value* and serve the better tool for standardization of the drug.

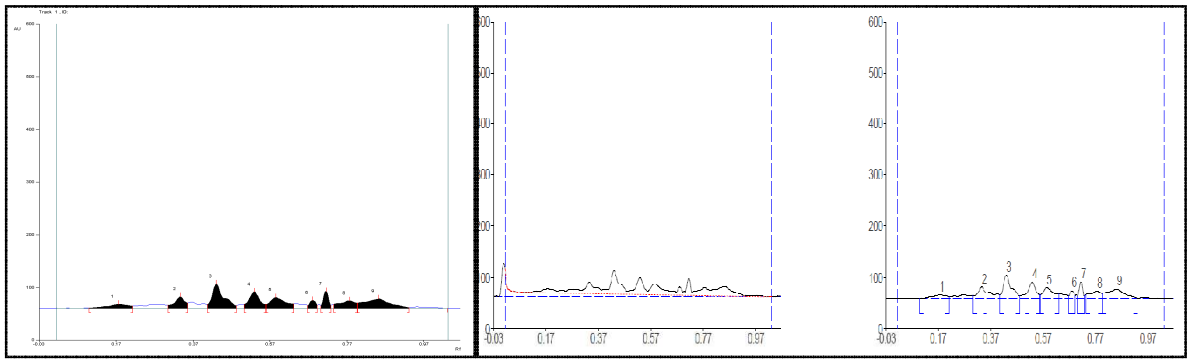
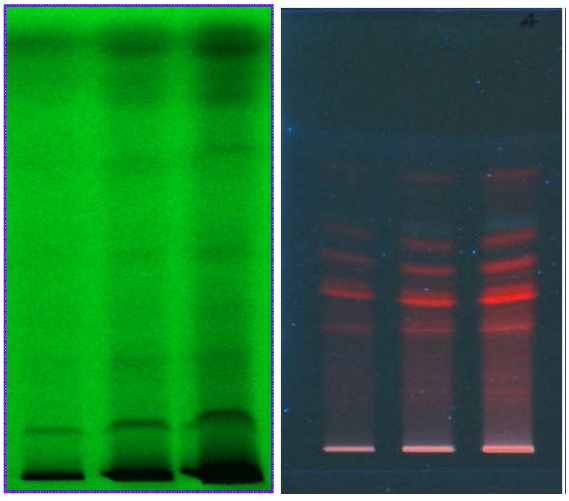


Fig.1 HPTLC Finger print of methanolic extract *L.variegata* scanned at 490 nm



(a) **(b)**

Fig. 2 a)HPTLC fluorescence image of methanolic extract of *L.variegata* observed at 254 nm
Fig.2 b) HPTLC fluorescence image of methanolic extract of L.variegata observed at 366 nm (bright) range

HPTLC fingerprinting is proved to be a liner, precise, accurate method for herbal identification and can be used further in authentication and characterization of the medicinally important plant [3]. Chromatographic fingerprint is a rational option to meet the need for more effective and powerful quality assessment to the traditional system of medicine throughout the world [1]. The optimized chromatographic finger print is not only an alternative analytical tool for authentication, but also an approach to express the various patterns of chemical ingredients distributed in the herbal drugs and to preserve such "database" for further multifaceal sustainable studies. HPTLC finger print analysis has become the most simple and reliable experiment.

HPTLC is feasible for development of chromatographic fingerprints to determine major active constituents of algae. The separation and resolution are much better, and the results are much more reliable and reproducible than TLC. Combined with digital scanning profiling, it has the main advantage of *in situ* quantitative measurement by scanning densitometry. Thus, the present study will provide sufficient information about the therapeutic efficacy of the drug and also in the identification, standardization and quality control of the experimental algae. From HPTLC studies, it has been found that the methanolic extract marine red *L.variegata* contains not only a single compound but a mixture of compounds.

CONCLUSION

The present study clearly gives evidence of the simultaneous bioactive quantitative of phytochemicals in methanol extract. Further, this method can be effectively used for routine quality control of algal materials as well as for formulations containing any or both of these compounds. In conclusion, the results obtained from the qualitative evaluation of HPTLC fingerprint images will be helpful in the identification and quality control of the drug and ensure therapeutic efficacy. HPTLC analysis of marine brown alga *Lobophora variegata* can provide standard finger prints and can be used as a reference for the identification and quality control of the drug.

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