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### Research Article

## HPTLC fingerprint profile of methanol extract of the marine red alga *Portieria hornemannii* (Lyngbye) (Silva)

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Authentication,  
*Portieria hornemannii*,  
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Standardization.

### Abstract

The present study was aimed to develop the high performance thin layer chromatography (HPTLC) fingerprint profile of methanol extracts of marine red alga *Portieria hornemannii* (Lyngbye) (Silva). A HPTLC method for the separation of the active constituents in extracts has been developed and TLC of these extracts on silica gel pre-coated aluminum plates of Merck by the automatic TLC applicator and using the solvent system n-hexane:ethyl acetate (60:40 v/v) was performed. HPTLC profiling of the extract confirm about the presence of various phytochemicals. HPTLC finger print scanned at 490 nm for methanol and ethyl acetate algal extracts revealed 13 peaks with  $R_f$  values in the range of 0.16 to 0.99 respectively. The HPTLC method for routine quality control of present species can be carried out using this method for extracts of plant and serve in qualitative, quantitative and was appropriate for standardization of the extract.

## 1. Introduction

The marine environment comprises of complex ecosystem with a plethora of organisms and many of these organisms are known to possess bioactive compounds. The marine biota of various kinds, are of immense ecological and of great socioeconomic importance. Chemical ecological investigations on marine organisms [1] have inspired the search for bioactive molecules of medicinal importance. Apart from human medicines, the research on marine natural products in the last three decades has also brought to the discoveries of many chemically and biologically interesting molecules, some of them are tetrodotoxin, manoalide, palytoxin, etc., have become indispensable tools in biochemical research and played significant roles in the recent advancement of life sciences [2]

Secondary metabolites produced by marine organisms could be the source of bioactive substance and useful in modeling compounds for drugs [3]. 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 [4]

Identification and quality evaluation of crude herbal extracts is a fundamental requirement. It is an accepted fact that the qualitative analysis of crude extracts constitutes an important and reliable part of quality control protocol. The HPTLC fingerprint profile of *Portieria hornemannii* as any change in the quality of extract directly affects the constituents.

Standardization and quality control of herbal drugs is very complicated because herbal products contain a group of phytoconstituents and are very capable of variation. There is the variability within the same plant material or between the different parts of the same plant. The variability may be from grower to grower, algae to algae and also depends on the harvest and post harvest handling. On the other hand herbal drugs have multiple phytoconstituents including active, inactive, unknown which are dietary rather than therapeutic [5]

Hence, methodologies that can generate a fingerprint of each extract in large collections would be useful to detect stability of the same extract over time. Preferably, the method should be based on electronic storage, retrieval and analysis of the data [6]. Various extraction methods and analytical methods as spectrophotometry are developed for the study about plant active compounds [7]. High-performance thin layer chromatography (HPTLC) based methods could be considered as a good alternative, as they are being explored as an important tool in routine drug analysis. The major advantage of HPTLC is its ability to analyze several samples simultaneously using a small quantity of mobile phase. This reduces the time and cost of analysis. In addition, it minimizes exposure risks and significantly reduces disposal problems of toxic organic effluents, thereby reducing the possibilities of environment pollution. HPTLC also facilitates repeated detection of chromatogram with same or different parameters [7-10]. *Portieria hornemannii* belongs to the class Rhodophyceae. This alga to treat various ailments and it has been reported to possess hepatoprotective, antioxidant, anticancer, antidiabetic, antifungal and antibacterial properties [11-12]. The present research deals with the development of HPTLC fingerprints of methanol extracts of marine red alga *Portieria hornemannii* which can be used for identification, authentication and characterization.

## 2. Materials and Methods

### 2.1 Algal Materials

The marine red alga *Portieria hornemannii* (Lyngbye) (Silva) was collected from intertidal regions of Leepuram, Kanyakumari, 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-water mixture 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 the methanol extract of *P.hornemannii* in powder form (yield 8%). A stock solution was prepared at a concentration of 25 mg/mL and used for the analysis. The sample was redissolved in 1 mL of chromatography grade (99.8%) chloroform, (99.8%) ethyl acetate and 90% ethanol, which was used in the 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 by following the method of Harborne [13] and Kpoviessia *et al.*, [14]

#### 2.3.1 Chromatographic Conditions

Chromatogram was developed on 5 x 10 cm aluminium TLC plate precoated with a 0.2 mm layer of silica gel 60F254 (E. Merck Ltd, Darmstadt, Germany) stored in a desiccator. 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 technique. The sample was applied in duplicate on precoated silica gel 60F254 aluminum sheets (5 x 10 cm) with the help of Linomat 5 applicators attached to CAMAG HPTLC system, which was programmed through WIN CATS software (Version 1.3.0) at  $\lambda_{max}$  254 and 366 nm is using Deuterium light source, the slit dimensions were 6.00 X 0.45 mm and at  $\lambda_{max}$  620 nm using Tungsten light source. The chromatograms were recorded.

#### 2.3.2 Developing Solvent System

The 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 with solvent vapour for 30 mins. The concentration of the sample (2.5  $\mu$ L) was applied in the track as 8 mm bands at a spraying rate of 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 3 equipped with the win CATS Software.

#### 2.3.3 Development of Chromatogram

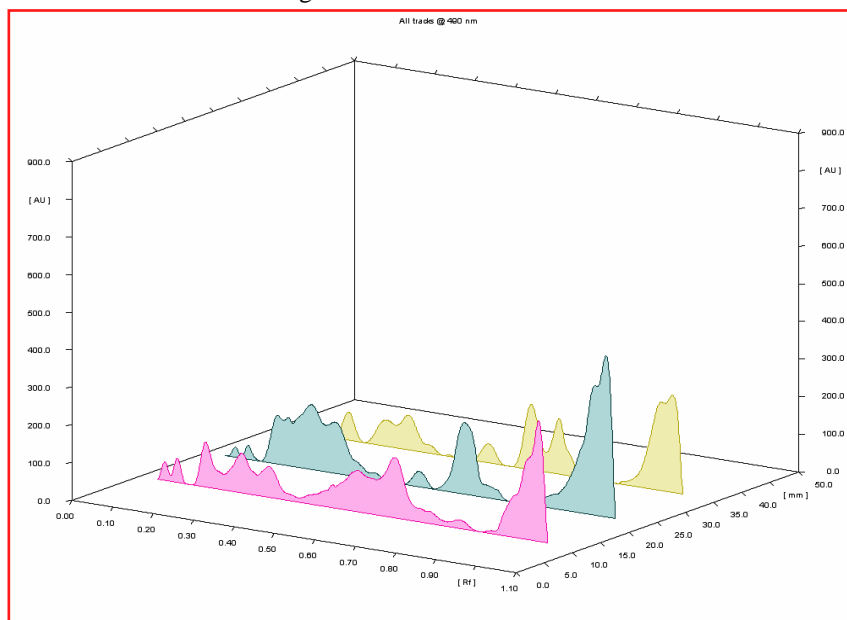
After the application of the sample, the chromatogram was developed in Twin trough glass chamber 10 x 10 cm saturated with solvent n-Hexane:ethyl acetate (60:40) for 15 mins.

#### 2.4.4 Detection of Spots

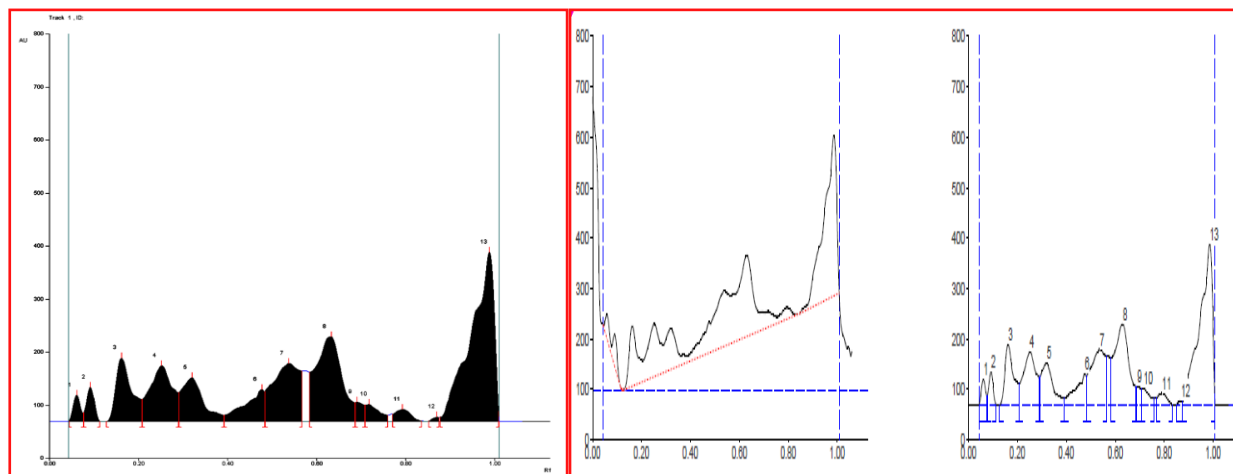
The air-dried plates were viewed under ultraviolet radiation in midday light. The chromatograms were scanned by the densitometer at 254 and 366 nm with or without staining with Permanganate, Potassium dichromate, Phosphomolybdic acid, Anisaldehyde-sulphuric acid stains, and Iodine vapour. The *R<sub>f</sub>* 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.

### 3. Results

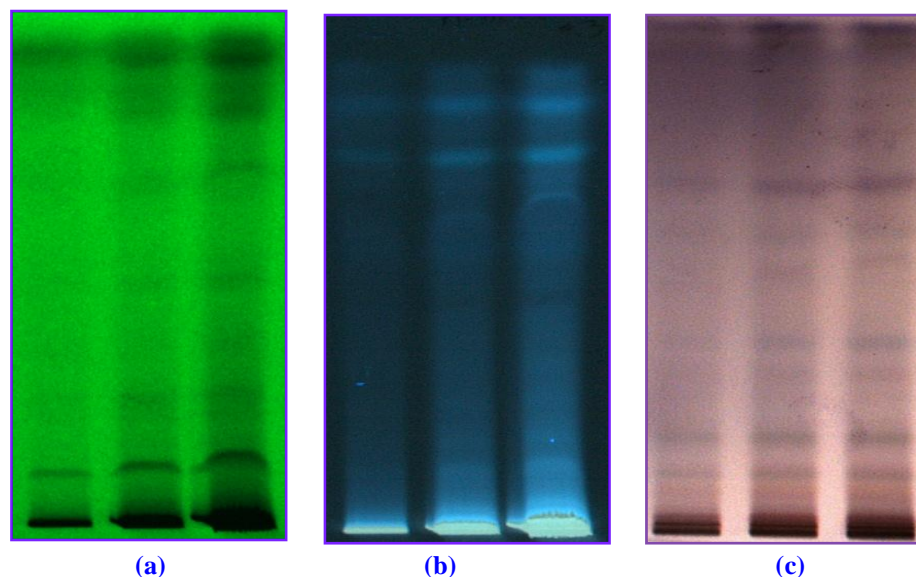
The chromatogram shown in Figs.1-3 indicates that all samples constitute were clearly separated without any tailed and diffuseness. It is evident from Fig.2, that there are 13 spots were visualized from the developed chromatogram of *Phornemannii* scanned at 490 nm. Table.1 indicates the occurrence of at least 13 different components in the ethanol extract. Maximum  $R_f$  values 0.16, 0.25, 0.32, 0.48, 0.54, 0.63, 0.69, 0.72, 0.79, 0.87, 0.99 were found to be more prominent. As the percentage area was more with 5.06%, 7.45%, 7.88%, 9.96%, 12.26%, 17.54%, 1.18%, 1.92%, 1.53%, 0.22%, 31.34% (Table.1). The remaining components are less in quantity as the percentage area of all spots was 3.65%. 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. 3Dimensional finger print of methanolic extract of *P. hornemannii* showing different peaks of phytoconstituents**



**Fig.2 HPTLC Finger print of methanolic extract of *P. hornemannii* scanned at 490 nm.**



**Fig.3a):** HPTLC fluorescence image of methanol extract of *P. hornemannii* observed at 254 nm.

**Fig.3b):** HPTLC fluorescence image of methanol extract of *P. hornemannii* observed at 366 nm (bright) range.

**Fig.3c):** HPTLC image after derivatization

#### 4. Discussion

Nowadays, 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 elicit pharmacological or toxicological effects in human beings and animals. Due to natural variability, the qualitative and quantitative composition of seaweeds may vary considerably. This preliminary study was carried out with HPTLC and the results showed that there are many compounds in *P. hornemannii*. The present study may help to detect and identify phytohormones which can be used to characterize the methanol extract of experimental algae for further therapeutic use.

Characteristic TLC/HPTLC finger printing of particular algal species will not only help in the identification and quality control of a particular species, but also provide basic information useful for the isolation, purification, characterization and identification of marker chemical compounds of the species.

HPTLC profile differentiation is such an important and powerful procedure which has often been employed for this purpose. HPTLC fingerprinting is proved to be a lenier, precise, accurate method for herbal identification and can be used further in authentication and characterization of the important medicinal plant [14]. The HPTLC method can be used for phytochemical profiling of plants and quantification of compounds present in plants. With an increasing demand for herbal products as medicines and cosmetics, there is an urgent need for standardization of plant products. Chromatographic fingerprint is a rational option to meet the need for more effective and powerful quality assessment of the traditional system of medicine throughout the world [15]. 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 multifaceted sustainable studies. HPTLC finger print analysis has become the most simple and reliable experiment.

The development of chromatogram will be specific with selected solvent system -n-hexane with ethyl acetate (70:30) and hexane: ethyl acetate (60:40),  $R_f$  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 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. Furthermore, the colourful pictorial HPTLC image provides extra, intuitive visible colour and/or fluorescence parameters for parallel assessment on the same plate. From HPTLC studies, it has been found that the methanol extract marine red alga *P. hornemannii* contains not a single compound, but a 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 effect of all compounds in composite.

## 5. 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. The present study establishes the fact that HPTLC fingerprinting profile can be used as the diagnostic tool to identify and determine the quality and purity of experimental algae *P. hornemannii* in future studies.

**Conflict of interest statement:** The authors do not have any conflict of interest.

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