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Research Article

Evaluation of Phytochemicals in Methanolic Extract of *Hypnea musciformis* (Wulf.) Lamouroux Collected from Manapad in the South East Coast of Tamil Nadu, India

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ABSTRACT

The present study was carried out to evaluate the phytochemicals in the methanolic extract of *Hypnea musciformis* (Wulf.) Lamouroux collected from Manapad in the south east coast of Tamil Nadu, India. The phytochemical screening of methanolic extract was studied using the standard procedure by UV-Visible spectroscopic, HPLC and FTIR. The UV-Visible spectrum showed the compounds separated at the nm of 300, 350, 420, 460, 534.5, 538.5, 606.35 646 and 667.5 with the absorption 0.361, 0.249, 0.675, 4.000, 0.967, 0.098, 1.260, 2.460 and 2.571 respectively. The qualitative HPLC fingerprint profile displayed six compounds at different retention time of 1.923min, 2.140min, 2.533min, 3.017min, 3.260min and 3.525min. The result of FTIR analysis was found the presence of functional groups such as amides, phosphorus compound, alcohols, phenols and halogen compounds.

Keywords: Phytochemicals, *Hypnea musciformis,* UV-Visible, HPLC, FTIR

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INTRODUCTION

Marine sources especially marine macro algae commonly known as seaweeds are receiving much attention mainly because of the contents of functional ingredients such as polyunsaturated acids, carotene and their pigments carotenoids, sulphated polysaccharide and sterols. Majority of the red algae have been found to have phenolic substances[1]. Marine macro algae are the excellent source of bioactive compounds such as carotenoids, dietary fibre, protein, essential fatty acids, vitamins and minerals[2,3]. Phytochemical studies with extracts from fresh thallus of *Gracilaria andersoniana* showed the following isolates: oleic acid, linoleic acid, cholesterol, prostaglandin A_2, prostaglandin E_2, leukotriene B_4 and phytol. Studies with *Gracilaria asiatica* reported the diterpenes cis and trans-phytol. A variety of lactones are present in *Gracilaria* from the Pacific Ocean, such as aplysiatoxin isolated from *Gracilaria confervoides*, polycavernoside B, polycavernoside B_2 and polycavernoside A_2 and A_3 isolated from *Gracilaria crassa.* Other constituents are also contained in this genus such as proteins r-phycoerythrin from *Gracilaria salicornia* and *Gracilaria longa*, gigartinine from *Gracilaria chilensis* and proteoglycan from *Gracilaria longa*.

The possibility of finding of new molecules from natural products is immeasurable. For this reason the marine algae and their derivatives are major sources of all drugs, affecting about 30% of pharmaceutical market. In the years 1981 and 2002, 877 new molecules were introduced into the market, with 49% of substances isolated from natural sources followed by semi-synthetic derivatives or synthesized molecules taking the structures of natural origin as models. The search for new effective medicines remains a challenge for scientists. Therefore around the world, many researchers have focused on natural sources for new molecules with algae among the targets of these studies. Hence, the present study was concerned with the screening of phytochemicals present in the methanolic extract of *Hypnea musciformis* (Wulf.) Lamouroux collected from Manapad in the south east coast of Tamil Nadu, India.

MATERIALS AND METHODS

Collection of Plant sample

The plant materials used in the present study was *Hypnea musciformis* (Wulf.) Lamouroux, belonging to Rhodophyceae (red algae) was made during the low tidal and subtidal regions (up to 1m depth) by hand picking. The collected materials were washed thoroughly with marine water in the field itself to remove the epiphytes and sediment particles. Then the samples were packed separately in polythene bags in wet conditions and brought to the laboratory, then thoroughly washed in tap water followed by distilled water to remove the salt on the surface of the thalli. They were stored in 5% formalin solution[4].

Preparation of extracts

For the preparation of methanolic extract, the plant specimens were washed thoroughly and placed on blotting paper and spread out at room temperature in the shade condition for drying. The shade dried samples were grounded to fine powder using a tissue blender. The powdered samples were then stored in the refrigerator for further use. 30g powdered samples were packed in Soxhlet apparatus and extracted with methanol for 8h separately[5].

UV-Vis spectral analysis

The methanolic crude extract containing the bioactive compound was analyzed UV-Visible spectroscopically for further confirmation. The methanolic crude extract of *Hypnea musciformis* (Wulf.) Lamouroux was scanned in a wavelength ranging from 200-900nm using a Shimazdu spectrophotometer and characteristic peaks were detected[6].

HPLC Analysis

The HPLC method was performed on a Shimadzu LC-10AT VP HPLC system, equipped with a model LC-10AT pump, UV-Vis detector SPD-10AT, a Rheodyne injector fitted with a 20µl loop and an auto injector SIL-10AT. A Hypersil® BDS C-18 column (4.6 × 250mm, 5µm size) with a C-18 guard column was used. The elution was carried out with gradient solvent systems with a flow rate of 1ml/min at ambient temperature (25-28°C). The mobile phase consisted of 0.1% v/v methanol (solvent A) and water (solvent B). The mobile phase was prepared daily, filtered through a 0.45µm and sonicated before use. Total running time was 15min. The sample injection volume was 20µl while the wavelength of the UV-Vis detector was set at 254nm[7].

Instrumentation

An isocratic HPLC (Shimadzu HPLC Class VP series) with two LC- 0 AT VP pumps (Shimadzu), a variable wave length programmable photo diode array detector SPD-M10A VP (Shimadzu), a CTO- 10AS VP column oven (Shimadzu), a SCL-10A VP system controller (Shimadzu), a reverse phase Luna 5µl C18 (2) and Phenomenex column (250 mm X 4.6mm) were used. The mobile phase components methanol: water (45:55) were filtered through a 0.2µ membrane filter before use and were pumped from the solvent reservoir at a flow rate of 1ml/min which yielded column backup pressure of 260-270kgf/cm^2. The column temperature was maintained at 27°C. 20µl of the respective sample and was injected by using a Rheodyne syringe (Model 7202, Hamilton).

FTIR analysis

The methanolic extract of *Hypnea musciformis* (Wulf.) Lamouroux was shade dried and FTIR analysis was performed using Perkin Elmer Spectrophotometer system, which was used to detect the characteristic peaks and their functional groups. The peak values of the FTIR were recorded. Each and every analysis was repeated twice and confirmed the spectrum [42].

RESULTS AND DISCUSSION

UV-Visible spectrum analysis

The UV-VIS fingerprint profile of the methanolic extract of *Hypnea musciformis* (Wulf.) Lamouroux was selected at the wavelength of 400nm to 700nm due to the sharpness of the peaks and proper baseline. The profile showed the compounds separated at the nm of 300, 350, 420, 460, 534.5, 538.5, 606.35 646 and 667.5 with the absorption 0.361, 0.249, 0.675, 4.000, 0.967, 0.098, 1.260, 2.460 and 2.571 respectively (Figure-1 and Table-1).

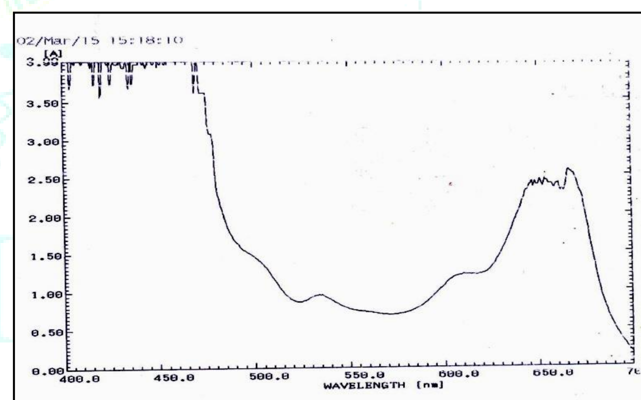

Figure-1: UV-Visible Spectrum of methanolic extract of *Hypnea musciformis* (Wulf.) Lamouroux.

Table-1: UV-Visible Spectrum of methanolic extract of *Hypnea musciformis* (Wulf.) Lamouroux.

Nm	300	350	420	460	534.5	538.5	606.35	646	667.5
Abs	0.361	0.249	0.675	4.000	0.967	0.098	1.260	2.460	2.571

HPLC analysis

The qualitative HPLC fingerprint profile of the methanolic extract of *Hypnea musciformis* (Wulf.) Lamouroux was selected at a wavelength of 660nm due to the sharpness of the peaks and proper baseline. The methanolic extract prepared by cold extraction was subjected to HPLC for the separation and identification of constituents present in the *Hypnea musciformis* (Wulf.) Lamouroux. Six compounds were separated at different retention time of 1.923min, 2.140min, 2.533min, 3.017min, 3.260min and 3.525min. The profile displayed five prominent peaks at the retention time of 1.923min, 2.140min, 2.533min 3.017min and 3.260, followed by one moderate peak was also observed at the retention time of 1.000min (Figure-2).

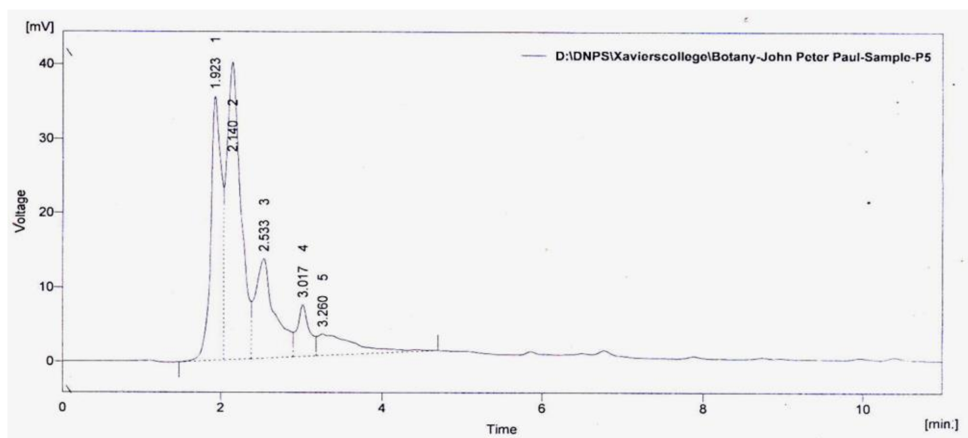

Figure-2: HPLC spectrum of methanolic extract of *Hypnea musciformis* (Wulf.) Lamouroux

FTIR Analysis

The FTIR spectrum was used to identify the functional group of the active components based on the peak value in the region of infra red radiation. The crude methanolic extract of *Hypnea musciformis* (Wulf.) Lamouroux was passed into the FTIR and the functional groups of the components were separated based on its peak ratio. The results of FTIR analysis showed different peaks at 825.48, 1080.06, 1149.50, 1382.87 1637.45 and 3419.56. It was confirmed the presence of functional groups such as amides, phosphorus compound, alcohols, phenols and halogen compounds (Figure-3).

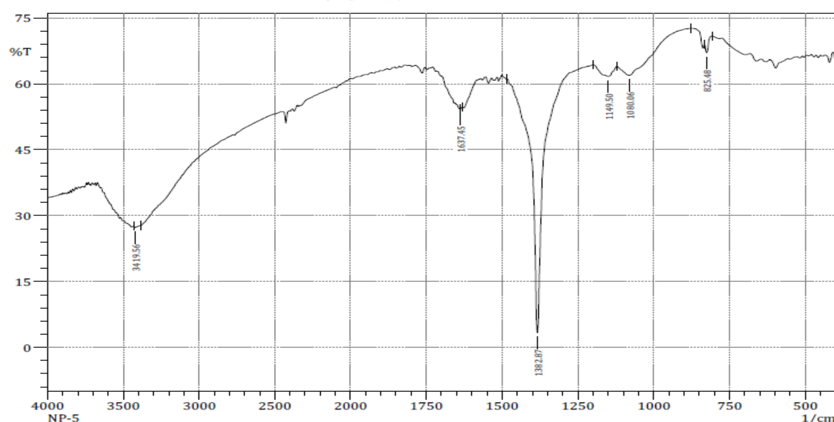

Figure-3: FTIR spectrum of methanolic extract of *Hypnea musciformis* (Wulf.) Lamouroux

CONCLUSION

From the present study, it was concluded that UV-Visible spectrum showed the compounds separated at the nm of 300, 350, 420, 460, 534.5, 538.5, 606.35 646 and 667.5 with the absorption 0.361, 0.249, 0.675, 4.000, 0.967, 0.098, 1.260, 2.460 and 2.571 respectively. The qualitative HPLC fingerprint profile displayed six compounds at different retention time of 1.923min, 2.140min, 2.533min, 3.017min, 3.260min and 3.525min. The profile displayed five prominent peaks at the retention time of 1.923min, 2.140min, 2.533min 3.017min and 3.260 followed by one moderate peak was also observed at the retention time of 1.000min. The result of FTIR analysis was found the presence of functional groups such as amides, phosphorus compound, alcohols, phenols and halogen compounds.

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CONFLICT OF INTEREST

The author declares that he has no conflict of interest.

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