Original Article

Phytochemical analysis of Acanthophora najadiformis using High-Resolution Liquid Chromatography Mass Spectrometry (HR-LCMS) and FTIR

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Abstract

A substantial source of marine natural products is oceanic macroalgae. The pharmaceutical industry contends that a wide variety of bioactive secondary metabolites produced by seaweeds can be used to create novel medications. It has been established that it is crucial to analyse complex bioactive chemicals using analytical methods like HR-LCMS. The goal of the current work was to use HR-LCMS and FTIR to analyse the bioactive metabolites found in the marine alga Acanthophora najadiformis.

Bioactive substances such as alkaloids, steroid, fatty acids, ester, phenol ethers, fatty alcohols, triterpenoids, polypeptides, glycoside, xanthenes, coumarin, flavonoid, diterpenoid, and xanthone are revealed by the HR-LCMS and FTIR analyses.

The current study's findings support the existence of significant phytocompounds in Acanthophora najadiformis and are helpful for further in-depth research to produce drugs from marine algae to treat a range of illnesses. Discovering all-natural remedies for a wide range of diseases and disorders is the focus of current research.

Keywords: Seaweeds, HR-LCMS, FTIR, Bioactive compounds.

1. INTRODUCTION

Oceans encompass more than 70% of Earth's surface, and up to 90% of the planet's life is found there (Pereira 2018).

Bioactive natural products with chemical and structural characteristics not present in terrestrial natural compounds can be found in abundance in the marine environment. They create a chemical defence mechanism to protect themselves from other species in their environment and to secure their survival (Aida H Shobier et al. 2016).

The seaweed species investigated up to this point have demonstrated changes in their chemical make-up (lipids, vitamins, proteins, minerals, and carbs influenced by environmental factors including seasonality, temperature, light, salinity, location, and storage circumstances (Rodeiro et al. 2015).

Algae and their by-products have been in high demand recently, both for use in manufacturing various agro-industrial goods and for human consumption. As per data from the FAO, the globe produced 23.8 million tonnes of algae in 2012, and it is expected that around 25 million tonnes of seaweed are produced year for use in cosmetics, food, and fertilisers (Monsalve-Bustamante et al. 2019).

Algae have traditionally been employed by riverine, estuarine, and coastal cultures to create homemade treatments that were subsequently used to cure a variety of health issues. Since these applications are based on the practical knowledge of previous generations and most of their mechanisms of action are unknown, fewer scientific investigations beyond basic data collecting and ethnographic recording would have been documented (Pereira 2018).

Algae in marine environments serve as a significant source of vital biomolecules that are utilised across all sectors, including the food, pharmaceutical, nutraceutical, and biomedical industries. Marine algae are divided into three main types based on their ability to survive and their nutritional content, including green algae (Chlorophyta), red algae (rhodophyta), and brown algae (phaeophyta) (Alharbi et al. 2020).

It is well known that marine macroalgae are producers of a variety of biogenic substances, such as terpenoids, polyunsaturated fatty acids, alkaloids, flavonoids, polyketides, polysaccharides,

sterols, phlorotannins, lipids, glycerols, and peptides that have anti-inflammatory, antimicrobial, antiviral, antifouling, anticancer, anticoagulant, antioxidant, anti-allergic, and anticancer activities (Aida H. Shobier et al. 2016).

Since bioactive compounds have low side effects and were obtained from natural resources in order to provide novel medicines, researchers are drawn to studying them (Atanas G. Atanaso 2021).

Extraction, analysis, chromatographic separation, and spectroscopic identification are examples of traditional techniques for characterising bioactive compounds. However, despite spending a lot of time and effort, most research only succeed in characterising a few of the known phytochemicals since it is difficult to find suitable phytochemical criteria. Unravelling the complex chemistry of bioactive crude extracts using high throughput and high-resolution methods is crucial in order to find the pharmaceutically potent bio-actives and facilitate the process of understanding their influence on the target (Marulasiddaswamy et al. 2021).

The current study sought to investigate the phytoconstituents contained in Acanthophora najadiformis using HRLC-MS and FTIR analyses.

2. MATERIALS AND METHODS

2.1 Collection of seaweed material

The coastal seaweed Acanthophora najadiformis was procured from the Ramanathapuram shore of the Gulf of Mannar, in Tamil Nadu, on the Southeast coast of India. The seaweed sample got numerous rinses with fresh water to flush off salts, and then it had a few rinses with distilled water. The sample of washed seaweed was shade-dried to completely remove the moisture. A mixer grinder was used to produce seaweed powder from the dried sample (Duraikannu et al. 2014).

2.2 Preparation of seaweed extracts

The extraction procedure was started by adding 80% ethanol solvent to a maceration jar with dried seaweed powder. This mixture was then let to stand for 7 consecutive days while being stirred regularly before being filtered. Using the same solvent, every sample underwent a 48-hour re-maceration process. To get crude extract, filtrate was collected and then evaporated with a rotary evaporator (Sami et al. 2021).

2.3 HR-LCMS analysis of Acanthophora najadiformis

Sample was sent to the SAIF at the Indian Institute of Technology, Bombay (IIT Bombay), India, and facility was acquired for HR-LCMS analysis. With the help of HPLC, the phytochemicals present in the ethanolic extract of Acanthophora najadiformis were separated in this case on a one-dimensional basis, consisting of a guard column and an analytical column packed with Thermo Acclaim Pepmap C18 material as a stationary phase and a mixture of water- acetonitrile (A) (90%) - 0.1% formic acid in water (B) as a mobile phase at a flow rate of 100 µl/min. The extract was then subjected to dual (positive and negative) ion mode LC-ESI-MS analysis utilising a 1290 Infinity UHPLC System further connected with a 6550 iFunnel Q-TOF, Agilent Technologies, USA, where Agilent iFunnel Technology increased ion sampling and transmission by electrospraying ions and focusing them on Agilent Jet Stream Technology using a hexabore capillary sample array. This instrument's iFunnel Q-TOF Mass Spectrometer section included parameters including capillary tension 3500 V, 35-psi nebulizer gas flow pressure and gas flow rate 13 L/min at a temperature of 250 0C, sheath gas flow rate 11 L/min at a temperature of 300 0C (Patil 2020).

2.4 FT-IR analysis of Acanthophora najadiformis

The different peaks and their functional groups were identified via FTIR analysis utilising a Perkin Elmer Spectrophotometer system with a transmittance range of 400–4000 cm-1. The FTIR's peak values were noted. The results of each analysis were verified twice (Salunke et al. 2022).

3. Result and Discussion

3.1 High Resolution-Liquid Chromatography-Mass spectrometry analysis (HR-LCMS) analysis of Acanthophora najadiformis

The approximate quantities of different chemicals that are present in Acanthophora najadiformis that are eluted in accordance with the retention period are shown on a chromatogram in Figure 1. The relative concentration of the bioactive compounds discovered in the plants was measured by the peak's height. To determine the composition and structure of the compounds, the mass spectrometer examines the molecules that were eluted at various intervals. The molecule's distinct fingerprint in the data repository is represented by these mass spectra. Formic acid was added to the mobile phase to improve peak resolution. It was found that each resolved peak may belong to more than one phytocompound because of similarities in polarity and chemical characteristics due to having the same retention period. Following the separation of the constituents, Agilent iFunnel technology employed electrospray to create various ions fragments, which were then concentrated using Agilent Jet Stream technology for improved ion sampling and transmission.

In accordance with the retention duration, mass, and molecular formula, as stated in Table 1, 31 substances were determined to be present in the ethanolic extract of Acanthophora najadiformis. The primary compounds predicted belonged to several categories of secondary metabolites, including alkaloids, steroid, fatty acid, ester, phenol ethers, fatty alcohols, triterpenoids, polypeptides, glycoside, xanthenes, coumarin, flavonoid, diterpenoid, and xanthone, according to the HR-LCMS investigation and thorough literature search. Figure 2 displays the distinct mass spectra of the separated bioactives from Acanthophora najadiformis.

3.2 FT-IR analysis Acanthophora najadiformis

The existence of bioactive chemicals that were hypothesised by HR LCMS experiments was validated by FTIR analysis. As seen in Figure 3, many peaks found by FTIR analysis were used to identify the functional groups contained in the ethanol extract of Acanthophora najadiformis. Based on the peak value in the infrared radiation band, the FTIR spectrum was utilised to identify the functional group of the active components. Acanthophora najadiformis ethanolic extract's FTIR study shows distinct peaks at 3414.33 due to presence of O–H stretching of polyphenolic alcohols, peak at 3000.14 indicates C=C-C stretch of Alkenes, C–H stretch at 2915.90 due to Alkanes, Presence of Aldehydes, ketone, and ester (C=O stretch) create the peak at 1656.18, peak at 1436.58 and 1406.60 due to presence of Alkene methylene group C–H bending, peak at 1312.89 indicates C-N stretch of aromatic amines, Aliphatic amines (C-N stretch) are responsible for the peak at 1017.83, Primary & secondary amines shows peak at 952.14 and 899.45 and the peak at 701.09 and 669.57 (C-H Stretch) suggest the presence of alkyl halide.

4. Conclusion

Secondary metabolites produced from natural sources are used in the efficient approach to drug discovery and development. The current study has demonstrated that Acanthophora najadiformis may be a source of advantageous compounds, according to the phytochemical analysis. This is the first article to employ the HR-LCMS method to identify and characterise bioactive compounds from Acanthophora najadiformis.

The ethanolic extract of Acanthophora najadiformis was found to contain therapeutically important bioactive compounds such as alkaloids, steroid, fatty acid, ester, phenol ethers, fatty alcohols, triterpenoids, polypeptides, glycoside, xanthenes, coumarin, flavonoid, diterpenoid, and xanthone using FTIR analysis and HR-LCMS high-resolution liquid chromatography. According

to findings of this studies, seaweeds are a great source of biogenic chemicals that have both structural and biological activity. The ancient use of seaweeds for a range of ailments is justified by the discovery of various bioactive compounds in this inquiry. However, research is currently being done to separate the components and analyse their pharmacological effects.

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Conflict of Interest

There are no hidden conflicts of interest from the writers. This article's text and content are solely the responsibility of the authors.

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