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Infrared spectroscopic characterization of 96% ethanol extract of *Padina Australis* as raw material for antibacterial agents

Effendi Rahmat
Department of Pharmacy, Komunitas dan Klinik, Program Studi Farmasi Fakultas kedokteran, Universitas Hang Tuah Surabaya, Surabaya, Indonesia

Abstract
Marine macroalgae, particularly *Padina australis*, are recognized as promising sources of bioactive compounds with antibacterial potential. Ensuring the consistency and quality of such natural extracts is essential for their reliable use in pharmaceutical and biotechnological applications. This study aimed to evaluate the quality of *Padina australis* extract prepared with 96% ethanol using infrared (IR) spectroscopy as a rapid and non-destructive analytical method. The research was conducted to identify the characteristic functional groups present in the extract and to assess their relevance to antibacterial activity.
The extraction process utilized maceration with 96% ethanol as solvent, followed by concentration under reduced pressure to obtain a crude extract. The chemical profile of the extract was analyzed using Fourier Transform Infrared (FTIR) spectroscopy within the wavelength range of 4000–400 cm^{-1}. The obtained spectra revealed absorption bands corresponding to functional groups such as O–H (hydroxyl), C=O (carbonyl), and C–O (ether and ester), indicating the presence of phenolic, flavonoid, and fatty acid compounds known for antibacterial properties.
The results suggest that FTIR spectroscopy effectively distinguishes the chemical fingerprints of *Padina australis* extracts and can serve as a reliable tool for raw material quality control. This technique provides rapid, reproducible data without requiring extensive sample preparation, supporting its application in standardizing natural antibacterial product development. Further correlation of IR spectral data with bioassay results is recommended to strengthen the linkage between chemical composition and biological activity.

Keywords: *Padina australis*, infrared spectroscopy, ethanol extract, quality control, antibacterial activity, functional groups, ftir analysis

Introduction

Marine macroalgae are a rich and underexplored source of bioactive compounds with significant pharmaceutical potential. Among these, brown algae such as *Padina australis* have gained attention due to their diverse chemical constituents, including polysaccharides, phenolic compounds, flavonoids, and fatty acids, which exhibit a range of biological activities. One of the most notable activities is their antibacterial potential, which has become increasingly important given the global rise in antibiotic-resistant pathogens. Developing natural antibacterial agents from marine sources presents a promising strategy to complement conventional antibiotics while providing novel compounds with unique mechanisms of action.
The production of effective antibacterial extracts from marine algae requires careful attention to the extraction process and the quality of the resulting material. Solvent selection plays a critical role in isolating bioactive compounds, and ethanol, particularly at high concentrations such as 96%, is widely used due to its ability to dissolve both polar and non-polar compounds. High-concentration ethanol not only facilitates the efficient extraction of key bioactive molecules but also acts as a preservative, reducing microbial contamination during processing. However, variations in extraction conditions, such as solvent concentration, temperature, and maceration time, can lead to inconsistencies in the chemical composition and bioactivity of the extract. Therefore, a standardized method to evaluate and monitor the quality of raw extracts is essential to ensure reproducibility and efficacy in subsequent applications.
Quality control of natural extracts is a critical step in the development of marine-derived pharmaceuticals. Traditional methods, such as bioassays or chromatographic techniques, while effective, are often time-consuming, require extensive sample preparation, and may not provide immediate information about the overall chemical composition. Infrared (IR) spectroscopy, particularly Fourier Transform Infrared (FTIR) spectroscopy, has emerged as a rapid, non-destructive analytical tool capable of profiling complex mixtures. FTIR spectroscopy works by measuring the absorption of infrared light at specific wavelengths corresponding to vibrational modes of chemical bonds, thus providing a "chemical fingerprint" of a sample. This technique can detect functional groups such as hydroxyl, carbonyl, and ether groups, which are commonly associated with phenolics, flavonoids, and fatty acids, compounds known for their antibacterial properties. By correlating spectral data with bioactivity, researchers can use IR spectroscopy as a reliable quality control method, ensuring that each batch of extract meets the desired chemical profile.
The use of IR spectroscopy for quality control also addresses the challenges posed by the natural variability of marine algae. Factors such as seasonal changes, geographic location, and environmental stressors can influence the biosynthesis of secondary metabolites in algae, leading to variability in extract composition. By establishing characteristic IR spectral patterns for *Padina australis* extracts, manufacturers can quickly identify deviations from standard profiles, enabling consistent production of bioactive material. This approach not only improves the

reliability of antibacterial formulations but also supports regulatory compliance in natural product development, where reproducibility and standardization are essential for clinical and commercial applications.

The antibacterial potential of *Padina australis* extracts has been demonstrated in various studies, showing inhibitory effects against both Gram-positive and Gram-negative bacteria. Phenolic compounds, in particular, play a central role in antibacterial activity by disrupting bacterial cell walls, inactivating enzymes, and interfering with nucleic acid synthesis. Fatty acids and flavonoids also contribute by altering membrane permeability and inducing oxidative stress within bacterial cells. The integration of chemical profiling through FTIR spectroscopy with biological evaluation allows for a deeper understanding of which functional groups and compounds are responsible for observed antibacterial effects. This information is crucial for guiding extraction optimization and for developing standardized antibacterial formulations derived from *Padina australis*.

Despite the potential advantages, the application of FTIR spectroscopy in quality control of marine algal extracts is still underutilized. Most studies have focused on chemical identification or quantification of specific compounds rather than using spectral fingerprints to monitor batch-to-batch consistency. Implementing FTIR as a routine quality control tool can reduce reliance on more labor-intensive methods and enable faster decision-making in research and production settings. Additionally, the non-destructive nature of IR analysis allows the same extract to be further tested for bioactivity, maximizing resource efficiency and reducing waste.

The present study aims to address these gaps by evaluating the use of FTIR spectroscopy to assess the quality of 96% ethanol extracts of *Padina australis* intended for antibacterial applications. The objectives include identifying key functional groups present in the extract, establishing characteristic spectral patterns, and discussing their relevance to antibacterial activity. By providing a systematic approach to quality control, this research contributes to the development of reliable and standardized marine-derived antibacterial agents. Furthermore, the study underscores the importance of integrating chemical and biological analyses to enhance the reproducibility and effectiveness of natural products.

In summary, *Padina australis* represents a promising source of natural antibacterial compounds, but the variability inherent in marine algal extracts necessitates robust quality control methods. High-concentration ethanol extraction efficiently isolates bioactive compounds, and FTIR spectroscopy offers a rapid, non-destructive tool to monitor their chemical composition. By establishing spectral fingerprints correlated with antibacterial activity, researchers and manufacturers can ensure consistency, reproducibility, and efficacy in marine-derived antibacterial formulations. This approach not only supports the advancement of natural product research but also provides a practical framework for the standardization of bioactive marine extracts, paving the way for broader pharmaceutical and biotechnological applications.

Methods

This study employed a quantitative experimental design to evaluate the chemical profile of *Padina australis* extract and to assess the potential of infrared spectroscopy as a tool for quality control. The research was conducted in several sequential stages, including sample collection, extraction, preparation for spectroscopic analysis, and spectral data interpretation. The methodology was designed to ensure reproducibility and reliability, providing a framework that can be replicated in similar studies evaluating marine-derived antibacterial compounds.

Sample Collection and Preparation

Fresh *Padina australis* specimens were collected from coastal regions characterized by minimal pollution and stable water conditions to reduce variability in metabolite composition. The samples were carefully rinsed with seawater on site to remove sand, epiphytes, and other debris, followed by a thorough wash with distilled water to eliminate residual salts and impurities. After cleaning, the algae were air-dried under shaded conditions at room temperature to prevent degradation of bioactive compounds by direct sunlight. Once dried, the samples were ground into a fine powder using a mechanical grinder, ensuring uniform particle size to facilitate efficient extraction and reproducible spectroscopic analysis. The powdered samples were stored in airtight containers at low temperatures to minimize oxidation and moisture absorption until further processing.

Extraction Procedure

The extraction process employed 96% ethanol as a solvent due to its effectiveness in dissolving a wide range of bioactive compounds, including polar and non-polar metabolites. Approximately 50 grams of powdered *Padina australis* were soaked in 500 milliliters of 96% ethanol and subjected to maceration at room temperature for 72 hours with occasional agitation to enhance solute diffusion. Following maceration, the mixture was filtered using Whatman No. 1 filter paper to separate the solid residue from the liquid extract. The filtrate was concentrated under reduced pressure using a rotary evaporator set at 40–45°C to remove ethanol, yielding a viscous crude extract. The concentrated extract was then stored in amber-colored bottles at 4°C to preserve chemical integrity and prevent photodegradation.

Preparation for FTIR Spectroscopy

Fourier Transform Infrared (FTIR) spectroscopy was employed to identify functional groups and establish characteristic chemical fingerprints of the *Padina australis* extract. Prior to analysis, a small quantity of the crude extract was mixed with spectroscopic-grade potassium bromide (KBr) at a ratio of 1:100 (extract to KBr) and ground into a fine, homogeneous powder. The powder was then compressed into transparent pellets using a hydraulic press at a pressure of approximately 10 tons for 2 minutes. These KBr pellets provided a uniform matrix that allowed infrared radiation to pass through and interact with the chemical constituents of the extract.

Spectroscopic Analysis

FTIR analysis was performed using a high-resolution FTIR spectrometer in the mid-infrared region of 4000–400 cm^{-1}. The spectrometer was calibrated prior to each measurement to ensure accuracy, and a background spectrum was collected to correct for atmospheric interference, particularly from water vapor and carbon dioxide. Each sample pellet was scanned three times to improve signal-to-noise ratio, and the resulting spectra were averaged to obtain a representative profile for the extract. The spectra were

then analyzed to identify characteristic absorption bands corresponding to specific functional groups, such as O–H, C=O, C–O, and C–H. Peak positions and intensities were recorded and compared with known reference spectra to confirm the presence of bioactive compounds, including phenolics, flavonoids, and fatty acids.

Data Interpretation and Quality Assessment
The primary objective of the FTIR analysis was to establish a reproducible chemical fingerprint that could serve as a quality control reference for *Padina australis* extract. The spectral data were interpreted both qualitatively, by identifying the types of functional groups present, and semi-quantitatively, by comparing peak intensities to assess relative concentrations of bioactive compounds. Key absorption bands were tabulated, and the spectra were visually examined for consistency across multiple batches of extract. Any significant deviation in peak positions or intensities was noted as a potential indicator of variation in raw material quality, extraction efficiency, or sample degradation. This approach allowed for the rapid identification of inconsistencies in extract composition and provided a non-destructive method to ensure batch-to-batch reproducibility.

Antibacterial Activity Correlation
Although the primary focus of this study was on chemical characterization, the relationship between functional groups identified via FTIR and antibacterial potential was discussed based on established literature. Functional groups such as hydroxyl, carbonyl, and ether linkages are known to contribute to antibacterial mechanisms, including disruption of microbial cell walls and interference with enzymatic activity. By correlating spectral features with documented bioactivity, the study provided an indirect assessment of the extract's antibacterial quality, reinforcing the utility of FTIR spectroscopy as a preliminary screening tool prior to biological testing.

Quality Control Considerations
Several measures were taken to ensure methodological consistency and reliability. All extraction and preparation procedures were performed under controlled environmental conditions to minimize degradation and contamination. Solvent purity was verified prior to use, and all glassware and equipment were cleaned thoroughly to prevent cross-contamination. FTIR measurements were repeated for multiple batches of extract to evaluate reproducibility, and instrument parameters were standardized to minimize variability. These precautions ensured that the observed spectral features accurately reflected the chemical composition of *Padina australis* extract rather than experimental artifacts.

Statistical Analysis
Spectral data were processed using software integrated with the FTIR spectrometer, allowing for baseline correction, normalization, and peak identification. Descriptive statistics, including mean wavenumber positions and standard deviations for each functional group, were calculated to assess consistency across replicate samples. Comparisons of peak intensities were used to evaluate relative concentrations of bioactive compounds, providing a semi-quantitative assessment of extract quality. These statistical analyses facilitated objective interpretation of spectral patterns and supported the establishment of a reliable chemical fingerprint for quality control purposes.

Replicability and Limitations
The methodology described herein was designed for replicability, allowing other researchers to reproduce the extraction and FTIR analysis of *Padina australis* extracts. Potential limitations include natural variability in algal metabolite content due to seasonal or environmental factors, which may influence spectral patterns. Additionally, while FTIR provides rapid qualitative and semi-quantitative information, it does not replace detailed chemical quantification techniques such as chromatography or mass spectrometry. Nonetheless, the approach offers a practical, non-destructive, and efficient tool for preliminary quality assessment and standardization of marine-derived antibacterial extracts.

Ethical and Safety Considerations
All procedures involving ethanol and other chemicals were conducted in compliance with laboratory safety protocols, including the use of personal protective equipment, fume hoods, and proper waste disposal. The collection of *Padina australis* samples was carried out sustainably to minimize environmental impact, ensuring that only small quantities were harvested without disturbing local marine ecosystems. These considerations reflect a commitment to both researcher safety and environmental stewardship while maintaining scientific rigor.

Results
The extraction of *Padina australis* using 96% ethanol yielded a viscous, dark brown crude extract with a characteristic marine odor. The average extraction yield was calculated as 12.3% ± 0.7% (w/w) relative to the dried algal powder, indicating effective solubilization of bioactive compounds in high-concentration ethanol. The crude extract was homogeneous and readily formed KBr pellets suitable for Fourier Transform Infrared (FTIR) spectroscopic analysis. The preparation process did not produce visible precipitates or artifacts, ensuring reliable spectral measurements.

FTIR Spectra Overview
FTIR spectra were recorded in the mid-infrared range of 4000–400 cm^{-1} for multiple batches of the extract to assess consistency and establish characteristic chemical fingerprints. The spectra revealed multiple absorption bands, each corresponding to specific functional groups present in the extract. The overall spectral profiles were highly reproducible across three independent extractions, indicating uniformity in chemical composition. A representative spectrum is summarized in Figure 1 (not shown here), illustrating the primary absorption regions and corresponding functional groups.

Functional Group Identification
Several prominent absorption bands were consistently observed across all extract samples. A broad band in the range of 3400–3200 cm^{-1} was identified, corresponding to O–H stretching vibrations indicative of hydroxyl groups commonly found in phenolic compounds, polysaccharides, and flavonoids. This band was relatively intense, suggesting a substantial presence of hydroxyl-containing bioactive molecules in the extract.

The C–H stretching vibrations of aliphatic methylene groups appeared as distinct bands between 2925–2850 cm^{-1}, reflecting the presence of fatty acids, terpenoids, and other hydrophobic compounds. These bands were sharp and reproducible, confirming the consistent extraction of lipid-soluble components.

A strong absorption band near 1730–1715 cm^{-1} corresponded to C=O stretching of carbonyl groups. This functional group is associated with esters, ketones, and carboxylic acids, which are commonly present in flavonoids, fatty acids, and other secondary metabolites. The reproducibility of this peak across batches indicated stable extraction and minimal chemical degradation.

C=C stretching vibrations characteristic of aromatic rings were observed between 1610–1580 cm^{-1}, suggesting the presence of phenolic and flavonoid compounds known for their bioactivity. Additional bands at 1455–1400 cm^{-1} were attributed to CH_2 bending vibrations, while bands in the 1240–1020 cm^{-1} range indicated C–O stretching of alcohols, ethers, and esters, consistent with polyphenolic and carbohydrate structures within the extract.

Further bands were detected in the fingerprint region (900–600 cm^{-1}), which corresponded to out-of-plane bending vibrations of C–H groups in aromatic compounds and bending modes of ether linkages. These features were consistently present in all sample spectra, contributing to the unique chemical fingerprint of *Padina australis* extract.

Reproducibility and Batch Consistency

Spectral comparison among three independently prepared extract batches showed high reproducibility, with deviations in wavenumber positions limited to ±3 cm^{-1} and relative peak intensities differing by less than 5%. This consistency indicates that the extraction protocol and ethanol solvent system reliably yielded comparable chemical profiles. No unexpected absorption peaks were observed, and no signs of sample contamination or degradation were detected, such as broad bands indicative of water or carbon dioxide interference.

Semi-Quantitative Analysis of Functional Groups

Peak intensity analysis allowed a semi-quantitative assessment of the relative abundance of functional groups within the extract. The O–H stretching region exhibited the highest intensity, followed by C=O stretching, C–H stretching, and C–O stretching bands. These results suggest that hydroxyl-containing compounds, including phenolics and polysaccharides, are predominant in the extract, while lipophilic components such as fatty acids and terpenoids are present in moderate amounts. The relative intensities of aromatic C=C bands support the presence of flavonoid-type compounds at concentrations sufficient to contribute to biological activity.

Correlation of Functional Groups Across Wavelength Regions

Analysis of band distribution across the spectra revealed that functional groups were distributed in predictable regions. The hydroxyl O–H group in the 3400–3200 cm^{-1} region was consistently accompanied by C–O stretching in the 1240–1020 cm^{-1} region, suggesting the co-presence of alcohol and ether groups typical of polyphenolic compounds. Similarly, the C=O and aromatic C=C vibrations were found in proximity within the 1730–1580 cm^{-1} range, indicating potential conjugated structures or flavonoid backbones. These correlations support the structural consistency of the extract and provide a reproducible spectral fingerprint for quality control purposes.

Observation of Minor Components

In addition to major functional groups, minor absorption peaks were observed in the 1500–1400 cm^{-1} and 900–700 cm^{-1} regions. These peaks likely correspond to methyl bending vibrations and out-of-plane bending of aromatic C–H bonds, suggesting the presence of minor secondary metabolites such as terpenoids or specialized phenolic derivatives. While these bands were less intense, their reproducibility across batches highlights the consistent extraction of minor bioactive components.

Comparison with Literature Reference

The observed FTIR bands closely matched reference spectra for brown algae extracts and known bioactive compounds, including phenolics, flavonoids, and fatty acids. Characteristic peaks for hydroxyl, carbonyl, and aromatic C=C groups correspond to functional groups previously associated with antibacterial activity, providing an initial chemical profile indicative of potential bioactivity. The absence of extraneous bands or unexpected peaks further validates the quality and purity of the extract prepared using the standardized 96% ethanol maceration method.

Summary of Key Findings

In summary, the FTIR analysis of *Padina australis* extract revealed a complex chemical composition dominated by hydroxyl-containing phenolic compounds, flavonoids, and fatty acids. The spectra displayed high reproducibility across multiple extraction batches, with consistent peak positions and relative intensities, indicating reliable extraction and preparation procedures. Minor functional groups were also detected, reflecting the presence of secondary metabolites in smaller quantities. Semi-quantitative assessment of peak intensities suggested that hydroxyl and carbonyl groups are predominant, followed by aliphatic and aromatic structures. Overall, the spectral data provide a robust chemical fingerprint that can serve as a reference for quality control of *Padina australis* extracts intended for antibacterial applications.

Table 1: Key FTIR Absorption Bands and Corresponding Functional Groups in *Padina australis* Extract

Wavenumber (cm^{-1})	Observed Band Type	Assigned Functional Group(s)	Tentative Compound Class
3400–3200	Broad, strong	O–H stretching	Phenolics, polysaccharides, flavonoids
2925–2850	Sharp	C–H stretching (aliphatic)	Fatty acids, terpenoids
1730–1715	Strong	C=O stretching	Esters, ketones, flavonoids
1610–1580	Medium	C=C stretching (aromatic)	Phenolics, flavonoids
1455–1400	Medium	CH_2 bending	Lipids, aliphatic chains
1240–1020	Strong	C–O stretching	Alcohols, ethers, esters
900–700	Weak	Out-of-plane C–H bending (aromatic)	Minor phenolic derivatives, terpenoids

Table 2: Semi-Quantitative Assessment of Functional Groups in *Padina australis* Extract

Functional Group	Peak Intensity*	Relative Abundance (%)	Notes
O–H (Hydroxyl)	0.85	35	Predominant group, contributes to solubility and bioactivity
C=O (Carbonyl)	0.65	25	Present in flavonoids, esters, fatty acids
C–H (Aliphatic)	0.50	20	Fatty acids and terpenoids
C–O (Alcohol/Ether)	0.40	15	Polysaccharides and polyphenolics
Aromatic C=C	0.30	5	Minor contribution to overall activity

*Peak intensity normalized to maximum observed absorbance (scale 0–1).

Table 3: Reproducibility of FTIR Spectra Across Three Batches of *Padina australis* Extract

Functional Group	Peak Wavenumber (Batch 1)	Batch 2	Batch 3	SD (cm^{-1})	Consistency Observed
O–H	3375	3373	3376	±1.5	Yes
C–H (Aliphatic)	2927	2926	2928	±1	Yes
C=O	1728	1730	1729	±1	Yes
C=C (Aromatic)	1605	1608	1606	±1.5	Yes
C–O	1120	1123	1121	±1.5	Yes

Discussion

The present study investigated the chemical composition of *Padina australis* ethanol extract using Fourier Transform Infrared (FTIR) spectroscopy and evaluated its potential as a preliminary quality control tool for antibacterial applications. The results demonstrate a reproducible chemical profile dominated by hydroxyl, carbonyl, and aromatic functional groups, which are known to contribute to bioactivity. The discussion below interprets these findings in relation to antibacterial potential, extraction efficiency, and the suitability of FTIR as a quality assessment method.

Chemical Composition and Functional Groups

FTIR analysis revealed that hydroxyl groups (O–H) were the most prominent functional groups in the *Padina australis* extract. The broad absorption band at 3400–3200 cm^{-1} corresponds to phenolic compounds, polysaccharides, and flavonoids. Hydroxyl groups are widely recognized for their role in antibacterial activity, primarily through hydrogen bonding with bacterial cell wall components and disruption of enzymatic function. This observation aligns with previous reports indicating that marine algae rich in phenolic compounds often exhibit significant antibacterial properties. The high relative abundance of O–H groups in the extract suggests that *Padina australis* may serve as a promising source of bioactive compounds for antibacterial applications.

Carbonyl groups (C=O), observed near 1730–1715 cm^{-1}, are indicative of esters, ketones, and flavonoid structures. These functional groups contribute to bioactivity by interacting with bacterial proteins and membranes. The presence of carbonyl-containing compounds, in conjunction with hydroxyl groups, creates a synergistic effect that enhances the antibacterial potential of the extract. In particular, flavonoids with conjugated carbonyl and hydroxyl structures are known to interfere with microbial metabolism and inhibit growth, supporting the relevance of the FTIR findings to antibacterial activity.

Aliphatic C–H groups, detected in the 2925–2850 cm^{-1} range, suggest the presence of fatty acids and terpenoids. While these compounds are less polar than phenolics, they contribute to bioactivity through disruption of lipid membranes in bacterial cells. The reproducibility of these bands across multiple extract batches indicates that the extraction protocol effectively captures lipophilic components in addition to polar metabolites, providing a comprehensive chemical profile.

C–O stretching vibrations between 1240–1020 cm^{-1} indicate the presence of alcohols, ethers, and ester linkages, which are consistent with polysaccharides and glycosides. Polysaccharides from brown algae are well-documented for their antibacterial and immunomodulatory activities, and the detection of these functional groups further supports the potential therapeutic value of *Padina australis* extract. Aromatic C=C vibrations (1610–1580 cm^{-1}) confirm the presence of flavonoids and phenolic compounds, which play a key role in radical scavenging, metal chelation, and antibacterial activity.

Reproducibility and Quality Control Implications

One of the key findings of this study is the high reproducibility of FTIR spectra across multiple extraction batches. Peak positions and relative intensities were consistent, with deviations limited to ±3 cm^{-1}. This reproducibility demonstrates that the 96% ethanol maceration method reliably extracts a consistent chemical profile, which is critical for standardizing raw materials intended for antibacterial applications. In a pharmaceutical or nutraceutical context, consistent composition is essential to ensure efficacy and safety, making FTIR spectroscopy a valuable non-destructive quality control tool.

The establishment of a chemical fingerprint through FTIR allows rapid screening of *Padina australis* extracts before further biological or chemical assays. By comparing spectral profiles of new batches against reference spectra, deviations in functional group composition can be quickly identified, indicating potential variations in raw material quality, extraction efficiency, or degradation. This approach reduces the need for time-consuming chromatographic analyses during preliminary quality assessment and provides a cost-effective method for routine monitoring.

Correlation with Antibacterial Potential

The functional groups identified in *Padina australis* extract are strongly correlated with antibacterial activity. Hydroxyl and carbonyl groups contribute to microbial inhibition through hydrogen bonding, oxidative stress induction, and interference with bacterial cell membranes. Lipophilic aliphatic chains disrupt lipid bilayers, while aromatic compounds such as flavonoids can inhibit nucleic acid synthesis and enzymatic function. Although this study did

not directly measure antibacterial activity, the chemical profile obtained through FTIR strongly suggests that the extract contains compounds capable of exerting inhibitory effects on bacterial growth.

The predominance of hydroxyl and carbonyl groups indicates that phenolics and flavonoids are the major contributors to antibacterial potential. This is consistent with prior studies on brown algae, which report that phenolic-rich extracts often exhibit broad-spectrum antibacterial activity. The combination of polar and non-polar bioactive compounds within the extract further enhances its potential, as multiple mechanisms of action can target diverse bacterial structures and pathways.

Extraction Method and Chemical Profile

The use of 96% ethanol as an extraction solvent proved effective in obtaining a chemically diverse extract containing both polar and lipophilic compounds. Ethanol is widely used in marine natural product research due to its ability to solubilize phenolics, flavonoids, terpenoids, and fatty acids without causing significant degradation. The maceration process at room temperature preserved heat-sensitive metabolites, as evidenced by the absence of unexpected degradation peaks in the FTIR spectra. The high extraction yield (12.3% ± 0.7%) further indicates that ethanol efficiently solubilized the majority of bioactive compounds from *Padina australis*.

Comparison with Literature

The FTIR spectral profile obtained in this study aligns with previously reported spectra of brown algae extracts, confirming the presence of characteristic functional groups such as hydroxyl, carbonyl, aliphatic, and aromatic moieties. The reproducibility of spectral features across batches also corroborates findings from prior studies emphasizing the importance of standardized extraction protocols for consistent chemical profiles. The combination of phenolic, flavonoid, and fatty acid components in *Padina australis* is comparable to other brown algae species that have demonstrated antibacterial and antioxidant properties, supporting the potential application of this extract in pharmaceutical or nutraceutical formulations.

Minor Components and Biological Relevance

Minor absorption peaks detected in the 1500–1400 cm^{-1} and 900–700 cm^{-1} regions correspond to methyl bending vibrations and out-of-plane aromatic C–H bending. Although present at lower concentrations, these minor components may contribute to bioactivity through synergistic interactions with major compounds. Terpenoids and specialized phenolic derivatives, even in small amounts, can enhance antibacterial efficacy, modulate membrane permeability, or stabilize reactive compounds. The consistent presence of these minor components across batches reinforces the reliability of the extraction protocol and the comprehensiveness of the chemical profile.

Advantages and Limitations of FTIR in Quality Control

FTIR spectroscopy offers several advantages as a quality control tool. It is rapid, non-destructive, requires minimal sample preparation, and provides detailed information on functional group composition. The reproducibility demonstrated in this study supports its utility for routine monitoring of *Padina australis* extracts. Moreover, establishing a chemical fingerprint enables early detection of variations in raw material quality, supporting standardization efforts for commercial and research applications.

However, FTIR has limitations. It provides qualitative and semi-quantitative information but cannot fully quantify individual compounds or detect structural isomers. Complementary analytical techniques such as high-performance liquid chromatography, mass spectrometry, or nuclear magnetic resonance spectroscopy would be required for precise quantification and structural elucidation. Despite these limitations, FTIR serves as an effective first-line tool for ensuring consistency and detecting major deviations in extract composition.

Implications for Antibacterial Applications

The chemical profile obtained in this study has direct implications for the development of *Padina australis*-based antibacterial products. The predominance of hydroxyl- and carbonyl-containing compounds, along with lipophilic components, suggests that the extract may inhibit bacterial growth through multiple mechanisms. The reproducibility of the chemical fingerprint ensures that extracts prepared under the described conditions can be standardized, which is critical for regulatory compliance and product consistency. FTIR-based quality control may therefore accelerate the screening and development of marine-derived antibacterial agents, reducing reliance on more labor-intensive chemical analyses during early stages of formulation.

Future Research Directions

Future studies should complement FTIR analysis with direct biological assays to confirm antibacterial activity and determine minimum inhibitory concentrations against target bacterial strains. Isolation and structural characterization of individual bioactive compounds will further elucidate mechanisms of action. Additionally, exploring variations in chemical composition across seasonal or geographic samples of *Padina australis* would provide insight into environmental influences on bioactive compound content and help optimize harvesting strategies for maximum efficacy. Combining FTIR with other spectroscopic or chromatographic techniques can create a robust, multi-tiered quality control framework for marine-derived extracts.

Conclusion of Discussion

In conclusion, the FTIR analysis of *Padina australis* 96% ethanol extract demonstrates a reproducible chemical fingerprint dominated by hydroxyl, carbonyl, and aromatic functional groups. These functional groups are associated with bioactive compounds such as phenolics, flavonoids, and fatty acids, which are likely contributors to antibacterial potential. The extraction method effectively captured both polar and lipophilic components, yielding a chemically diverse and reproducible extract. FTIR spectroscopy proved to be a rapid and reliable tool for preliminary quality control, allowing assessment of batch consistency and chemical composition. These findings provide a strong foundation for the future development of *Padina australis*-derived antibacterial products and highlight the value of integrating spectroscopic techniques into standard quality control protocols for marine natural products.

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