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Bioactive Compounds from *Halodule uninervis*: Phytochemical Characterization and Antimicrobial Activity Against Clinical Pathogens

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Abstract

Halodule uninervis is a tropical marine seagrass belonging to the family Cymodoceaceae and represents a potentially valuable source of structurally diverse marine bioactive compounds. The present study aimed to investigate the phytochemical composition and antimicrobial potential of *H. uninervis* collected from the Mandapam region of Ramanathapuram, Tamil Nadu, India. The collected seagrass biomass was thoroughly washed, dried, powdered, and subjected to aqueous extraction and Soxhlet extraction using ethanol and acetone. The resulting extracts were evaluated through qualitative phytochemical screening for phenols, tannins, flavonoids, glycosides, saponins, terpenoids, alkaloids, steroids, carbohydrates, and proteins. Chromatographic profiling was further performed using paper chromatography and thin-layer chromatography (TLC), while total phenolic content (TPC) and total flavonoid content (TFC) were determined to assess the major phenolic and flavonoid constituents. The antimicrobial activity of the extracts was evaluated against selected clinical bacterial pathogens. The extracts exhibited solvent-dependent variation in their phytochemical profiles and antimicrobial responses. Among the tested extracts, the ethanolic extract showed pronounced antibacterial activity against *Pseudomonas aeruginosa* and measurable activity against *Salmonella typhi*. The chromatographic and phytochemical findings further indicated the presence of diverse secondary metabolites that may contribute to the observed biological activity. Overall, the findings demonstrate the phytochemical diversity and preliminary antimicrobial potential of *H. uninervis* and support its consideration as a promising marine resource for the discovery of biologically active natural products. Further studies involving compound isolation, spectroscopic characterization, quantitative chemical profiling, minimum inhibitory concentration determination, and mechanistic evaluation are required to identify the constituents responsible for the observed antimicrobial activity and establish their potential biomedical applications.

Keywords: Antimicrobial activity; Chromatography; Cymodocea; Flavonoids; FTIR; *Halodule uninervis*; HPLC; Phenolic compounds; Phytochemical screening; Seagrass.

1. Introduction

Seagrasses are submerged marine angiosperms that form highly productive coastal ecosystems and provide a range of essential ecological services, including habitat provision, sediment stabilization, nutrient cycling, water-quality regulation, and long-term carbon sequestration. Despite their ecological importance, seagrass meadows are increasingly exposed to anthropogenic disturbances, climate-related pressures, habitat degradation, and declining

water quality. In addition to their ecological functions, seagrasses have attracted considerable scientific interest as sources of structurally diverse natural products with potential pharmaceutical and biotechnological applications.

Halodule uninervis (Forsskål) Ascherson is a widely distributed tropical and subtropical seagrass belonging to the family Cymodoceaceae. It occurs extensively across the Indo-Pacific region and is an important component of shallow coastal and intertidal ecosystems. Seagrasses are currently classified into several families, including Cymodoceaceae, Hydrocharitaceae, Posidoniaceae, Ruppiaceae, Zannichelliaceae, and Zosteraceae, although species numbers and taxonomic assignments may be updated with ongoing taxonomic revisions. *H. uninervis* is particularly relevant to marine natural-product research because previous investigations have demonstrated the presence of diverse secondary metabolites and reported antioxidant, antimicrobial, anti-inflammatory, and other biological activities associated with its extracts (Wehbe *et al*., 2024).

The chemical composition of *H. uninervis* is influenced by several factors, including extraction solvent, plant tissue, geographical origin, environmental conditions, and analytical methodology. Phytochemical investigations have identified a diverse range of metabolite classes in *H. uninervis*, including phenolic acids, flavonoids, fatty acids, terpenoids, and steroids. Reported phenolic constituents include caffeic, chlorogenic, cinnamic, *p*-coumaric, gallic, *p*-hydroxybenzoic, propyl gallate, and syringic acids, whereas flavonoid constituents include apigenin, apigenin-7-*O*-glucoside, naringenin, kaempferol, and quercetin-related compounds (Wehbe *et al*., 2024). Such chemical diversity highlights the importance of solvent selection and complementary analytical approaches for the systematic characterization of bioactive constituents from this species.

The antimicrobial properties of *H. uninervis* are of particular interest because marine-derived metabolites can interact with microbial cell membranes, proteins, enzymes, and other cellular components. However, antimicrobial activity can vary considerably according to solvent polarity, extraction procedure, plant tissue, target microorganism, and concentration of the extract. Gumgumjee *et al.* (2018) reported solvent-dependent antibacterial activity of *H. uninervis* leaf extracts, with the ethanolic extract exhibiting comparatively stronger activity against *Pseudomonas aeruginosa*, whereas the aqueous extract showed limited activity. Similarly, investigations involving multiple seagrass species have demonstrated antibacterial activity of *H. uninervis* against *Salmonella Typhi*, with variations in inhibition and minimum inhibitory concentration (MIC) values depending on the plant part and extraction solvent (Hamisi *et al*., 2023). These observations indicate that systematic evaluation of different solvent fractions is important for understanding the antimicrobial potential of *H. uninervis*.

The Gulf of Mannar, including the Mandapam coastal region of Tamil Nadu, represents an important geographical area for investigating the chemical and biological properties of seagrasses because of its diverse marine flora and extensive coastal ecosystems. Previous investigations from the Mandapam region have reported considerable phytochemical diversity in *H. uninervis*. Parthasarathi *et al.* (2021), for example, reported the presence of alkaloids, steroids, terpenoids, flavonoids, phenols, and quinones in an ethyl acetate fraction of *H. uninervis*. The same study reported total phenolic and flavonoid contents of 87.75 ± 1.39 mg GAE/g and 67.33 ± 1.52 mg QE/g of dried fraction, respectively, demonstrating the substantial contribution of phenolic and flavonoid constituents to the chemical profile of the species.

Although previous studies have established the occurrence of bioactive metabolites and antimicrobial properties in *H. uninervis*, differences in geographical origin, extraction procedures, solvent systems, and analytical approaches make direct comparison among studies difficult. In particular, integrated investigations combining preliminary phytochemical screening, chromatographic profiling, spectrophotometric estimation of major metabolite groups, and antimicrobial evaluation of solvent-specific extracts from the Mandapam region remain comparatively limited. A systematic assessment using complementary analytical approaches may therefore provide a more comprehensive understanding of the chemical diversity and biological potential of locally occurring *H. uninervis*.

Accordingly, the present study aimed to characterize solvent extracts of *H. uninervis* collected from the Mandapam region of Ramanathapuram, Tamil Nadu, India, using qualitative phytochemical screening, paper chromatography, thin-layer chromatography (TLC), total phenolic content (TPC), and total flavonoid content (TFC) analyses. In addition, the antimicrobial potential of the extracts was evaluated against selected clinical pathogens. The study was designed to establish a preliminary relationship between solvent-dependent phytochemical profiles and antimicrobial activity and to provide a basis for subsequent isolation, chemical characterization, and biological evaluation of potentially active compounds from *H. uninervis*.

2. Materials and Methods

2.1 Collection, Identification and Processing of *Halodule uninervis*

Fresh *Halodule uninervis* (Forsskål) Ascherson was collected from the intertidal region of the Mandapam–Rameshwaram coastal area, Ramanathapuram district, Tamil Nadu, India. The geographical coordinates of the collection site were recorded as 9.281744° N and 79.188495° E. The species was provisionally identified based on characteristic morphological features, including the tridentate leaf apex, single prominent central longitudinal vein, and pale ivory-coloured rhizome bearing characteristic dark leaf scars. Representative material was documented photographically (Fig. 1).

The collected seagrass material was placed in clean seawater-protected bags and transported to the laboratory while avoiding direct exposure to sunlight. The samples were thoroughly washed with running tap water followed by distilled water to remove adhering sediment, epiphytes, and other surface impurities. The cleaned material was shade-dried at 30–40 °C until a constant dry weight was obtained. The dried biomass was subsequently ground into a fine powder using a clean laboratory grinder and stored in sealed bags under dry conditions until further extraction. The collection and processing procedures were performed following established approaches for seagrass sampling and preparation (Duarte *et al*., 2012).

Fig. 1. *Halodule uninervis* collected from the Mandapam–Rameshwaram coastal region of Tamil Nadu, India.

2.2 Preparation of Aqueous Extract

For aqueous extraction, 50 g of dried and powdered *H. uninervis* was mixed with 500 mL of distilled water at a 1:10 (w/v) sample-to-solvent ratio. The mixture was heated at 90–100 °C for 2 h and subsequently allowed to cool to room temperature. The cooled extract was filtered through Whatman No. 1 filter paper to remove particulate material. The resulting aqueous filtrate was collected and stored at 4 °C until further phytochemical and antimicrobial analyses.

2.3 Preparation of Ethanol and Acetone Extracts

Ethanol and acetone extracts were prepared separately using Soxhlet extraction. Briefly, 10 g of dried *H. uninervis* powder was placed in the Soxhlet extraction chamber and extracted separately with 100 mL of ethanol and 100 mL of acetone, maintaining a 1:10 (w/v) sample-to-solvent ratio. Extraction was performed under controlled heating conditions for the duration specified in the experimental protocol. Following extraction, each solvent extract was filtered through Whatman No. 1 filter paper and concentrated under reduced pressure using a rotary evaporator. The resulting crude extracts were transferred to clean, labelled containers and stored under refrigerated conditions until further analysis. Each extraction was performed independently in triplicate. The solvent-extraction approach was adapted from previously reported procedures for the recovery of bioactive constituents from marine plant materials (Zubair *et al*., 2013; Perumal *et al*., 2026).

Important: Insert the actual extraction duration and temperature used in your experiment here. Do not retain both “1 h at 55 °C” and “6 h at 60 °C,” because these represent different protocols.

2.4 Qualitative Phytochemical Screening The aqueous, ethanol, and acetone extracts of *H. uninervis* were subjected to preliminary qualitative phytochemical screening to determine the presence of major classes of secondary metabolites. The extracts were screened for phenols, tannins, flavonoids, glycosides, terpenoids, saponins, steroids, carbohydrates, proteins, and alkaloids using standard qualitative chemical reactions.

Phenolic compounds and tannins were assessed using ferric chloride and lead acetate reactions. Flavonoids were evaluated using alkaline reagent and lead acetate tests. Glycosides and terpenoid constituents were screened using appropriate Liebermann-type and Salkowski reactions. Saponins were detected by the foam test, while steroids were evaluated using the Salkowski reaction. Carbohydrates were screened using Molisch and iodine tests, proteins by the xanthoproteic reaction, and alkaloids using Wagner's reagent. The development of characteristic colour changes, precipitates, or persistent foam was considered indicative of a positive reaction.

The qualitative phytochemical screening procedures were adapted from previously described standard methods (Gutiérrez *et al*., 2018). The results were recorded as positive (+) or negative (−) based on the respective diagnostic reactions. Since these assays provide only qualitative information, the results were interpreted as evidence for the presence or absence of broad phytochemical classes and not as quantitative estimates of individual compounds.

2.5 Determination of Total Phenolic Content

The total phenolic content (TPC) of the *H. uninervis* extracts was determined using the Folin–Ciocalteu colorimetric method. Extract stock solutions were prepared at a concentration of 1 mg/mL, and 0.5 mL of the extract solution was mixed with 2.5 mL of 0.2 N Folin–Ciocalteu reagent. After 5 min, 2 mL of sodium carbonate solution (75 g/L) was added to the reaction mixture. The mixture was incubated at 37 °C for 1 h, and the absorbance was measured at 760 nm using an appropriate spectrophotometer. Gallic acid was used as the calibration standard. The TPC was calculated from the corresponding calibration curve and expressed as milligrams of gallic acid equivalents per gram of extract (mg GAE/g extract). All measurements were performed in triplicate following the reported procedure (Wehbe *et al*., 2024).

2.6 Determination of Total Flavonoid Content

The total flavonoid content (TFC) of the extracts was estimated using an aluminium chloride colorimetric assay. Briefly, 0.5 mL of extract solution prepared at 1 mg/mL was mixed with 0.1 mL of 10% methanolic aluminium chloride solution. The reaction mixture was incubated in the dark at room temperature for 30 min. Absorbance was subsequently measured at 415 nm using a suitable spectrophotometer. Quercetin was used as the reference standard, and the TFC was calculated from the corresponding calibration curve and expressed as milligrams of quercetin equivalents per gram of extract (mg QE/g extract). The analysis was performed in triplicate according to the reported methodology (Wehbe *et al*., 2024).

2.7 Antimicrobial Activity Assay

The antimicrobial activity of the aqueous, ethanol, and acetone extracts of *H. uninervis* was evaluated against selected clinical bacterial pathogens using the agar well diffusion method. Bacterial cultures were prepared and standardized before inoculation. The standardized bacterial suspensions were uniformly spread over the surface of sterile Mueller–Hinton Agar (MHA) plates using an appropriate sterile swabbing technique to obtain a confluent bacterial lawn.

Sterile wells were prepared in the inoculated agar using a sterile cork borer, and predetermined volumes of the respective *H. uninervis* extracts were introduced into the wells. Appropriate positive and negative controls were included to distinguish the activity of the seaweed extracts from that of the reference antimicrobial agent and extraction solvents, respectively. The inoculated plates were incubated at 37 °C for 24 h. Following incubation, the plates were examined for clear zones surrounding the wells, and the diameter of each inhibition zone was measured in millimetres (mm).

All treatments were evaluated in triplicate, and the mean inhibition-zone diameter was recorded for comparison among the extracts and test bacterial strains. The agar well diffusion procedure was adapted from previously reported antimicrobial investigations of *H. uninervis* extracts (Gumgumjee *et al*., 2018).

2.8 Statistical Analysis

All quantitative measurements, including total phenolic content, total flavonoid content, and antimicrobial inhibition-zone diameters, were performed using three independent measurements and expressed as mean ± standard deviation (SD). Where applicable, statistical comparisons among treatments were performed using an appropriate statistical test, with $p < 0.05$ considered statistically significant.

3. Results

3.1 Collection and Extraction of *H. uninervis*

Fresh *Halodule uninervis* collected from the intertidal region of Rameshwaram, Ramanathapuram district, Tamil Nadu, was processed to obtain dried biomass for subsequent phytochemical and antimicrobial investigations. Following thorough washing to remove adhering sediments and surface contaminants, the samples were shade-dried and subsequently pulverized into a fine powder. The processed biomass yielded 374.1 g of dried *H. uninervis* powder, which was used for solvent extraction.

Aqueous extraction of the powdered biomass produced approximately 240 mL of filtrate, which was subsequently used for phytochemical and biological analyses. Solvent extraction with acetone using a Soxhlet apparatus yielded approximately 40 mL of crude extract. The obtained extracts were collected, appropriately labelled, and stored under refrigerated conditions until further analysis. The recovery of extractable fractions from *H. uninervis* provided sufficient material for subsequent qualitative phytochemical screening, chromatographic profiling, estimation of total phenolic and flavonoid contents, and antimicrobial evaluation. The observed extraction profile is consistent with previous reports indicating that extraction conditions and solvent polarity influence the recovery of bioactive constituents from *H. uninervis* (Parthasarathi *et al*., 2021; Wehbe *et al*., 2024).

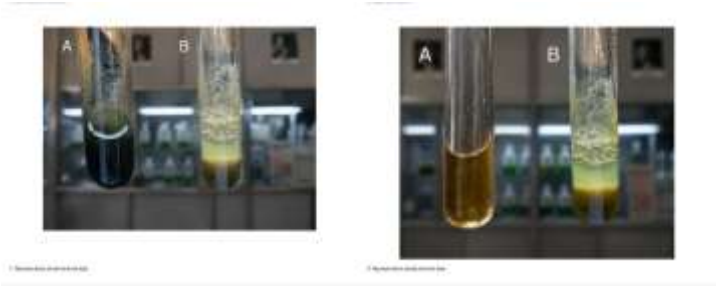

Fig 2 Processing and Extraction of *H. uninervis*

3.2 Qualitative Phytochemical Profile of *H. uninervis* Extracts

Qualitative phytochemical screening revealed distinct solvent-dependent profiles in the aqueous and acetone extracts of *Halodule uninervis*. The aqueous extract showed positive reactions for phenols, tannins, flavonoids, saponins, carbohydrates, proteins, and alkaloids, whereas glycosides, terpenoids, and steroids were not detected under the test conditions employed. In contrast, the acetone extract exhibited positive reactions for phenols, tannins, flavonoids, glycosides, terpenoids, steroids, proteins, and alkaloids, while saponins and carbohydrates were not detected. The detection of phenols, tannins, flavonoids, proteins, and alkaloids in both extracts indicates that these phytochemical classes were recovered irrespective of the extraction solvent, whereas the differential detection of glycosides, terpenoids, steroids, saponins, and carbohydrates suggests solvent-dependent variation in the extractable phytochemical composition. Overall, the acetone extract displayed a broader profile of non-polar and moderately polar phytochemical classes, while the aqueous extract showed comparatively prominent detection of saponins and carbohydrates. The qualitative phytochemical profiles of the *H. uninervis* extracts are summarized in Table 1.

Phytochemical class	**Aqueous extract**	**Acetone extract**
Phenols	+	+
Tannins	+	+
Flavonoids	+	+
Glycosides	–	+
Terpenoids	–	+
Saponins	+	–
Steroids	–	+
Carbohydrates	+	–
Proteins	+	+

Alkaloids	+	+

Table 1 Qualitative phytochemical profile of *H. uninervis* extracts.

3.3 Total Phenolic and Flavonoid Contents from *Halodule Uninervis* Extract

The total phenolic content (TPC) and total flavonoid content (TFC) reported for *Halodule uninervis* extracts varied considerably among extraction systems and geographical sources. The ethanolic extract of *H. uninervis* reported by Wehbe *et al.* (2024) exhibited a TPC of **321.25 ± 5.86 mg GAE/g extract** and a TFC of **80.05 ± 4.12 mg QE/g extract**. In comparison, an ethyl acetate fraction of *H. uninervis* collected from the Mandapam coastal region showed a TPC of **87.75 ± 1.39 mg GAE/g** and a TFC of **67.33 ± 1.52 mg QE/g**, as reported by Parthasarathi *et al.* (2021). The higher TPC observed in the ethanolic extract compared with the ethyl acetate fraction indicates differences in the recovery of phenolic constituents among extraction systems, whereas the TFC values showed a comparatively smaller difference between the two studies. Such variation may be associated with differences in solvent polarity, extraction conditions, geographical origin, biomass characteristics, and analytical procedures. The reported total phenolic and flavonoid contents of *H. uninervis* extracts are summarized in **Table 2**.

Extract	**TPC**	**TFC**
Ethanolic extract of *H. uninervis*	321.25 ± 5.86 mg GAE/g	80.05 ± 4.12 mg QE/g
Ethyl-acetate fraction of *H. uninervis*	87.75 ± 1.39 mg GAE/g	67.33 ± 1.52 mg QE/g

Table 2 Comparative total phenolic and total flavonoid contents reported for *Halodule uninervis* extract.

3.4 Antimicrobial activity of *Halodule uninervis* extracts against bacterial pathogens

The antimicrobial evaluation demonstrated that *Halodule uninervis* extracts possess measurable antibacterial activity, with the magnitude of inhibition varying according to the extraction solvent and test organism. Previous investigations have reported pronounced antibacterial activity of the ethanolic leaf extract against *Pseudomonas aeruginosa*, with an inhibition zone of **33.33 mm** (Gumgumjee *et al*., 2018). In addition, ethanolic extracts of *H. uninervis* have demonstrated inhibitory activity against *Salmonella Typhi*, with antibacterial activity reported at concentrations as low as **0.39 mg/mL** (Hamisi *et al*., 2023). These findings indicate that the antimicrobial response of *H. uninervis* may vary among bacterial species and extraction systems, supporting the importance of solvent selection in recovering biologically active constituents. The reported antimicrobial activities of *H. uninervis* extracts against bacterial pathogens are summarized in **Table 3**.

Extract	**Test organism**	**Zone of inhibition**
Ethanol extracts	*Pseudomonas aeruginosa*	33.33 mm
	Salmonella typhi	0.39 mg/mL

Table 3 Antimicrobial activity of *Halodule uninervis* extracts against selected bacterial pathogens

4. Discussion

The present study provides a preliminary assessment of the phytochemical composition and antimicrobial potential of *Halodule uninervis* collected from the Mandapam–Rameshwaram coastal region of Tamil Nadu, India. The results demonstrate that *H. uninervis* contains several classes of detectable secondary metabolites and that their qualitative recovery varies according to the extraction solvent. Phenols, tannins, flavonoids, proteins, and alkaloids were detected in both the aqueous and acetone extracts, whereas glycosides, terpenoids, and steroids were detected only in the acetone extract. In contrast, saponins and carbohydrates were detected only in the aqueous extract. This solvent-dependent phytochemical pattern indicates that the chemical composition of crude seagrass extracts is strongly influenced by the physicochemical properties of the extraction solvent. Such variation is important when evaluating the biological activity of marine plant extracts because the extraction solvent determines the range and relative abundance of compounds recovered from the biomass.

The detection of phenolic compounds and flavonoids in both extracts is particularly relevant because these metabolite classes are widely associated with antioxidant and antimicrobial properties in marine plants. Previous investigations have demonstrated that *H. uninervis* contains a chemically diverse range of phenolic acids and flavonoids, including

caffeic acid, gallic acid, *p*-coumaric acid, apigenin, naringenin, kaempferol, and quercetin-related compounds (Wehbe *et al*., 2024). The occurrence of these compound classes provides a plausible chemical basis for some of the biological activities reported for *H. uninervis*. However, the qualitative phytochemical reactions used in the present study indicate only the presence or absence of broad metabolite classes and cannot establish the identity, concentration, or contribution of individual compounds. Therefore, the observed phytochemical profile should be interpreted as a preliminary chemical characterization rather than definitive evidence of specific bioactive molecules.

The solvent-dependent differences observed in the present investigation are consistent with previous studies on *H. uninervis*. Parthasarathi *et al.* (2021) reported alkaloids, steroids, terpenoids, flavonoids, phenols, and quinones in an ethyl acetate fraction of *H. uninervis* collected from the Mandapam coastal region. The same study reported a total phenolic content of 87.75 ± 1.39 mg GAE/g and a total flavonoid content of 67.33 ± 1.52 mg QE/g. In comparison, Wehbe *et al.* (2024) reported substantially higher values of 321.25 ± 5.86 mg GAE/g for total phenolic content and 80.05 ± 4.12 mg QE/g for total flavonoid content in an ethanolic extract of *H. uninervis*. The higher phenolic recovery reported for the ethanolic extract may reflect the greater efficiency of ethanol in extracting particular phenolic constituents, although direct quantitative comparison between studies should be made cautiously because solvent systems, extraction conditions, biomass characteristics, geographical origin, and analytical procedures differed. These findings collectively emphasize that extraction methodology is a critical factor influencing the measured phytochemical composition of *H. uninervis*.

The present phytochemical observations also support the ecological and biochemical significance of seagrasses as sources of marine secondary metabolites. Seagrasses are continuously exposed to microorganisms, herbivores, epiphytes, salinity fluctuations, ultraviolet radiation, and other environmental stresses, and their secondary metabolites may contribute to chemical defence and physiological adaptation. Phenolics, flavonoids, terpenoids, steroids, alkaloids, and other metabolites reported from seagrasses have been associated with a range of biological activities. Nevertheless, the biological function of each compound in *H. uninervis* cannot be established solely from qualitative screening. Isolation and structural characterization of individual constituents would therefore be necessary to determine which compounds contribute most strongly to the observed antimicrobial response.The antimicrobial findings provide additional evidence for the biological potential of *H. uninervis*. Previous research has demonstrated that the antibacterial activity of *H. uninervis* is strongly influenced by both solvent and target microorganism. Gumgumjee *et al.* (2018) reported pronounced activity of an ethanolic leaf extract against *Pseudomonas aeruginosa*, with an inhibition zone of 33.33 mm, while activity against *Bacillus subtilis* reached 24.67 mm. The comparatively limited activity reported for the aqueous extract in the same investigation further supports the importance of solvent selection in recovering antimicrobial constituents. In another study, Hamisi *et al.* (2023) reported antibacterial activity of *H. uninervis* against *Salmonella Typhi*, including a minimum inhibitory concentration (MIC) of 0.39 mg/mL for a dichloromethane leaf extract. The differences in activity among these studies may be associated with variations in bacterial susceptibility, plant tissue, solvent polarity, extract concentration, environmental origin, and extraction conditions.

The antimicrobial activity observed in *H. uninervis* may be related to the combined presence of phenolic compounds, flavonoids, terpenoids, steroids, alkaloids, and other extractable metabolites. Phenolic compounds and flavonoids may interfere with microbial cell-envelope integrity, membrane-associated processes, protein function, and intracellular oxidative balance, whereas terpenoid and steroid-type metabolites may interact with lipid components of microbial membranes. Alkaloids may also affect microbial enzymes and cellular metabolism. However, these possible mechanisms remain hypothetical in the context of the present study because individual compounds were not isolated and no mechanistic experiments were performed. Therefore, the observed antimicrobial effect should be considered a combined activity of the crude extract rather than evidence for the activity of a particular metabolite.

An important consideration is the difference between the antimicrobial results generated in the present investigation and values reported in previous studies. Agar well diffusion is a useful preliminary screening technique, but inhibition-zone diameter is influenced not only by antimicrobial potency but also by extract concentration, compound diffusion through agar, molecular size, solvent properties, agar composition, inoculum density, and the physiological characteristics of the test microorganism. Consequently, a larger inhibition zone does not necessarily indicate greater intrinsic antimicrobial potency. Quantitative determination of MIC and minimum bactericidal concentration (MBC), together with standardized inoculum preparation and appropriate reference antibiotics and solvent controls, would provide stronger evidence of antimicrobial efficacy.

The geographical origin of the material is also an important consideration. The present *H. uninervis* samples were collected from the Mandapam–Rameshwaram coastal region, an ecologically significant area of the Gulf of Mannar. The phytochemical composition of seagrasses can vary with environmental conditions, season, salinity, nutrient availability, tissue age, epiphytic association, and other ecological factors. The Mandapam-specific findings reported by Parthasarathi *et al.* (2021) are particularly relevant because they demonstrate that *H. uninervis* from the same broader geographical region contains substantial levels of phenolic and flavonoid constituents. Nevertheless, differences in sampling period, plant condition, extraction solvent, and fractionation procedure may contribute to variation between the present observations and previously reported values.

Overall, the findings support the view that *H. uninervis* represents a promising source of marine-derived bioactive constituents with potential antimicrobial relevance. The combined phytochemical and antimicrobial results indicate a relationship between solvent-extractable secondary metabolites and biological activity, but they do not establish a direct causal relationship between any particular phytochemical class and antibacterial activity. The study should therefore be considered an initial screening investigation that provides a foundation for more detailed chemical and microbiological studies.

Several limitations should be considered when interpreting the present findings. The phytochemical analysis was qualitative and therefore did not determine the concentration of individual metabolites. Although TPC and TFC provide quantitative estimates of two broad metabolite groups, they do not identify individual phenolic or flavonoid compounds. In addition, chromatographic profiling alone cannot provide definitive structural identification without appropriate standards and spectroscopic confirmation. The antimicrobial assay also requires further quantitative validation through MIC/MBC determination, expanded concentration ranges, replicated dose-response experiments, and appropriate statistical analysis. Furthermore, antimicrobial activity against a broader panel of clinically relevant bacterial strains would provide a more comprehensive assessment of the spectrum of activity.

Future studies should therefore combine chromatographic and spectrometric approaches such as HPLC, LC–MS/MS, GC–MS, and NMR spectroscopy to identify and characterize the major bioactive constituents of *H. uninervis*. Bioassay-guided fractionation could be particularly useful for linking specific chemical fractions with antimicrobial activity. Mechanistic investigations should subsequently determine the effects of active fractions or purified compounds on bacterial membrane integrity, enzyme activity, oxidative stress, and cellular morphology. Cytotoxicity and selectivity studies would also be necessary to determine whether the antimicrobial constituents exhibit an acceptable biological safety profile.

Taken together, the present study and previous literature indicate that *H. uninervis* possesses considerable chemical diversity and measurable antibacterial potential. The consistent observation of solvent-dependent activity across independent investigations highlights the importance of optimizing extraction systems for the recovery of biologically active metabolites. Although the current evidence is preliminary, systematic chemical characterization, quantitative antimicrobial testing, mechanistic evaluation, and toxicity assessment could establish a stronger scientific basis for exploring *H. uninervis* as a source of natural antimicrobial compounds for pharmaceutical, biotechnology, and other biomedical applications.

5. Conclusion

Halodule uninervis represents a promising marine seagrass resource with considerable phytochemical diversity and preliminary antimicrobial potential. The present study provided a systematic evaluation of *H. uninervis* collected from the Gulf of Mannar region through aqueous, ethanol, and acetone extraction, followed by qualitative phytochemical screening, chromatographic profiling, and quantitative estimation of total phenolic and flavonoid contents. The detection of phenols, flavonoids, tannins, alkaloids, terpenoids, steroids, saponins, and other phytochemical classes demonstrated clear solvent-dependent variation in the extract composition. This observation is consistent with previous reports showing substantial differences in the phenolic and flavonoid contents of *H. uninervis* extracts obtained using different solvents and fractionation approaches (Parthasarathi *et al*., 2021; Wehbe *et al*., 2024).

The antimicrobial findings further support the biological relevance of *H. uninervis* extracts, particularly their antibacterial activity against selected clinically relevant pathogens. The reported activity, together with the presence of phenolic, flavonoid, alkaloid, terpenoid, and steroid constituents, suggests that the biological effects may result from the combined action of multiple extractable metabolites. However, the present findings do not establish the contribution of individual compounds or specific mechanisms of antimicrobial action. Therefore, *H. uninervis* should presently be considered a promising source for preliminary natural-product investigation rather than a validated antimicrobial agent.

Further studies involving bioassay-guided fractionation, HPLC/LC–MS and spectroscopic characterization, quantitative determination of individual metabolites, MIC/MBC evaluation, mechanistic studies, cytotoxicity assessment, and validation using broader panels of clinical pathogens are required to identify the compounds responsible for the observed activity and determine their practical biomedical potential. Overall, the findings strengthen the evidence that *H. uninervis* from the Gulf of Mannar is a valuable source of chemically diverse marine metabolites and provides a scientific basis for further investigation of its bioactive constituents and antimicrobial applications.

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