



ANTIFUNGAL EFFICACY OF GRACILARIA EDULIS EXTRACTS AGAINST FUSARIUM PATHOGENS OF SUGARCANE

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Abstract

Sugarcane production is adversely affected by fungal diseases, highlighting the need for effective and environmentally compatible disease-management strategies. The present study investigated the *in vitro* antifungal activity of the red seaweed *Gracilaria edulis* against *Fusarium* isolates obtained from diseased sugarcane collected from three districts of Tamil Nadu, India. Diseased stalk tissues from Ramanathapuram, Namakkal, and Karur were cultured on Potato Dextrose Agar (PDA), and the resulting fungal isolates were characterized based on cultural and microscopic features using lactophenol cotton blue staining. The isolates were morphologically identified as *Fusarium verticillioides* from Ramanathapuram and *Fusarium sacchari* from Namakkal and Karur. *G. edulis* collected from Pudumadam was washed, shade-dried, powdered, and extracted using distilled water and acetone. Qualitative phytochemical screening revealed the presence of flavonoids, saponins, alkaloids, carbohydrates, and proteins in the aqueous extract, whereas the acetone extract contained flavonoids, carbohydrates, alkaloids, and phytosterols. The antifungal activity of the acetone extract was evaluated using the agar well diffusion assay at 10, 20, and 50 μ L. The extract exhibited inhibitory activity against all tested isolates, with inhibition generally increasing with extract volume. The maximum inhibition zone of 20 mm was recorded against the Ramanathapuram-associated *F. verticillioides* isolate at 50 μ L, followed by 15 mm and 10 mm against the Karur- and Namakkal-associated *F. sacchari* isolates, respectively. These findings provide preliminary *in vitro* evidence for the antifungal potential of *G. edulis* extract against sugarcane-associated *Fusarium* fungi. However, the fungal isolates were identified solely on the basis of phenotypic characteristics, and the antifungal assessment was restricted to an *in vitro* assay. Molecular confirmation of pathogen identity, quantitative determination of antifungal potency, chemical characterization of active constituents, replicated dose-response studies, mechanistic investigations, and greenhouse and field evaluations are necessary to establish the efficacy, safety, and practical potential of *G. edulis*-derived antifungal agents for sustainable sugarcane disease management.

Keywords: Antifungal activity, Bioactive compounds, *Fusarium* spp, *Halodule uninervis*, Seagrass; Sugarcane diseases, Sustainable disease management.

1. Introduction

Sugarcane (*Saccharum* spp.) is a major tropical C4 crop supporting sugar, ethanol, biomass and multiple value-added industries. Its high photosynthetic efficiency and capacity for sucrose accumulation make it economically important, while its vegetative propagation and long crop cycle expose production systems to persistent pathogen pressure. The supplied thesis identifies disease as a major constraint to sugarcane productivity and describes fungal diseases including red rot, smut, pineapple disease, wilt, Pokkah boeng and eye spot. (Dinesh Babu et al., 2022; Grivet et al., 2004; Wang et al., 2023). Among sugarcane diseases, *Fusarium*-associated disorders are important because infection can compromise plant establishment, stalk development and cane quality. In the present study, diseased stalks from three Tamil Nadu districts yielded isolates assigned morphologically to *F. verticillioides* and *F. sacchari*. The thesis records distinct colony characteristics among districts and emphasizes that colony morphology alone is insufficient for definitive species-level identification. (Ekwomadu & Mwanza, 2023; Raza et al., 2019). Conventional disease management frequently relies on chemical inputs. Although fungicides can provide effective suppression, repeated use can increase selection pressure, residue concerns and environmental burden. This motivates investigation of biologically derived compounds that could complement integrated disease management. Marine macroalgae are particularly attractive because they contain diverse polysaccharides, phenolics, flavonoids, sterols, proteins and other secondary metabolites with antimicrobial activities. The thesis specifically highlights the broad bioactive potential of red seaweeds and the agricultural relevance of seaweed-derived compounds. (Ambika & Sujatha, 2015; Yang et al., 2021; Mandal et al., 2025)

Gracilaria edulis is an economically important red alga distributed in tropical and subtropical coastal habitats and is widely used as an agarophyte. The supplied document describes its occurrence along the south-eastern Indian coast and reports a biochemical profile rich in carbohydrates, protein and dietary fibre, together with secondary metabolites and sulfated polysaccharides. (Baweja et al., 2016; Torres et al., 2019; Bhushan et al., 2023). Seaweed extracts can exhibit antifungal activity against plant pathogens, providing a rationale for testing *G. edulis* against sugarcane-associated fungi. The specific gap addressed here is the preliminary evaluation of *G. edulis* extracts against *Fusarium* isolates obtained from geographically separated sugarcane samples in Tamil Nadu. The objectives were therefore to isolate and characterize fungal pathogens, prepare aqueous and acetone extracts of *G. edulis*, screen their phytochemical constituents, and evaluate antifungal activity under in vitro conditions.

2. Materials and Methods

2.1 Collection of Diseased Sugarcane Samples and Isolation of Fungal Pathogens

Diseased sugarcane stalk samples exhibiting visible symptoms of fungal infection were collected from Ramanathapuram, Namakkal, and Karur districts of Tamil Nadu, India. The infected stalk portions were aseptically collected in sterile bags and transported to the laboratory for pathogen isolation. Symptomatic tissue segments measuring approximately 1.0–1.5 cm were excised from the diseased portions and initially washed to remove adhering soil and debris. The tissue segments were surface sterilized with 15% sodium hypochlorite for 30 s and subsequently rinsed thoroughly with sterile distilled water to remove residual disinfectant. The sterilized segments were placed on Potato Dextrose Agar (PDA) plates under aseptic conditions and incubated at 28 ± 2 °C for 7 d. Emerging fungal colonies were monitored periodically, and actively growing colonies were transferred to fresh PDA plates. Repeated subculturing was performed to obtain morphologically uniform pure cultures. The isolation procedure was carried out following standard approaches used for the isolation and characterization of plant-associated filamentous fungi (Booth, 1971; Leslie and Summerell, 2006).

2.2 Cultural and Microscopic Characterization of Fungal Isolates

The purified fungal isolates were characterized based on their cultural and microscopic features. Macroscopic characteristics, including colony colour, texture, margin, elevation, growth rate, aerial mycelial development, and pigmentation, were recorded during growth on PDA. For microscopic examination, fungal structures were prepared using lactophenol cotton blue (LPCB) staining. A small portion of actively growing mycelium was transferred to a clean glass slide containing LPCB, gently teased, covered with a coverslip, and examined microscopically. Conidial morphology and dimensions were evaluated using ocular and stage micrometers. The observed characteristics were compared with standard taxonomic descriptions of *Fusarium* species (Booth, 1971; Leslie and Summerell, 2006).

Based on the combined cultural and microscopic characteristics, the isolates were provisionally assigned as *Fusarium verticillioides* and *Fusarium sacchari*. Since molecular identification was not performed in the present study, these species assignments are considered morphology-based identifications and were not treated as molecularly confirmed taxonomic determinations. Molecular confirmation using appropriate genetic markers, sequencing, and phylogenetic analysis would be required for definitive species-level identification.

2.3 Collection and Processing of *Gracilaria edulis*

Fresh thalli of the red seaweed *Gracilaria edulis* were collected from natural populations at Pudumadam, Tamil Nadu, India (9.27262° N, 78.995362° E). The collected material was transported to Thondi for further processing. The seaweed samples were thoroughly washed with seawater followed by tap water to remove adhering sediments, epiphytes, and excess salts. The cleaned biomass was subjected to repeated sun-bleaching for approximately two weeks as described in the source methodology, followed by air-drying. The dried material was packed in clean, sealed bags and stored under suitable conditions until extraction. Prior to extraction, the dried seaweed was cut into small pieces and pulverized into a coarse/fine powder to facilitate efficient solvent contact.

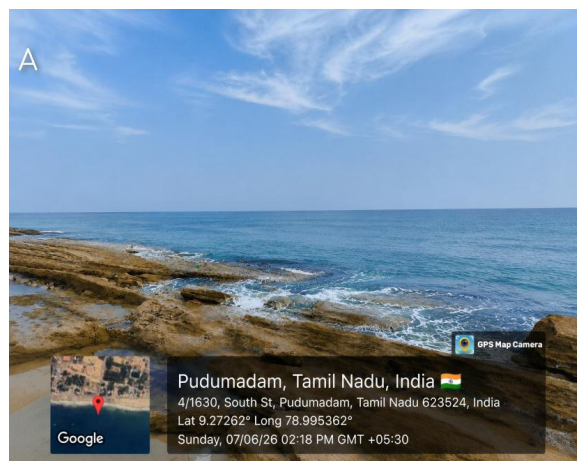


Fig 1. Collection site of *Gracilaria edulis* at Pudumadam, Tamil Nadu.

2.4 Preparation of Aqueous and Acetone Extracts

Aqueous and acetone extracts of *G. edulis* were prepared using Soxhlet-assisted extraction. For the aqueous extraction, approximately 10 g of dried and powdered seaweed material was loaded into a Soxhlet apparatus containing distilled water as the extraction solvent. Following extraction, the aqueous extract was concentrated and stored at 4 °C until further analysis. For acetone extraction, approximately 10 g of powdered *G. edulis* was extracted with acetone using a sample-to-solvent ratio of 1:10 (w/v). Soxhlet extraction was performed for 1 h. Following extraction, the solvent was removed to obtain the crude acetone extract, which was subsequently stored at 4 °C until phytochemical screening and antifungal evaluation. The use of solvent extraction for recovering biologically active constituents from seaweeds is consistent with established approaches for marine algal phytochemical investigations (Harborne, 1998; Gressler et al., 2011).

2.5 Qualitative Phytochemical Screening

Preliminary qualitative phytochemical screening was performed on the aqueous and acetone extracts to detect major classes of secondary metabolites. The extracts were screened for phenols, tannins, flavonoids, glycosides, terpenoids, saponins, steroids, carbohydrates, proteins, alkaloids, and phytosterols using standard qualitative chemical reactions. The presence or absence of individual phytochemical groups was determined based on characteristic colour development, precipitate formation, or persistent foam formation, as applicable to the respective tests.

The qualitative screening procedures were based on standard phytochemical methods described by Harborne (1998) and Trease and Evans (2002), with appropriate modifications for seaweed extracts. The aqueous extract showed positive reactions for flavonoids, saponins, carbohydrates, proteins, and alkaloids. The acetone extract showed positive reactions for flavonoids, carbohydrates, alkaloids, and phytosterols. The results were recorded qualitatively as positive (+) or negative (–). Since these assays are preliminary qualitative tests, the results were interpreted only as evidence for the detectable presence of broad phytochemical classes and were not considered quantitative measurements of individual compounds.

2.6 In Vitro Antifungal Activity Assay

The antifungal activity of the *G. edulis* acetone extract was evaluated against the isolated *Fusarium* pathogens using the agar well diffusion method. The fungal isolates were maintained on PDA and actively growing cultures were used for inoculation. PDA plates were inoculated with the respective fungal isolates to obtain relatively uniform fungal growth. Sterile wells were subsequently prepared in the inoculated agar using a sterile cork borer. Different volumes of the crude acetone extract, namely 10, 20, and 50 µL, were separately introduced into the respective wells. The inoculated plates were incubated at 25 ± 2 °C for 72 h under suitable conditions. Following incubation, the plates were examined for clear zones surrounding the wells. The diameter of each inhibition zone was measured in millimetres and recorded as the indicator of antifungal activity. A larger inhibition-zone diameter was considered to represent greater growth inhibition under the experimental conditions. The agar well diffusion approach was used as a preliminary screening method for evaluating the antifungal activity of the seaweed extract.

2.7 Experimental Interpretation and Methodological Limitations

The antifungal assay was used to establish preliminary growth-inhibitory activity of the crude *G. edulis* acetone extract against the selected sugarcane-associated *Fusarium* isolates. Because the study employed a crude extract and an agar diffusion assay, the observed inhibition cannot be directly attributed to a specific phytochemical constituent or interpreted as a minimum inhibitory concentration. Furthermore, qualitative phytochemical screening does not establish the concentration or chemical identity of the detected metabolites. Therefore, future investigations should incorporate appropriate solvent and positive controls, biological replication, statistical analysis, MIC/MFC determination, quantitative phytochemical estimation, chromatographic and spectroscopic characterization, and molecular confirmation of the fungal isolates. These additional approaches would strengthen the evidence for the antifungal potential and facilitate assessment of the practical applicability of *G. edulis*-derived bioactive compounds.

3. Results

3.1 Isolation and Phenotypic Characterization of *Fusarium* Isolates

Distinct fungal colonies were successfully recovered from diseased sugarcane stalk samples collected from Ramanathapuram, Namakkal, and Karur districts of Tamil Nadu, India. Based on cultural and microscopic characteristics, the isolates were provisionally assigned to the genus *Fusarium*. The isolate obtained from Ramanathapuram was designated S1 and identified morphologically as *Fusarium verticillioides*, whereas the isolates recovered from Namakkal and Karur were designated S2 and S3, respectively, and identified as *Fusarium sacchari*. Visible fungal growth developed within approximately 3–8 d of incubation, with the isolates exhibiting predominantly fluffy to cottony colony textures (Table.1).

Considerable variation in cultural characteristics was observed among the isolates. The Ramanathapuram isolate (S1) produced a predominantly white colony on the upper surface, accompanied by yellow pigmentation on the reverse side. The Namakkal isolate (S2) exhibited violet to white coloration on the colony surface, with distinct violet pigmentation on the reverse. In contrast, the Karur isolate (S3) developed a cottony white colony with comparatively less pronounced pigmentation and a white reverse. Differences were also observed in colony margin and elevation among the isolates, indicating phenotypic variability between the fungal populations obtained from different geographical locations. These cultural characteristics, together with microscopic observations, formed the basis for the preliminary morphological identification of the isolates as *F. verticillioides* and *F. sacchari*. However, as molecular characterization was not performed, the species-level assignments should be considered morphology-based and require molecular confirmation for definitive identification.

Table 1. Phenotypic characteristics of the three sugarcane-associated fungal isolates recorded in the source study

District	Isolate assignment	Growth	Texture	Margin	Colony colour
Ramanathapuram	<i>F. verticillioides</i> (S1)	3–5 d	Fluffy	Irregular	Front white; reverse yellow
Namakkal	<i>F. sacchari</i> (S2)	3–5 d	Fluffy/cotton	Filiform	Front violet/white; reverse violet
Karur	<i>F. sacchari</i> (S3)	4–8 d	Fluffy/cotton	Irregular	Front cottony/white; reverse white

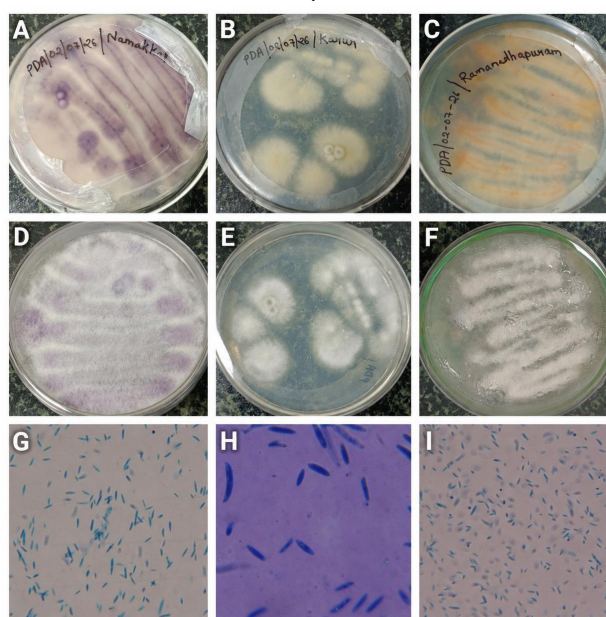


Fig 2. Representative colony and microscopic observations of isolates S1, S2, S3.

Microscopic examination of the fungal isolates using lactophenol cotton blue (LPCB) preparations revealed morphological structures consistent with the genus *Fusarium*. Light microscopic observations provided additional support for the preliminary identification based on the cultural characteristics observed on PDA. The isolates exhibited well-developed aerial mycelia with variations in colony appearance, pigmentation, opacity, and surface texture among the respective cultures. These phenotypic differences may reflect isolate-level variation associated with their geographical origin and growth characteristics. Similar variations in cultural and morphological characteristics have been reported among *Fusarium* isolates associated with plant diseases (Shamly et al., 2014). However, since molecular characterization was not performed in the present study, the observed microscopic features should be regarded as supportive rather than definitive evidence for species-level identification (Fig.2).

Table 2. Qualitative phytochemical screening of aqueous and acetone extracts of *G. edulis*.

Phytochemical class	Aqueous	Acetone
Phenols	—	—
Tannins	—	—

Flavonoids	+	+
Glycosides	–	–
Terpenoids	–	–
Saponins	+	–
Steroids	–	–
Carbohydrates	+	+
Proteins	+	–
Alkaloids	+	+

3.2 Phytochemical Profile of *Gracilaria edulis* Extracts

Qualitative phytochemical screening revealed distinct differences between the aqueous and acetone extracts of *Gracilaria edulis*. The aqueous extract showed positive reactions for flavonoids, saponins, carbohydrates, proteins, and alkaloids, indicating the presence of these phytochemical classes. In contrast, phenols, tannins, glycosides, terpenoids, and steroids were not detected under the qualitative test conditions employed. The acetone extract exhibited a different phytochemical profile, with positive reactions for flavonoids, carbohydrates, alkaloids, and phytosterols, whereas phenols, tannins, glycosides, terpenoids, saponins, steroids, and proteins were not detected. The variation between the two extracts indicates that solvent selection influenced the qualitative recovery of detectable phytochemical constituents from *G. edulis*. The presence of flavonoids and alkaloids in both extracts suggests that these metabolite classes may have relatively broad extractability, while the detection of saponins and proteins exclusively in the aqueous extract may reflect their greater affinity for the polar solvent. Conversely, the detection of phytosterols only in the acetone extract may be associated with their comparatively higher solubility in organic solvents. These findings provide preliminary evidence of solvent-dependent phytochemical variation in *G. edulis* and support further quantitative and chemical characterization of the extracts to determine the specific compounds potentially associated with their biological activity (Table.2).

3.3 In Vitro Antifungal Activity of *G. edulis* Acetone Extract

The acetone extract of *Gracilaria edulis* exhibited inhibitory activity against all tested sugarcane-associated *Fusarium* isolates in the agar well diffusion assay (Table.3). The Namakkal-associated *F. sacchari* isolate showed inhibition zones of 3, 5, and 10 mm at extract volumes of 10, 20, and 50 μ L, respectively. The Ramanathapuram-associated *F. verticillioides* isolate exhibited inhibition zones of 6, 5, and 20 mm at the corresponding extract volumes. The Karur-associated *F. sacchari* isolate showed inhibition zones of 5, 10, and 15 mm, respectively. The maximum inhibition zone of 20 mm was recorded against the Ramanathapuram-associated *F. verticillioides* isolate at 50 μ L. Overall, the extract demonstrated a measurable inhibitory effect against all three isolates, although the magnitude of inhibition varied among isolates and extract volumes. The comparatively higher inhibition observed against *F. verticillioides* suggests greater susceptibility of this isolate to the acetone extract under the tested conditions. The variation in inhibition may be associated with differences in fungal susceptibility and the interaction and diffusion characteristics of extract constituents within the agar medium. These findings provide preliminary in vitro evidence for the antifungal potential of *G. edulis* acetone extract against sugarcane-associated *Fusarium* pathogens (Fig 3&4).

Table 3. Zones of inhibition produced by *G. edulis* acetone extract against the fungal isolates.

Sampling district	Extract	10 μ L (mm)	20 μ L (mm)	50 μ L (mm)	Change, 10 $\rightarrow$ 50 μ L
Ramanathapuram <i>F. verticillioides</i> S1	Acetone	6	5	20	+14 mm
Namakkal <i>F. sacchari</i> S2	Acetone	3	5	10	+7 mm
Karur <i>F. sacchari</i> S3	Acetone	5	10	15	+10 mm

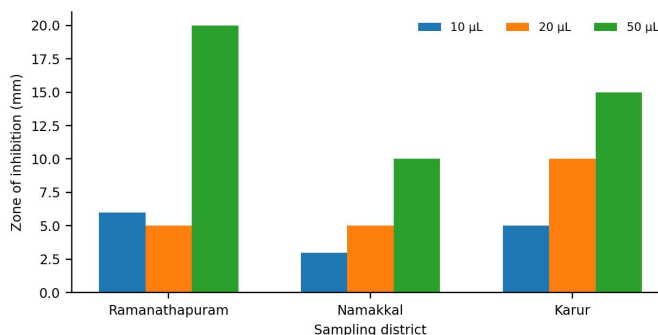


Fig 3. Dose-associated inhibition zones recorded for *G. edulis* acetone extract. The 50 μ L treatment produced the largest zone for each isolate.

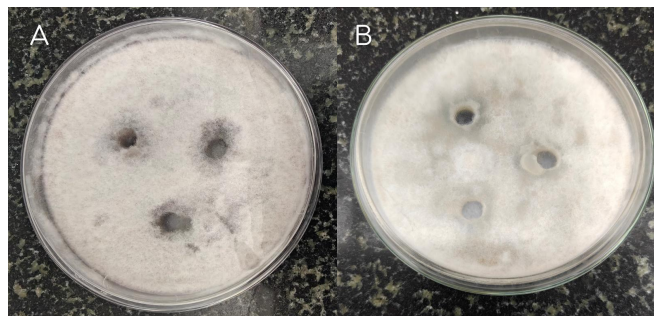


Fig 4. Representative agar-well diffusion plate showing inhibition produced by *G. edulis* acetone extract

4. Discussion

The present study demonstrated that the crude acetone extract of the red seaweed *Gracilaria edulis* exhibited inhibitory activity against all three *Fusarium* isolates associated with diseased sugarcane. The maximum inhibition zone of 20 mm was recorded against the Ramanathapuram-associated *Fusarium verticillioides* isolate at an extract volume of 50 μ L. The two *Fusarium sacchari* isolates obtained from Namakkal and Karur exhibited inhibition zones of 10 and 15 mm, respectively, at the same extract volume. These results indicate that the acetone-soluble fraction of *G. edulis* contains constituents capable of inhibiting the growth of the tested sugarcane-associated *Fusarium* isolates under in vitro conditions. The differential response among the isolates further suggests that susceptibility to the extract may vary according to the physiological and biochemical characteristics of individual fungal isolates.

The inhibitory response generally increased with increasing extract volume. For the Namakkal-associated *F. sacchari* isolate, the inhibition zone increased from 3 mm at 10 μ L to 5 mm at 20 μ L and 10 mm at 50 μ L. Similarly, the Karur-associated *F. sacchari* isolate showed an increase from 5 to 10 and 15 mm at 10, 20, and 50 μ L, respectively. The Ramanathapuram-associated *F. verticillioides* isolate showed inhibition zones of 6, 5, and 20 mm at the respective extract volumes. Although the overall response was greatest at 50 μ L, the slight reduction from 6 to 5 mm between 10 and 20 μ L for the *F. verticillioides* isolate indicates that the response was not strictly proportional at every concentration. This variation may arise from differences in diffusion behaviour, local extract distribution within the agar, or isolate-specific susceptibility. Because only three extract volumes were evaluated and replicate-level measurements were not available, the present observations should be interpreted as a preliminary concentration-response pattern rather than evidence of statistically established dose dependence.

The greater inhibition observed against *F. verticillioides* compared with the two *F. sacchari* isolates may reflect differences in the sensitivity of the fungal isolates to the constituents present in the crude extract. Even within the same fungal species, differences in strain physiology, membrane properties, growth characteristics, extracellular enzymes, and stress-response mechanisms may influence susceptibility to natural products. In the present study, the two *F. sacchari* isolates also showed different responses at the highest extract volume, with 10 mm inhibition for the Namakkal isolate and 15 mm for the Karur isolate. This observation indicates that geographical origin or isolate-level variation may contribute to differences in antifungal response. However, the current dataset is insufficient to determine whether the variation is attributable to species, isolate genotype, environmental origin, or experimental variability. Additional isolates and replicated experiments would therefore be necessary to investigate this variation more rigorously.

The antifungal activity observed in the present investigation is consistent with previous reports describing the biological potential of red seaweeds. Ambika and Sujatha (2015) and Kolanjinathan and Manigandan (2014) reported antimicrobial activities associated with red seaweed extracts, supporting the broader potential of Rhodophyta as a source of biologically active metabolites. Red seaweeds contain structurally diverse compounds, including phenolic compounds, flavonoids, sterols, pigments, fatty acids, and sulfated polysaccharides, which have been associated with different biological activities (Rosemary et al., 2019; Gunathilaka et al., 2019; Jayalakshmi et al., 2021). The present observation therefore adds to the existing evidence that *G. edulis* may possess constituents with antimicrobial potential, although the activity observed here cannot be directly attributed to any particular compound.

The qualitative phytochemical profile obtained in the present study provides a possible basis for interpreting the antifungal response. Flavonoids and alkaloids were detected in both aqueous and acetone extracts, whereas saponins and proteins were detected only in the aqueous extract and phytosterols were detected in the acetone extract. The presence of flavonoids and alkaloids in the acetone extract is of particular interest because both groups comprise chemically diverse compounds with reported antimicrobial properties. Similarly, phytosterols and other lipophilic constituents recovered during organic-solvent extraction may contribute to the biological properties of seaweed extracts. However, the qualitative screening performed in this study identifies only broad classes of metabolites and does not establish their abundance, chemical structures, or individual biological contributions.

The phytochemical differences between the aqueous and acetone extracts also demonstrate the influence of solvent selection on the recovery of detectable metabolites. The detection of saponins and proteins in the aqueous extract but not in the acetone extract may reflect their greater compatibility with the polar extraction system, whereas phytosterols detected in the acetone extract are consistent with the recovery of relatively lipophilic constituents. Similar solvent-dependent differences in phytochemical recovery have been reported in investigations of marine macroalgae and other natural resources. Consequently, the stronger antifungal response observed with the acetone extract should not be interpreted simply as evidence that acetone extraction produces a universally more active extract. Rather, it suggests that

constituents recovered under the specific extraction conditions used in the present study may have contributed to the observed inhibition.

The biological activity of red seaweeds has been associated with a complex mixture of secondary metabolites and structurally diverse marine polysaccharides. Rosemary et al. (2019), Gunathilaka et al. (2019), and Jayalakshmi et al. (2021) have highlighted the presence and biological significance of compounds such as polyphenols, sterols, flavonoids, and sulfated polysaccharides in red seaweeds. These constituents may exhibit antimicrobial effects through different mechanisms, including interaction with microbial cell surfaces, alteration of membrane-associated processes, interference with cellular enzymes, and modulation of oxidative processes. Nevertheless, such mechanisms cannot be confirmed from the present agar-well diffusion results. The current study establishes only growth inhibition and does not determine the cellular target or mode of action of the *G. edulis* extract.

The agar-well diffusion method is appropriate for preliminary screening of crude extracts because it allows rapid comparison of inhibitory activity among test organisms and extract concentrations. However, inhibition-zone diameter is influenced by both antimicrobial activity and the physical diffusion properties of the compounds through agar. Compounds with strong activity but limited diffusion may produce relatively small zones, whereas highly diffusible compounds may produce larger zones. Therefore, the 20 mm zone observed against *F. verticillioides* should not be interpreted directly as a quantitative measure of antifungal potency. Determination of minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC) would provide more reliable quantitative estimates of the concentration required to inhibit or eliminate the fungal isolates.

Another important limitation concerns the identification of the fungal pathogens. The isolates were assigned morphologically as *F. verticillioides* and *F. sacchari* based on cultural and microscopic characteristics. Morphological characterization is useful for preliminary identification; however, species within the *Fusarium* complex may show overlapping phenotypic features. Molecular identification using suitable sequence-based markers, followed by phylogenetic analysis, would therefore provide stronger evidence for species-level assignment. Previous studies have emphasized the importance of molecular approaches for accurate identification and discrimination of closely related *Fusarium* taxa (Raza et al., 2019; Ekwomadu and Mwanza, 2023). Molecular confirmation would also strengthen comparisons between the present isolates and previously reported sugarcane-associated *Fusarium* populations.

The agricultural relevance of *G. edulis* is another factor supporting further investigation of its bioactive potential. *G. edulis* is an economically important red seaweed associated with agar production and has been cultivated and utilized as a marine biomass resource (Jayasankar and Ramamoorthy, 1997; Kalkman et al., 1991; Ganesan et al., 2011; Bhushan et al., 2023). The availability of biomass is an important consideration when evaluating marine resources for agricultural biotechnology. A naturally available and cultivable seaweed could potentially provide a renewable source of biomass for extraction of bioactive fractions. However, scalability alone does not establish suitability as a crop-protection agent. Seasonal variation, geographical origin, cultivation conditions, biomass quality, extraction efficiency, and stability of the active constituents must be considered before developing a standardized formulation.

From a sustainable disease-management perspective, seaweed-derived extracts may have potential as complementary components of integrated disease management rather than immediate replacements for conventional fungicides. The present findings demonstrate activity against selected *Fusarium* isolates, but they do not establish disease suppression in sugarcane plants. An extract that inhibits fungal growth in agar may behave differently when applied to plant tissues because of differences in absorption, stability, metabolism, environmental degradation, and interactions with the host plant and soil microbiota. Therefore, the next stage of investigation should involve bioassay-guided fractionation of the acetone extract to identify the active fraction(s), followed by chemical profiling using techniques such as liquid chromatography–mass spectrometry (LC–MS) and, where appropriate, gas chromatography–mass spectrometry (GC–MS). Quantitative phytochemical analysis should also be performed to determine the abundance of the major metabolite classes.

Mechanistic studies would further clarify the basis of the observed antifungal activity. Selected fractions or purified compounds could be evaluated for their effects on fungal spore germination, mycelial growth, hyphal morphology, membrane integrity, cellular leakage, and oxidative stress. Such investigations would help distinguish general growth inhibition from specific antifungal mechanisms. In addition, appropriate positive and negative controls, independent biological replicates, and statistical analysis should be incorporated into subsequent experiments. A broader concentration range would allow construction of a more reliable dose-response relationship and facilitate calculation of MIC and MFC values.

Before agricultural application, the safety and phytotoxicity of the extract should also be evaluated. In vitro antifungal activity alone is insufficient to demonstrate suitability for crop protection. Controlled greenhouse experiments should first determine whether effective antifungal concentrations can be applied without adversely affecting sugarcane germination, growth, biomass accumulation, or physiological performance. Disease-severity assessments in artificially inoculated plants would then provide stronger evidence of biological efficacy. Promising treatments could subsequently be evaluated under field conditions to determine their stability and effectiveness under natural environmental and disease-pressure conditions.

Overall, the present study provides preliminary evidence that the crude acetone extract of *Gracilaria edulis* possesses inhibitory activity against sugarcane-associated *Fusarium* isolates, with the strongest observed response against the Ramanathapuram-associated *F. verticillioides* isolate. The detection of flavonoids, alkaloids, carbohydrates, and phytosterols in the extracts provides a preliminary indication of the chemical diversity of the seaweed biomass, but the specific constituents responsible for antifungal activity remain unresolved. The findings are therefore best regarded as an initial screening outcome rather than evidence of a defined antifungal compound or validated biocontrol product. Future

studies integrating molecular pathogen identification, standardized and replicated antifungal assays, quantitative phytochemical analysis, LC–MS/GC–MS profiling, MIC/MFC determination, mechanism-of-action studies, phytotoxicity assessment, and greenhouse and field validation will be essential to determine whether *G. edulis*-derived bioactive fractions can be developed as practical and environmentally compatible components of sustainable sugarcane disease management.

5. Conclusions

The present study provides preliminary in vitro evidence for the antifungal potential of the red seaweed *Gracilaria edulis* against *Fusarium* isolates associated with diseased sugarcane. The phytochemical screening demonstrated that the aqueous and acetone extracts contained different classes of detectable metabolites, indicating solvent-dependent recovery of bioactive constituents. The crude acetone extract exhibited inhibitory activity against all three tested *Fusarium* isolates, although the magnitude of inhibition varied among isolates. The maximum inhibition zone of 20 mm was recorded at 50 µL against the Ramanathapuram-associated *Fusarium verticillioides* isolate, whereas the Namakkal- and Karur-associated *Fusarium sacchari* isolates exhibited inhibition zones of 10 and 15 mm, respectively, at the same extract volume.

These findings indicate that *G. edulis* represents a promising marine biomass source for further exploration of natural antifungal metabolites relevant to sugarcane disease management. However, the present investigation should be regarded as an initial in vitro screening study rather than evidence of a validated biocontrol agent. The observed antifungal activity cannot be attributed to any specific compound because the phytochemical analysis was qualitative and no chemical characterization of the active constituents was performed. Similarly, the *Fusarium* isolates were identified on the basis of cultural and microscopic characteristics and therefore require molecular confirmation for definitive species-level identification.

Further investigations should focus on standardized extraction and fractionation, quantitative phytochemical analysis, LC–MS/GC–MS-based chemical profiling, and identification of the compounds or fractions responsible for the observed antifungal activity. Determination of MIC and MFC values, together with replicated dose-response experiments, appropriate controls, and statistical analysis, would provide stronger quantitative evidence of antifungal efficacy. Mechanistic studies are also required to understand the cellular and physiological basis of fungal growth inhibition. Importantly, phytotoxicity assessment and greenhouse experiments should be conducted to determine crop safety and disease-suppressing efficacy before progressing to field trials. Overall, *G. edulis* shows potential as a renewable source of natural antifungal constituents, and systematic validation through chemical, microbiological, plant-based, and field-level studies may establish its future role as a complementary component of sustainable sugarcane disease management.

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