



ANTIFUNGAL POTENTIAL OF HALODULE UNINERVIS SEAGRASS EXTRACTS AGAINST SUGARCANE-ASSOCIATED FUSARIUM PATHOGENS

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Abstract

Sugarcane cultivation is adversely affected by fungal diseases, resulting in substantial losses in crop yield and quality and increasing reliance on chemical fungicides. The present study investigated the antifungal potential of the marine seagrass *Halodule uninervis* against fungal pathogens associated with sugarcane diseases. Diseased sugarcane samples were collected from Erode, Salem, and Tiruvannamalai districts of Tamil Nadu, India. Fungal pathogens were isolated on Potato Dextrose Agar and identified based on their cultural and microscopic characteristics as *Fusarium oxysporum*, *Fusarium sacchari*, and *Fusarium incarnatum*. The collected *H. uninervis* biomass was shade-dried, powdered, and extracted using aqueous and acetone-based extraction methods. Qualitative phytochemical analysis revealed the presence of phenols, tannins, flavonoids, proteins, and alkaloids in both extracts, whereas glycosides, terpenoids, and steroids were detected exclusively in the acetone extract. Based on the phytochemical profile, the acetone extract was further evaluated for antifungal activity using the agar well diffusion assay at concentrations of 10, 20, and 50 μ L. The extract exhibited concentration-dependent inhibitory activity against all three *Fusarium* isolates. The highest inhibition zone of 20 mm was observed against *F. oxysporum* at 50 μ L, followed by 14 mm against *F. sacchari* and 13 mm against *F. incarnatum*. These results demonstrate that *H. uninervis* possesses bioactive constituents with promising antifungal properties against important sugarcane-associated *Fusarium* pathogens. The findings highlight the potential of seagrass-derived bioactive compounds as a natural and environmentally compatible resource for developing sustainable approaches to fungal disease management in sugarcane. Further investigations involving compound identification, mechanism-of-action studies, and in planta and field-level validation are necessary to establish their practical efficacy and application potential.

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

1. Introduction

Sugarcane (*Saccharum* spp.) is a major tropical and subtropical crop and an important source of sugar, bioethanol, electricity from bagasse and other value-added products. Its high biomass productivity and C4 photosynthetic pathway make it an important component of food, energy and agro-industrial systems. Sugarcane is cultivated across a wide geographic range, with Brazil and India among the leading producers, and India supports a large sugarcane-based rural and industrial economy (Srivastava et al., 2012; Dinesh Babu et al., 2022; FAO, 2024; O'Connell et al., 2022). The crop is also increasingly discussed in the context of biorefinery and near-zero-waste utilization because bagasse, molasses and press mud can be converted into energy, industrial products and agricultural inputs (Mario de Matos et al., 2020; Sukunya Areeya et al., 2024; Sameera et al., 2025).

Disease remains a major constraint to sustainable sugarcane production. Fungal pathogens are particularly important because they can reduce cane growth, cane weight, juice quality and sugar recovery. Major diseases include red rot, wilt, Pokkah boeng, smut and sett rot, and disease patterns can be modified by temperature, humidity, rainfall, soil conditions, ratooning and continuous cultivation (Viswanathan, 2018; Viswanathan et al., 2017, 2021, 2022; Hossain et al., 2020; Usmana et al., 2020; Msomba et al., 2024). *Fusarium* species are important sugarcane pathogens, with *F. sacchari* associated with wilt and Pokkah boeng and other *Fusarium* taxa implicated in disease complexes. *F. oxysporum* is a vascular pathogen capable of root and xylem colonization, while *F. incarnatum* has been associated with Pokkah boeng symptoms (Srimali et al., 2021; Lin et al., 2025). Disease management therefore requires approaches that are effective while reducing dependence on intensive chemical inputs.

Marine seagrasses represent a comparatively underexplored source of bioactive natural products. These marine angiosperms have evolved biochemical and physiological adaptations to fluctuating salinity, light and other environmental stresses and contain phenolics, flavonoids, tannins, terpenoids, steroids and other secondary metabolites with reported biological activities (Al-Arbash et al., 2016; Dhivakar et al., 2019; Gono et al., 2022; Wehbe et al., 2024). *Halodule uninervis* is a widely distributed tropical seagrass of the family Cymodoceaceae and is reported from Indo-Pacific coastal habitats including India. Its phytochemical profile and reported antioxidant, antibacterial and other biological activities make it a candidate for investigation as a source of antifungal metabolites (Vanitha et al., 2020; Parthasarathi et al., 2021; Wehbe et al., 2024; Surroop et al., 2024). The present study was therefore designed to isolate and characterize fungal pathogens associated with diseased sugarcane from selected districts of Tamil Nadu and to evaluate the antifungal potential of *H. uninervis* extracts. The specific objectives were to isolate pure fungal cultures, characterize the isolates using morphological and microscopic observations, prepare aqueous and acetone extracts of *H. uninervis*, screen the extracts qualitatively for major phytochemical classes, and evaluate the *in vitro* antifungal activity of the acetone extract against the isolated *Fusarium* pathogens.

2. Materials and Methods

2.1 Collection of Diseased Sugarcane Samples and Fungal Isolation

Diseased sugarcane stalk samples exhibiting visible symptoms of fungal infection were collected from Erode, Salem, and Tiruvannamalai districts of Tamil Nadu, India. The samples were aseptically collected in sterile zip-lock bags and transported to the laboratory for fungal isolation. Small infected tissue segments measuring approximately 4–6 mm were excised from the advancing margins of diseased tissues and surface sterilized with 1% sodium hypochlorite for 1–2 min, followed by treatment with 70% ethanol for 1 min. The segments were subsequently rinsed 2–3 times with sterile distilled water to remove residual disinfectants and dried on sterile filter paper. The sterilized tissue pieces were placed on Potato Dextrose Agar (PDA) plates under aseptic conditions, sealed with Parafilm, and incubated at 28–30 °C. Emerging fungal colonies were monitored periodically, and actively growing colony margins were transferred to fresh PDA after approximately 5 days. The cultures were subcultured three times to obtain morphologically uniform and pure fungal isolates.

2.2 Morphological and Microscopic Characterization of Fungal Isolates

The purified fungal isolates were characterized based on their macroscopic and microscopic features. Colony characteristics, including growth period, colony texture, margin, elevation, surface colour, pigmentation, opacity, and aerial mycelial development, were recorded following incubation on PDA. For microscopic examination, a small portion of actively growing fungal mycelium was transferred to a clean glass slide containing a drop of lactophenol cotton blue (LPCB) stain. The preparation was gently covered with a coverslip and examined under a digital microscope at 40× magnification. The morphology of the mycelia, spores, conidia, and other characteristic fungal structures was recorded. Based on the combined cultural and microscopic characteristics, the isolates were designated S1, S2, and S3 and provisionally identified as *Fusarium oxysporum*, *Fusarium sacchari*, and *Fusarium incarnatum*, respectively. Morphological characterization was performed following established procedures for the preliminary identification of filamentous fungi (Booth, 1971; Leslie and Summerell, 2006).

2.3 Collection and Processing of *Halodule uninervis*

The seagrass *Halodule uninervis* was collected from the intertidal region of Rameshwaram, Ramanathapuram district, Tamil Nadu, India (9.28174° N, 79.18849° E). The collected 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 sediments, epiphytes, and other surface impurities. The cleaned biomass was shade-dried at approximately 30–40 °C until a constant dry condition was obtained. The dried material was subsequently ground into a fine powder using a clean laboratory grinder and stored in sealed zip-lock bags until extraction.

2.4 Preparation of Aqueous and Acetone Extracts

For preparation of the aqueous extract, 50 g of dried *H. uninervis* powder was mixed with 500 mL of distilled water at a 1:10 (w/v) 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, and the resulting filtrate was collected and stored at 4 °C until further analysis. For acetone extraction, 10 g of dried *H. uninervis* powder was loaded into a Soxhlet extraction apparatus with 100 mL of acetone, maintaining a 1:10 (w/v) sample-to-solvent ratio. Extraction was performed for approximately 1 h at around 55 °C. The recovered acetone extract was collected and stored in a sealed container at 4 °C until phytochemical and antifungal analyses. Solvent selection and extraction conditions were based on established approaches used for the recovery of bioactive constituents from marine and seagrass-derived materials (González et al., 2016; Gono et al., 2022).

2.5 Qualitative Phytochemical Screening

The aqueous and acetone extracts of *H. uninervis* were qualitatively screened for major classes of phytochemicals, including phenols, tannins, flavonoids, glycosides, terpenoids, saponins, steroids, carbohydrates, proteins, and alkaloids. Standard qualitative chemical tests were employed to determine the presence or absence of individual phytochemical groups. Phenolic compounds were assessed using the ferric chloride test, while tannins were evaluated using ferric chloride and lead acetate reactions. Flavonoids were screened using alkaline-reagent and lead acetate tests. Glycosides and terpenoid constituents were evaluated using Liebermann–Burchard-type reactions and related qualitative procedures, while steroids were examined using the Salkowski reaction. Saponins were detected by the foam test. Carbohydrates were assessed using Molisch and iodine tests, proteins using the xanthoproteic test, and alkaloids using Wagner's reagent. The development of characteristic colour changes, precipitates, or persistent foam, as applicable to each assay, was recorded as a positive reaction.

The qualitative screening procedures were adapted from established phytochemical screening protocols described by Harborne (1998) and Trease and Evans (2002), with modifications appropriate to the seagrass extracts. The results were recorded qualitatively as positive (+) or negative (–) based on the respective test reactions. As qualitative phytochemical assays provide only preliminary evidence of the presence of broad metabolite classes, the results were not interpreted as quantitative estimates of individual compounds.

2.6 Antifungal Activity Assay

The antifungal activity of the acetone extract was evaluated against the three sugarcane-associated *Fusarium* isolates using the agar well diffusion method. The isolates were grown on PDA for approximately 5–8 days at 25–30 °C to obtain active fungal growth. A portion of the actively growing fungal material was aseptically transferred and uniformly spread over sterile PDA plates using a sterile cotton swab. Three wells were prepared in each inoculated plate using a sterile cork borer. The acetone extract was added to the respective wells at volumes of 10, 20, and 50 µL. The plates were sealed with Parafilm and incubated at 25 ± 2 °C for 72 h.

After incubation, the plates were examined for clear zones surrounding the wells. The diameter of each inhibition zone was measured in millimetres and recorded as an indicator of antifungal activity. The response of each *Fusarium* isolate to the different extract volumes was compared to assess the concentration-dependent inhibitory effect of the *H. uninervis* acetone extract. The assay was conducted under aseptic conditions to minimize external contamination.

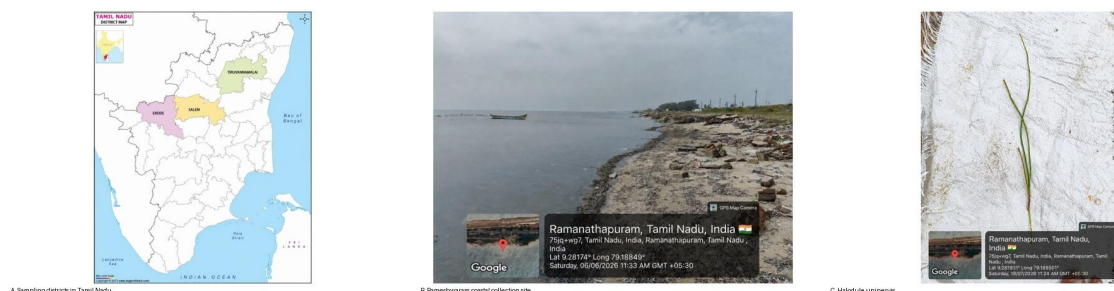


Fig 1. Sampling and collection of biological materials. (A) Selected sugarcane sampling districts in Tamil Nadu; (B) coastal collection region at Rameshwaram; (C) *H. uninervis*

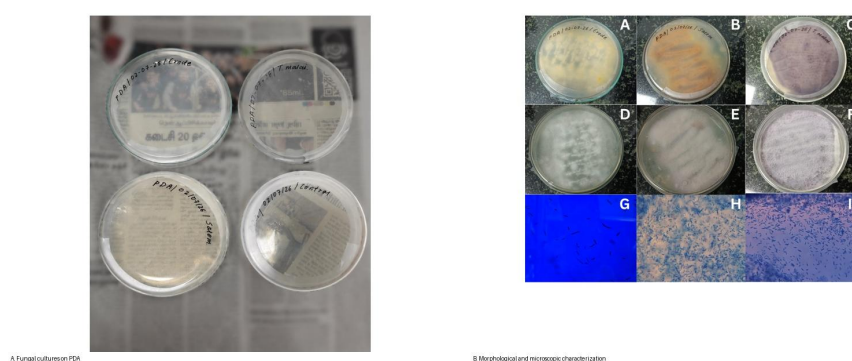


Fig 2. Isolation and characterization of sugarcane-associated fungi. (A) Representative fungal cultures on PDA; (B) morphological and microscopic observations used for identification of isolates S1, S2, S3

3. Results

3.1 Isolation and characterization of sugarcane-associated fungi

Diseased sugarcane samples collected from Erode, Salem, and Tiruvannamalai districts of Tamil Nadu were processed for the isolation of associated fungal pathogens. Following inoculation onto Potato Dextrose Agar (PDA), distinct fungal colonies developed within 3–5 days of incubation at 25–30 °C. The isolates exhibited noticeable differences in colony morphology, including colony colour, texture, margin characteristics, elevation, surface appearance, opacity, and pigmentation on the reverse side of the culture plates. Based on these cultural characteristics and subsequent microscopic observations, the three isolates were provisionally designated as *Fusarium* spp. and coded as S1, S2, and S3 according to their respective geographical origins.

The isolate obtained from the Erode diseased sugarcane sample (S1) developed a fluffy to cottony colony with a filiform margin and slightly raised elevation. The colony surface was predominantly white, with a velvety appearance and abundant aerial mycelium. Yellowish pigmentation was observed on the reverse side of the culture plate. The colony was opaque and showed progressive development during the incubation period. Microscopic examination of the isolate using lactophenol cotton blue (LPCB) preparation revealed fungal structures consistent with the morphological characteristics of *Fusarium*. Based on the combination of cultural and microscopic features, the Erode isolate (S1) was identified as *Fusarium oxysporum*.

The Salem isolate (S2) also produced a fluffy colony, but its morphological characteristics differed from those of S1. S2 exhibited an irregular colony margin with a raised elevation and a velvety surface texture. The colony front was predominantly white, while yellowish-orange pigmentation was observed on the reverse side. Unlike S1 and S3, the S2 colony was described as translucent. Abundant aerial mycelial growth was observed, and the overall colony morphology was characteristic of a filamentous fungus. LPCB microscopic examination revealed fungal structures supporting its preliminary identification as a *Fusarium* isolate. Based on the observed cultural and microscopic characteristics, S2 was designated as *Fusarium sacchari*.

The isolate obtained from Tiruvannamalai (S3) exhibited a fluffy to cottony colony with a filiform margin and slightly raised elevation. The colony surface was cottony white with prominent aerial mycelial development. Dark purple pigmentation was observed on the reverse side of the culture plate, providing a distinctive characteristic compared with the other two isolates. S3 was opaque and displayed a cottony surface texture. Microscopic examination following LPCB staining revealed fungal structures consistent with the morphological features expected for *Fusarium*. Considering the combined cultural and microscopic characteristics, the Tiruvannamalai isolate (S3) was identified as *Fusarium incarnatum*.

The observed variation in colony morphology among the three isolates demonstrates phenotypic differences among the fungal populations recovered from the different sugarcane-growing regions. Colony pigmentation, margin configuration, surface texture, aerial mycelium, and reverse pigmentation provided useful preliminary characteristics for differentiating the isolates. The differences in pigmentation were particularly evident, with S1 producing yellowish pigmentation, S2 showing yellowish-orange pigmentation, and S3 exhibiting dark purple pigmentation on the reverse side. Such morphological variation can provide preliminary taxonomic information; however, cultural characteristics may also vary depending on the growth medium, incubation conditions, age of the culture, and environmental factors.

Microscopic characterization using LPCB preparations further supported the preliminary identification based on colony morphology. The observation of fungal structures in the stained preparations provided additional evidence that the recovered isolates belonged to the genus *Fusarium*. The combination of macroscopic and microscopic characteristics was therefore used as the basis for the preliminary designation of S1 as *F. oxysporum*, S2 as *F. sacchari*, and S3 as *F. incarnatum*. Nevertheless, because several *Fusarium* species can exhibit overlapping morphological characteristics, species-level identification based exclusively on morphology should be considered provisional. Molecular characterization using appropriate genetic markers would provide greater confidence in the identification of the isolates.

The successful recovery of three morphologically distinct *Fusarium* isolates from diseased sugarcane provides a useful basis for subsequent evaluation of the antifungal activity of *H. uninervis* extracts. The isolates represented different sampling locations and exhibited different cultural characteristics, allowing their responses to the seagrass extract to be compared under the experimental conditions. The observed variation in colony morphology was also consistent with the differences subsequently recorded in their susceptibility to the acetone extract of *H. uninervis*. Among the three isolates, S1 (*F. oxysporum*) exhibited the highest inhibition response, whereas S2 (*F. sacchari*) and S3 (*F. incarnatum*) showed comparatively lower responses.

Overall, the cultural and microscopic examination successfully differentiated the three fungal isolates obtained from diseased sugarcane samples. S1 from Erode was identified as *F. oxysporum*, S2 from Salem as *F. sacchari*, and S3 from Tiruvannamalai as *F. incarnatum*. All isolates developed visible growth within 3–5 days at 25–30 °C and produced abundant aerial mycelia with distinct colony characteristics. These observations provided the preliminary morphological basis for selecting the isolates for subsequent antifungal screening. The detailed cultural and morphological characteristics of the fungal isolates obtained from diseased sugarcane are presented in Table 1.

Table 1 Cultural and morphological characteristics of fungal isolates obtained from diseased sugarcane

Characteristic	Erode-S1 <i>F. oxysporum</i>	Salem-S2 <i>F. sacchari</i>	Tiruvannamalai-S3 <i>F. incarnatum</i>
Growth period	3–5 days	3–5 days	3–4 days
Colony texture	Fluffy/cottony	Fluffy	Fluffy/cottony
Colony margin	Filiform	Irregular	Filiform

Elevation	Slightly raised	Raised	Slightly raised
Optimum temperature	25–30 °C	25–30 °C	25–30 °C
Front/reverse colour	White/yellow	White/orange-yellow	Cottony white/dark purple
Colony type	Aerial	Aerial	Aerial
Surface	Velvety	Velvety	Cottony

3.2 Processing and extraction of *H. uninervis*

H. uninervis was successfully collected from the intertidal region of Rameshwaram, Tamil Nadu. The collected seagrass material was thoroughly washed to remove adhering debris and surface contaminants. The cleaned biomass was shade-dried under suitable conditions to minimize degradation of heat-sensitive constituents. The dried material was subsequently ground into a fine powder for extraction. The final weight of the dried and powdered *H. uninervis* biomass was 374.1 g.

Aqueous and acetone extraction procedures were performed using the prepared seagrass powder. The aqueous extraction yielded approximately 248 mL of filtrate as reported in the figure caption. However, the corresponding results text reports an aqueous filtrate volume of 240 mL, representing a source-reported discrepancy. The acetone extraction performed using the Soxhlet method yielded approximately 40 mL of extract. The obtained extracts were suitable for subsequent qualitative phytochemical screening and antifungal activity evaluation.

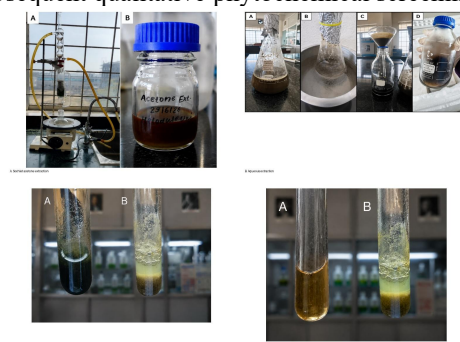


Fig 3 Processing and Extraction of *H. uninervis*

3.3 Qualitative phytochemical profile

The aqueous extract of *H. uninervis* showed positive results for several phytochemical classes. Phenols, tannins, flavonoids, saponins, carbohydrates, proteins, and alkaloids were detected in the aqueous extract. Glycosides, terpenoids, and steroids were not detected in the aqueous extract using the qualitative tests employed. The acetone extract exhibited a comparatively broader phytochemical profile. Phenols, tannins, flavonoids, glycosides, terpenoids, steroids, proteins, and alkaloids were detected in the acetone extract. Saponins and carbohydrates were not detected in the acetone extract. The differences between the extracts indicate solvent-dependent recovery of phytochemical constituents. The presence of phenols, tannins, and flavonoids in both extracts suggests their relatively broad extractability. The additional detection of glycosides, terpenoids, and steroids in the acetone extract may reflect their greater solubility in the selected solvent. The qualitative phytochemical profiles of *H. uninervis* aqueous and acetone extracts are summarized in Table 2.

Table 2 Qualitative phytochemical profile of *H. uninervis* extracts

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

The phytochemical profiles of the aqueous and acetone extracts showed noticeable differences. This contrasting profile indicates that the extraction solvent influenced the recovery of detectable phytochemical classes.

Such solvent-dependent variation may be related to differences in the polarity and solubility of bioactive constituents. Previous studies have also reported that extraction solvents can influence the chemical composition of seagrass extracts (González et al., 2016; Gono et al., 2022). Phenols, tannins, and flavonoids were detected in both aqueous and acetone extracts.

These compounds are widely associated with antimicrobial and antioxidant activities in plants and marine-derived resources. The presence of these metabolites may contribute to the biological activity observed in the present study. Previous reports have similarly highlighted the antimicrobial and antioxidant potential of phenolic compounds, tannins, and flavonoids (Supriadi et al., 2016; Kim et al., 2020; Wehbe et al., 2024). The acetone extract additionally showed glycosides, terpenoids, and steroids, indicating broader qualitative phytochemical recovery. Overall, the findings emphasize the importance of solvent selection when investigating the bioactive potential of *H. uninervis* extracts.

3.4 Antifungal activity of acetone extract

The acetone extract of *H. uninervis* exhibited inhibitory activity against all three *Fusarium* isolates. The inhibition increased with increasing extract volume in the agar well diffusion assay. The Erode isolate, identified as *F. oxysporum*, showed zones of 5, 8, and 20 mm at 10, 20, and 50 μ L, respectively. The Salem isolate, identified as *F. sacchari*, showed inhibition zones of 10, 11, and 14 mm at the respective concentrations. The Tiruvannamalai isolate, identified as *F. incarnatum*, exhibited zones of 6, 12, and 13 mm, respectively. The maximum inhibition zone of 20 mm was recorded against *F. oxysporum* at 50 μ L. This indicates comparatively greater sensitivity of *F. oxysporum* to the acetone extract. Both *F. sacchari* and *F. incarnatum* exhibited moderate inhibitory responses across the tested volumes. The concentration-dependent response suggests increased antifungal activity with higher extract loading under the assay conditions. Overall, *H. uninervis* acetone extract demonstrated promising preliminary in vitro activity against the tested sugarcane-associated *Fusarium* isolates.

Table 3. Zone of inhibition produced by *H. uninervis* acetone extract against *Fusarium* isolates.

Sampling district	Fungal isolate	10 μ L (mm)	20 μ L (mm)	50 μ L (mm)
Erode	<i>F. oxysporum</i> (S1)	5	8	20
Salem	<i>F. sacchari</i> (S2)	10	11	14
Thiruvannamalai	<i>F. incarnatum</i> (S3)	6	12	13

The antifungal activity of *H. uninervis* acetone extract increased with increasing extract concentration. The highest inhibition zone was recorded against *F. oxysporum* at 50 μ L, measuring 20 mm. This represented a four-fold increase in inhibition-zone diameter compared with the 10 μ L treatment (5 mm). Against *F. sacchari*, the inhibition zone increased from 10 mm at 10 μ L to 14 mm at 50 μ L. Similarly, *F. incarnatum* showed an increase from 6 mm to 13 mm across the tested concentrations. The results indicate a concentration-dependent inhibitory response of the extract against all three isolates. Among the tested pathogens, *F. oxysporum* exhibited the greatest susceptibility to the extract. The comparatively lower inhibition observed for *F. sacchari* and *F. incarnatum* suggests variation in fungal sensitivity. These differences may be associated with species-specific physiological characteristics and differential interactions with constituents.

Overall, the findings indicate that *H. uninervis* acetone extract has promising preliminary antifungal activity against sugarcane-associated *Fusarium* isolates under in vitro conditions

Fig4Antifungal Activity



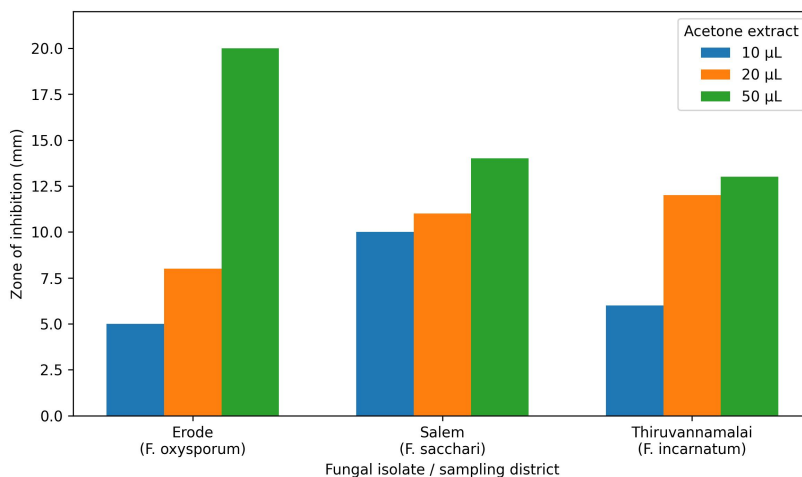


Fig 5 Concentration-dependent antifungal activity of *Halodule uninervis* extract

4. Discussion

The present study provides preliminary in vitro evidence for the antifungal potential of *Halodule uninervis* extracts against *Fusarium* isolates associated with diseased sugarcane. The acetone extract exhibited inhibitory activity against all three tested isolates, namely *Fusarium oxysporum*, *Fusarium sacchari*, and *Fusarium incarnatum*, although the magnitude of inhibition varied among the isolates. The highest inhibition zone of 20 mm was recorded against *F. oxysporum* at an extract volume of 50 µL, whereas lower inhibition zones of 14 and 13 mm were observed against *F. sacchari* and *F. incarnatum*, respectively. The observed differences indicate that the susceptibility of the tested *Fusarium* isolates to *H. uninervis* extract was not uniform. Such variation may be associated with differences in fungal physiology, cell-wall and membrane characteristics, growth rate, metabolic activity, or the ability of individual isolates to tolerate or respond to bioactive constituents present in the extract.

The concentration-dependent increase in inhibition observed in the present study suggests that greater extract loading may increase the amount of potentially active constituents available to interact with the growing fungal mycelium. In an agar diffusion system, the extent of inhibition is influenced not only by the biological activity of the compounds but also by their concentration, solubility, molecular size, polarity, and diffusion through the agar matrix. Therefore, the larger inhibition zone observed at 50 µL should be interpreted as evidence of increased inhibitory activity under the experimental conditions rather than as a direct measure of antifungal potency. Agar diffusion assays are useful for preliminary screening, but they do not provide definitive information regarding the minimum concentration required to inhibit or kill the pathogen. Accordingly, determination of minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC) would provide more quantitative information regarding the antifungal efficacy of the extract.

The phytochemical screening results provide a possible biochemical basis for the observed antifungal activity. Phenols, tannins, flavonoids, proteins, and alkaloids were detected in both aqueous and acetone extracts, while glycosides, terpenoids, and steroids were additionally detected in the acetone extract. The broader phytochemical profile observed in the acetone extract may partly explain its measurable antifungal activity. However, qualitative phytochemical screening only establishes the presence or absence of broad classes of compounds and does not determine their concentration or individual contribution to biological activity. Therefore, the relationship between the detected phytochemicals and antifungal activity should be considered tentative until supported by quantitative and chemical analyses.

Phenolic compounds and flavonoids have been widely investigated for their antimicrobial properties and may interfere with several cellular processes in microorganisms. Depending on their structure and concentration, phenolic constituents can affect membrane integrity, protein function, oxidative balance, and other essential cellular processes. Tannins may interact with proteins and extracellular or cell-associated components, potentially affecting fungal growth and development. Alkaloids and terpenoid compounds also represent chemically diverse groups with reported biological activities and may contribute to antimicrobial responses through different mechanisms. Nevertheless, the present study did not determine which specific compounds were responsible for the inhibition observed against the *Fusarium* isolates. It is therefore inappropriate to attribute the antifungal activity to any individual phytochemical class solely on the basis of qualitative screening.

The detection of multiple phytochemical groups suggests that the observed antifungal effect may result from the combined activity of several constituents rather than from a single dominant compound. Such interactions may be additive, synergistic, or, in some cases, antagonistic. The relative abundance of these constituents may also differ between extraction solvents, depending on their polarity and chemical properties. The comparatively detectable activity of the acetone extract may therefore reflect more effective extraction of certain moderately polar or non-polar constituents. However, solvent-dependent extraction efficiency alone cannot establish the identity of the active molecules. Bioassay-guided fractionation followed by chromatographic separation and spectroscopic characterization would be required to determine the chemical constituents associated with the antifungal response.

The antifungal potential observed in the present study is broadly consistent with previous reports describing the biological and phytochemical potential of *H. uninervis*. Earlier investigations have reported bioactive constituents and biological activities associated with *H. uninervis*, supporting the view that this seagrass may represent a valuable source

of natural metabolites with pharmaceutical, antimicrobial, or other biotechnological relevance (Subhashini et al., 2013; Supriadi et al., 2016; Parthasarathi et al., 2021; Gono et al., 2022; Wehbe et al., 2024). However, comparisons among studies should be made cautiously because differences in geographical origin, season, environmental conditions, biomass preparation, extraction solvent, extraction conditions, target microorganisms, and assay methodology can substantially influence phytochemical composition and biological activity. The present findings therefore extend the existing evidence by examining the antifungal activity of *H. uninervis* extracts specifically against *Fusarium* isolates recovered from diseased sugarcane.

The differences in inhibition observed among *F. oxysporum*, *F. sacchari*, and *F. incarnatum* may also reflect species- or isolate-specific responses to the extract. Fungal pathogens belonging to the same genus can differ considerably in their physiological characteristics and sensitivity to natural products. Variation in cell-wall composition, membrane permeability, detoxification mechanisms, enzymatic activity, growth rate, and stress-response pathways may influence susceptibility to plant- or marine-derived metabolites. In addition, the diffusion characteristics of individual compounds through agar may differ, meaning that inhibition-zone diameter cannot be interpreted exclusively as a measure of intrinsic antifungal potency. The comparatively larger inhibition zone recorded against *F. oxysporum* suggests greater sensitivity under the tested conditions, but additional quantitative assays are required to determine whether this isolate is genuinely more susceptible to the extract.

An important consideration is the identification of the fungal isolates. In the present study, the isolates were identified using cultural and microscopic characteristics. Although morphology-based identification can provide useful preliminary information, closely related *Fusarium* species may display overlapping morphological characteristics, making species-level identification challenging. Molecular confirmation using appropriate fungal genetic markers, together with sequence-based analysis, would strengthen the taxonomic reliability of the isolates. This is particularly important when the objective is to develop a pathogen-specific disease-management strategy. Accurate identification would also facilitate comparison with previously characterized sugarcane-associated *Fusarium* populations and improve the reproducibility of future investigations.

The current findings should also be interpreted within the limitations of the experimental design. The absence or limited description of appropriate positive and negative controls restricts the extent to which the antifungal effect can be attributed specifically to the *H. uninervis* extract. A suitable reference fungicide would provide a comparative benchmark, while solvent controls would help confirm that the observed inhibition was not caused by the extraction solvent itself. Adequate biological and technical replication, together with statistical analysis, would further establish the reproducibility and significance of differences among extract concentrations and fungal isolates. Quantitative analysis of inhibition zones should ideally be accompanied by appropriate measures of variation and statistical comparisons.

Further investigation of the extract should therefore include quantitative phytochemical estimation and chemical profiling using suitable analytical techniques. Chromatographic approaches can help separate individual constituents, while spectroscopic methods can provide information regarding their chemical structures. Fractionation of the acetone extract followed by antifungal screening would enable identification of the fractions responsible for the strongest activity. Subsequent purification and structural characterization of the active compounds could provide a clearer understanding of the chemical basis of the observed inhibition. Such an approach would also help determine whether the activity is associated with a single compound or a combination of metabolites.

Mechanistic studies represent another important area for future research. The present agar diffusion assay demonstrates growth inhibition but does not explain how the extract affects the fungal cells. Future studies could investigate effects on spore germination, hyphal development, membrane integrity, cellular leakage, oxidative stress, and other physiological processes. Microscopic examination of treated fungal hyphae could provide preliminary evidence of structural alterations, while biochemical or molecular approaches could further clarify potential cellular targets. Understanding the mechanism of action would be particularly valuable for assessing whether the extract has potential as a complementary disease-management agent and whether repeated exposure could result in reduced sensitivity.

Before considering agricultural application, the safety of the extract to sugarcane plants must also be established. Antifungal activity against a pathogen does not necessarily indicate that an extract is safe for the host plant. Phytotoxicity studies should therefore examine seed germination, root and shoot growth, biomass accumulation, chlorophyll content, and other relevant physiological parameters at concentrations showing antifungal activity. Following laboratory safety assessment, promising extract concentrations or purified fractions should be evaluated under controlled in planta conditions. Such experiments would determine whether the extract can reduce disease severity while maintaining normal plant growth and development.

Overall, the present findings support the potential of *H. uninervis* as a marine source of bioactive metabolites with antifungal activity against sugarcane-associated *Fusarium* pathogens. The concentration-dependent inhibition and the presence of diverse phytochemical constituents provide a useful basis for further investigation. However, the current evidence remains preliminary and should not yet be interpreted as proof of effective field-level disease control or as a replacement for conventional fungicides. Molecular confirmation of pathogen identity, appropriate experimental controls, quantitative MIC/MFC assays, adequate replication and statistical validation, quantitative phytochemical analysis, chemical characterization, mechanism-of-action studies, and phytotoxicity assessment are necessary to strengthen the evidence. Subsequent in planta and field experiments will be essential to determine efficacy under realistic agricultural conditions. Collectively, these investigations could establish whether *H. uninervis*-derived extracts or their purified bioactive fractions have practical value as environmentally compatible and sustainable components of integrated sugarcane disease management.

5. Conclusion

The present study provides preliminary evidence for the antifungal potential of the marine seagrass *Halodule uninervis* against fungal pathogens associated with sugarcane diseases. Diseased sugarcane samples collected from different districts of Tamil Nadu yielded three *Fusarium* isolates that were identified based on cultural and microscopic characteristics as *Fusarium oxysporum*, *Fusarium sacchari*, and *Fusarium incarnatum*. Phytochemical screening of aqueous and acetone extracts of *H. uninervis* confirmed the presence of several biologically relevant constituents. Phenols, tannins, flavonoids, proteins, and alkaloids were detected in both extracts, whereas glycosides, terpenoids, and steroids were additionally detected in the acetone extract. These findings suggest that *H. uninervis* represents a potential marine source of diverse secondary metabolites that may contribute to its biological activity.

The acetone extract exhibited measurable inhibitory activity against all three *Fusarium* isolates in the agar well diffusion assay, with inhibition increasing with increasing extract volume. The maximum inhibition zone of 20 mm was recorded against *F. oxysporum* at 50 μ L, followed by 14 mm against *F. sacchari* and 13 mm against *F. incarnatum*. The observed concentration-dependent response indicates that the acetone-soluble fraction contains constituents capable of suppressing the growth of sugarcane-associated *Fusarium* pathogens under in vitro conditions. Among the tested isolates, *F. oxysporum* showed comparatively greater sensitivity to the extract, suggesting possible differences in susceptibility among the target fungi.

Importantly, the present findings should be considered an initial assessment rather than definitive evidence of field-level disease control. The fungal isolates were identified using morphological characteristics, and therefore molecular confirmation using suitable genetic markers is necessary to establish their taxonomic identity with greater confidence. Similarly, future antifungal assays should incorporate appropriate positive and negative controls and determine quantitative parameters such as minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC). Quantitative estimation of the major phytochemical groups and chromatographic and spectroscopic characterization of the acetone extract would be valuable for identifying the compounds responsible for the observed antifungal activity.

Further research should also investigate the mechanism of antifungal action of *H. uninervis*-derived constituents, including their effects on fungal growth, membrane integrity, spore germination, and hyphal development. Assessment of phytotoxicity against sugarcane plants is essential before considering agricultural application, as antifungal activity alone does not establish crop safety. Subsequently, promising fractions or purified compounds should be evaluated through controlled in planta experiments to determine their ability to suppress disease development without adversely affecting plant growth. Field trials under different environmental and disease-pressure conditions would ultimately be required to assess consistency, efficacy, formulation stability, and practical applicability.

Overall, the study highlights *Halodule uninervis* as a promising but largely unexplored marine resource for the discovery of natural antifungal agents targeting sugarcane-associated *Fusarium* pathogens. The demonstrated in vitro inhibitory activity, together with the detection of diverse phytochemical constituents, provides a foundation for subsequent bioassay-guided fractionation and chemical characterization. With systematic validation through molecular, biochemical, phytotoxicity, in planta, and field studies, *H. uninervis*-derived bioactive fractions may contribute to the development of environmentally compatible alternatives or complementary approaches to conventional fungicide-based management of fungal diseases in sugarcane.

References

1. Aala Al-Arbash, D. A.-B. (2016). Structural and biochemical responses of the seagrass *Halodule uninervis* to changes in salinity in Kuwait Bay, Doha area. *Kuwait Journal of Science*, 43(4), 131–142.
2. Agus Supriadi, A. B. (2016). Antibacterial activity of methanol extract from seagrass of *Halodule uninervis* in the coastal of Lampung. *Der Pharmacia Lettre*, 8(4), 77–79.
3. Ashok K. Srivastava, M. K. (2012). Sugarcane production: Impact of climate change on sugarcane production. *Biodiversitas*, 13(4), 214–227.
4. Baehaki, A., H. L. (2020). Antidiabetic activity with N-hexane, ethyl-acetate and ethanol extract of *Halodule uninervis* seagrass. *Pharmacognosy Journal*, 12(4), 805–808.
5. Bahati Hakimu Msomba, P. M. (2024). Sugarcane sustainability in a changing climate: a systematic review on pests, diseases, and adaptive strategies. *Frontiers in Agronomy*, 6, 1423233.
6. Dhivakar, M. (2019). Biological approaches of *Halodule pinifolia* (Miki) Hartog on the growth and development of *Benincasa hispida* (Thunb.) Cogn. *Life Science Informatics Publications*, 5(3), 282.
7. Duli Zhao, Y.-R. L. (2015). Climate change and sugarcane production. Hindawi Publishing Corporation, 2015, 10.
8. Eneke Esoeyang Tambe Bechem, C. E. (2019). A survey of symptoms of fungal disease in sugarcane (*Saccharum officinarum* L.) in Buea, in the South West Region of Cameroon. *Annual Research & Review in Biology*, 31(3), 1–12.
9. FAO. (2024). Agricultural Outlook. OECD-FAO Agricultural Outlook 2024–2033.
10. FAO. (2026). Sugarcane. FAO Land, Water and Crop Information.
11. Fatma Sh. Abd El-Gwaid, A. S.-S. (2023). A comparative evaluation of phytochemical and antimicrobial properties of selected aquatic and terrestrial halophyte plants growing in Egypt. *International Journal of Theoretical and Applied Research*, 2(2), 169–182.
12. Fernanda Rodrigues Silva, M. L. (2024). Molecular characterization and pathogenicity of *Colletotrichum falcatum* causing red rot on sugarcane in Southern Florida. *Journal of Fungi*, 10, 742.
13. Gedela Venkata Hima Sameera, S. N. (2025). Morphological and cultural characterization of *Colletotrichum falcatum* isolates. *Plant Archives*, 25(1), 2031–2036.

14. Gono, C. M. P., A. P., Hertiani, T., Septiana, E., Putra, M., & Chianese, G. (2022). A comprehensive update on the bioactive compounds from seagrasses. *Marine Drugs*, 20, 406.
15. K. S. Dinesh Babu, V. J. (2022). A short review on sugarcane: its domestication, molecular manipulations and future perspectives. *Genetic Resources and Crop Evolution*, 69, 2623–2643.
16. Kethia L. González, R. G.-I. (2016). Determination of the antioxidant capacity of two seagrass species according to the extraction method. *Journal of Pharmacy & Pharmacognosy Research*, 4(5), 199–205.
17. Km. Nilima, A. S. (2024). A review on major diseases of sugarcane (*Saccharum officinarum*) and their management in India. *International Journal of Creative Research Thoughts*, 12(5), H828–H841.
18. Mario de Matos, F. (2020). Sugarcane biorefinery, technology and perspectives. *Elsevier*, 10, 1–19.
19. Md Imam Hossain, K. A. (2020). Current and prospective strategies on detecting and managing *Colletotrichum falcatum* causing red rot of sugarcane. *Agronomy*, 10, 1253.
20. Md Imam Hossain, K. A. (2021). Phylogenetic analysis and genetic diversity of *Colletotrichum falcatum* isolates causing sugarcane red rot disease in Bangladesh. *Biology*, 10(9), 862.
21. Md. Minnatullah, R. V. (2025). A comprehensive exploration with reference to sugarcane red rot caused by *Colletotrichum falcatum*. *Agriculture Association of Textile Chemical and Critical Reviews Journal*, 13(2), 1–10.
22. Muhammad Usmana, N. A. (2020). Sugarcane: Diseases due to pests, pest management strategies and factors influencing the production of sugarcane. *American Scientific Research Journal for Engineering, Technology, and Sciences*, 65(1), 126–139.
23. Nadine Wehbe, M. B.-S. (2024). A phytochemical analysis and the pharmacological implications of the seagrass *Halodule uninervis*: An overview. *Pharmaceuticals*, 17(8), 993.
24. Nidal Jaradat, F. H. (2015). Preliminary phytochemical screening, quantitative estimation of total flavonoids, total phenols and antioxidant activity of *Ephedra alata* Decne. *Journal of Materials and Environmental Sciences*, 6(6), 1771–1778.
25. O'Connell, A., D. J. (2022). Combining genomic selection with genome-wide association analysis identified a large-effect QTL and improved selection for red rot resistance in sugarcane. *Frontiers in Plant Science*, 13, 1–16.
26. P. Patel, R. S. (2019). Molecular identification and biocontrol activity of sugarcane rhizosphere bacteria against red rot pathogen *Colletotrichum falcatum*. *Elsevier*, 21, e00317.
27. P. Subhashini, E. D. (2013). Bioactive natural products from marine angiosperms: abundance and functions. *Natural Products and Bioprospecting*, 3(4), 129–136.
28. P. U. N. E. Srimali, D. S. (2021). Molecular characterization and pathogenicity of *Fusarium* species associated with Pokkah Boeng: An emerging disease of sugarcane in Sri Lanka. *Sugar Tech*, 24(5), 1357–1367.
29. Pakamart Sangdit, C. L. (2014). Cultural, morphological and pathological characterization of *Colletotrichum falcatum* causing red rot disease of sugarcane in Thailand. *Kasetsart Journal (Natural Science)*, 48(6), 880–892.
30. Perumal Parthasarathi, A. U. (2021). Phytochemical screening and in-vitro anticancer activity of ethyl acetate fraction of seagrass *Halodule uninervis*. *International Journal of Pharmaceutical Sciences and Drug Research*, 13(6), 677–684.
31. Posa, A. Skelton, G. R. (2006). Seagrass biodiversity of the Fiji and Samoa islands, South Pacific. *New Zealand Journal of Marine and Freshwater Research*, 40(2), 345–356.
32. Priyam Vandana, A. K. (2025). Red rot of sugarcane: A comprehensive review of pathogen biology, epidemiology and current management paradigms. *International Journal of Plant & Soil Science*, 37(12), 188–198.
33. R. Viswanathan, C. G. (2017). Epidemiology of *Fusarium* diseases in sugarcane: A new discovery of same *Fusarium sacchari* causing two distinct diseases, wilt and Pokkah Boeng. *Sugar Tech*, 19, 638–646.
34. Rajasekar, T., M. S. (2019). Screening of phytochemical, antioxidant activity and anti-bacterial activity of marine seaweeds. *International Journal of Pharmacy and Pharmaceutical Sciences*, 11(1), 61–66.
35. Rangaswani. (1958). An agar blocks technique for isolating soil microorganisms with special reference to phthiaceae fungi. *Science and Culture*, 24, 85.
36. Reynolds, P. L. (2013). Ocean: Find your blue—Seagrass and seagrass beds. *Smithsonian Ocean*.
37. Ribeiro, E. C., A., Penha, M., Nascimento, A., da Silva, D., & Mirand, R. (2025). Optimisation of Potato Dextrose Agar culture medium for Actinobacteria growth. *Microorganisms*, 13(3), 654.
38. Sharma, R., T. S. (2015). A review on red rot: The “cancer” of sugarcane. *Journal of Plant Pathology & Microbiology*, S1(003).
39. Sukunya Areeya, E. J. (2024). A review of sugarcane biorefinery: From waste to value-added products. *Applied Science and Engineering Progress*, 17(3), 7402.
40. Surroop, B., N. N.-B. (2024). Unlocking the therapeutic treasures of seagrasses: Antioxidant and antimicrobial activities of *Halophila stipulacea*, *Halodule uninervis*, and *Thalassodendron ciliatum*. *WIO Journal of Marine Science*, 23(2), 85–97.
41. Vanitha, V., L. R. (2020). A review on *Halodule uninervis* – A potent seagrass. *International Journal of Research in Pharmaceutical Sciences*, 11(1), 875–879.
42. Viswanathan. (2018). Changing scenario of sugarcane diseases in India since introduction of hybrid cane varieties: Path travelled for a century. *Journal of Sugarcane Research*, 8(1), 1–35.
43. Viswanathan, R. et al. (2021). Red rot of sugarcane (*Colletotrichum falcatum* Went). *CAB International*, 16, 23.
44. Viswanathan, R. (2022). Emerging diseases of sugarcane and new disease management strategies. *Journal of Sugarcane Research*, 12, 2–15.
45. Wenfeng Lin, C. Z. (2025). Characterization and genome analysis of *Fusarium oxysporum* provides insights into the

pathogenic mechanisms of the Pokkah Boeng disease in China. *Microorganisms*, 13(3), 573.