Original Article

Unlocking the therapeutic treasures of seagrasses: Antioxidant and antimicrobial activities of *Halophila stipulacea*, *Halodule uninervis*, and *Thalassodendron ciliatum*

Bhuvaneshwaree Surroop[1*], Nadeem Nazurally[1, 2], Deena Ramful-Baboolall[1], Arvind Ruggoo[3]

[1] Department of Agricultural and Food science, Faculty of Agriculture, University of Mauritius, Réduit, Mauritius, 80837

[2] Faculty of Fisheries, Hokkaido University, Japan, 060-0817

[3] Department of Agricultural Production and Systems, Faculty of Agriculture, University of Mauritius, Réduit, Mauritius, 80837

Western Indian Ocean JOURNAL OF Marine Science

Open access

Citation:
Surroop B, Nazurally N, Ramful-Baboolall D, Ruggoo A (2024) Unlocking the therapeutic treasures of seagrasses: Antioxidant and antimicrobial activities of *Halophila stipulacea*, *Halodule uninervis*, and *Thalassodendron ciliatum*. Western Indian Ocean Journal of Marine Science 23(2): 85-97 [doi: 10.4314/wiojms.v23i2.7]

Received:
May 25, 2024

Accepted:
October 2, 2024

Published:
December 10, 2024

*** Corresponding author:**
bsurroop28@gmail.com

Abstract
Seagrasses are essential to marine ecosystems and have been shown to possess pharmaceutical properties. This study evaluated the antioxidant and antimicrobial activities of the seagrass species, *Halophila stipulacea, Halodule uninervis*, and *Thalassodendron ciliatum* from a Mauritian lagoon. Two essential steps in the extraction process were investigated: drying method (oven-drying and freeze-drying) and maceration solvent (methanol and acetone), using a factorial design. The highest total phenolic content (60.1 mg GAE/g) was observed in oven-dried acetonic *T. ciliatum* extracts. Antioxidant activity was assessed through DPPH and ABTS assays, where all extracts demonstrated significant activities ($p < 0.05$). Oven drying and acetonic extractions resulted in greater activities (highest DPPH activity of 69.3 % with *T. ciliatum*). Significant interactions ($p < 0.05$) were found between species, drying methods, and solvents. *Escherichia coli* and *Bacillus cereus* were resistant to all extracts, while *Staphylococcus aureus* showed limited inhibition. *Pseudomonas aeruginosa* was more susceptible to freeze-dried extracts (greatest MIC of >1.25 mg/ml for acetonic *T. ciliatum*). *Candida albicans* was most susceptible to freeze dried acetonic *T. ciliatum* extracts (24.7 ± 4.06 mm). Consequently, this study encourages further exploration and use of seagrasses, especially, *T. ciliatum* in the pharmaceutical industry.

Keywords: Seagrass, Total phenolic content, Antioxidant, Antibacterial, Antifungal

Introduction
Nestled within the Indian Ocean's southern tropical belt, Mauritius is an island adorned with a diverse marine ecosystem, where seagrasses, crucial aquatic angiosperms, play a pivotal role. Montaggioni and Faure (1980) reported the presence of seven seagrass species in Mauritian lagoons, namely, *Syringodium isoetifolium, Thalassodendron ciliatum, Halophila ovalis, Halophila stipulacea, Halophila decipiens, Halodule uninervis* and *Cymodocea serrulata*. Seagrasses are vital marine species, providing nursery habitats for juvenile fish and supporting 20 % of the world's major fisheries (Madi Moussa *et al.*, 2020). They serve as key food sources for herbivores such as *Dugong dugon* and *Chelonia mydas* (Lin *et al.*, 2021). Additionally, they act as efficient carbon sinks, sequestering 27–44 Tg of organic carbon annually in sediments (Bijak *et al.*, 2023; Bedulli *et al.* 2020). Their ecological significance extends to the tourism industry in Mauritius through sediment filtration in water columns and enhanced coastline protection

from erosion by resilient rhizomes (Tandrayen-Ragoobur *et al.*, 2022; Amone-Mabuto *et al.*, 2023).

Seagrasses have demonstrated noteworthy pharmacological significance (Gono *et al.*, 2022; Lahay and Amiin, 2023; Punginelli *et al.*, 2023). In fact, the presence of phenolic compounds in seagrasses has shed light on their potential as sources of therapeutic agents (Astudillo-Pascual *et al.*, 2021; Gono *et al.*, 2022). Antioxidants counter oxidative stress from reactive oxygen species (ROS), which can contribute to cardiovascular diseases, inflammation, and cancer (Halliwell, 2024) and seagrasses have been determined to possess antioxidant potential (Sansone *et al.*, 2021; Divyashri *et al.*, 2021). However, the antioxidant potential of Mauritian seagrasses has been only been investigated by Ramah *et al.*(2014).

The scourge of infectious diseases has claimed countless lives over decades and antimicrobial resistance is further exposing mankind to risks (Manso *et al.*, 2021). In pursuit of solutions, researchers have explored natural reservoirs of antimicrobials, with seagrasses emerging as a subject of interest (Punginelli *et al.*, 2023; Ozbil *et al.*, 2024). For example, Hamisi *et al.*(2023) discovered the antimicrobial effect of seven seagrasses against a causative agent of typhoid fever, *Salmonella typhi*. Despite such studies, it remains salient that no drugs derived from seagrasses have obtained Food and Drug Administration (FDA) approval (Marine Pharmacology, 2023). Furthermore, the antimicrobial potential of seagrasses in Mauritian lagoons remain largely untapped. In the Western Indian Ocean (WIO) region itself, studies on the antimicrobial potential of seagrasses are quite limited. Hence, comprehensive studies are necessary to firmly establish seagrasses' antimicrobial potential and secure their recognition in the realm of antimicrobial drugs.

The evaluation of seagrass bioactivity requires phytochemical extraction, influenced by factors like drying methods, extraction techniques, and maceration solvent (Astudillo-Pascual *et al.*, 2021; Benjamin *et al.*, 2022). Although freeze-drying and oven-drying of seagrasses are commonly reported separately, limited research has investigated the combined effects of different drying methods and solvents, potentially leaving room for inaccuracies in the findings (Bharathi *et al.*, 2019; Susilo *et al.*, 2023).

Therefore, the primary aim of this paper is to highlight the pharmacological significance of seagrasses in Mauritius. This study delves into assessing the phenolic content, antioxidant and antimicrobial potential of three distinct seagrasses species found in the lagoons of Mauritius, namely, *Halophila stipulacea, Halodule uninervis*, and *Thalassodendron ciliatum*. Additionally, this study seeks to elucidate the influence of different drying methods (freeze-drying and oven drying) and various maceration solvents (acetone and methanol) on these identified properties.

Materials and methods

Study area and material collection and preservation

Pointe-aux-Feuilles, situated on the east coast of Mauritius at geographical coordinates 20° 18' 21" South, 57° 46' 24" East, was the collection site for fresh samples of *Halophila stipulacea, Halodule uninervis, and Thalassodendron ciliatum*. Snorkeling and diving techniques were employed to collect samples from a depth of 2-3 meters at the same location (Fig. 1). The species were identified based on their morphological characteristics, following the descriptions provided in the available literature for the WIO (Richmond, 2011). The hand-pulling technique with the aid of a small shovel was used, involving the gathering of leaves, shoots, and roots with minimum damage to the surrounding seagrass root systems. The samples were washed in seawater to remove sand particles, then placed in ice-cold conditions for transportation. Upon arrival at the University of Mauritius Zoology lab, samples underwent additional washing with tap water, then distilled water (Yuvaraj *et al.*, 2012).

Phytochemicals extraction

Fresh seagrass (70 g) was oven-dried and freeze-dried separately and ground in a grinder (PACIFIC PM600) to a fine powder. Five grams of the dried powder was soaked in 50 ml of 70 % acetone and 70 % methanol, separately, macerated for 48 hours in a shaker, followed by pump filtration and rotary evaporation. Both the dried extracts, obtained from acetone and methanol maceration, were resuspended in methanol. The percentage extract yield was then obtained using the following formula:

Percentage Yield= (W1×100)/W2
W1: Weight of extract after removing the solvent; W2-Dry weight of the sample

Quantitative estimation of Total Phenolic Content (TPC)

The procedure outlined by Nopi *et al.* (2018) was employed, using gallic acid as a standard. A mixture of

1 ml 20 % Folin Ciocalteau reagent and 200 µl seagrass extracts or gallic acid was vortexed and rested for 4 minutes. Then, 750 µl of 7 % Sodium Carbonate solution was added, vortexed, and kept in the dark for two hours. Absorbance was measured at 750 nm.

Antioxidant activity

DPPH Radical-Scavenging Assay

The procedure based on Kavitha *et al.*(2022) was followed. A 2 ml solution of methyl alcohol with DPPH (25ug/ml) was prepared. This mixture was combined with 0.5 ml of extracts or ascorbic acid, stirred, and incubated at 30 °-35 °C for 30 minutes in darkness. Absorbance was measured at 517 nm, using ascorbic acid as a standard. The percentage radical scavenging activities were then determined:

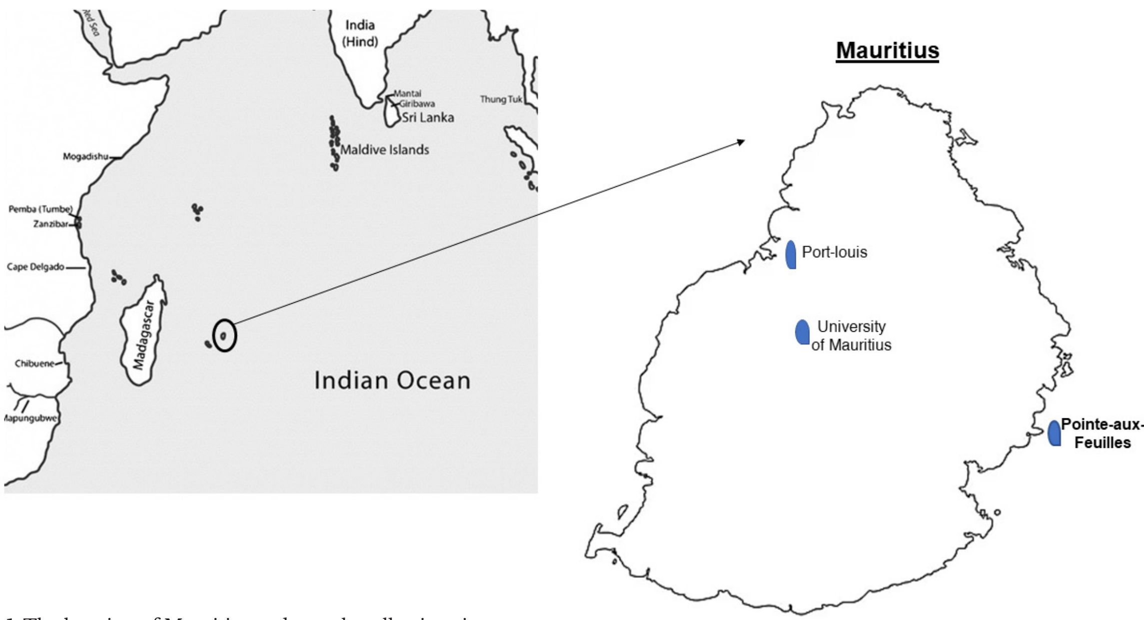

Figure 1. The location of Mauritius and sample collection site.

Percentage radical scavenging activity = 100 - ((Ac - As) / Ac) × 100
AC: absorbance of the control solution
AS: absorbance of the seagrass extracts.

ABTS Assay

The ABTS assay, based on Re *et al.* (1999) with modifications, was used, with ascorbic acid as a standard. A 7 mM ABTS stock solution was prepared by mixing ABTS with potassium persulfate, and stored in the dark for 12-16 hours. The stock solution was standardized to an absorbance of 0.700 at 734 nm. For the assay, 20 µl of crude extract or ascorbic acid was mixed with 2 ml of diluted ABTS solution, incubated for 7 minutes in the dark, and absorbance was measured at 734 nm. The absorbance values obtained were used to compute the percentage radical scavenging activities.

Antimicrobial susceptibility testing (ast)

The antimicrobial assay followed the standard Clinical and Laboratory Standards Institute (CLSI) protocol (CLSI, 2012). Pure cultures of *Bacillus cereus* ATCC 11778, *Staphylococcus aureus* ATCC 29213, *Pseudomonas aeruginosa* ATCC 27853, *Escherichia coli* ATCC 25922, and *Candida albicans* ATCC 10231 were used.

Disk diffusion assay

The Mueller-Hinton agar (MHA) disk diffusion technique was employed. Sterile 6 mm filter paper discs (Whatman #1) impregnated with 10µL of 10 mg/ml extracts were air-dried and placed on the microbial-inoculated MHA. Methanol was included as the negative control as it was previously used as the solvent for resuspending the dried extracts, while ampicillin was included as the positive control. The plates were incubated at 37 °C overnight, and the inhibition zones around the discs were measured using a vernier caliper.

Broth microdilution assay

The Broth microdilution was performed in a 96-well plate, utilizing a two-fold dilution technique, according to the method described by Eloff (1998). Methanol and nutrient broth were included as negative control, and Chloramphenicol as positive control.

Statistical design and analysis

All the tests were carried out in three replicates and the results displayed as means for the antioxidant, antimicrobial and Total Phenolic Content tests. The disk diffusion assay was displayed as mean and standard deviation. A completely randomized design with a 3x2x2 factorial treatment structure was used for the antioxidant and TPC tests. The 12 treatments were a combination of the three seagrasses, two drying methods and two maceration solvents and each were replicated thrice. The resulting data were subjected to analyses of variance (ANOVA) and the treatment sums of squares were split up into the main and interaction effects. All statistical analyses were carried out using the software package JAMOVI 2.5 (Jamovi, 2024). A significance level of 5 % was used for all the statistical tests.

Results

Percentage yield

The mass of the dry extracts was measured, and yield percentages were calculated (Fig. 2). Higher yields were obtained when the samples were oven dried and methanol-macerated (highest for *Halophila stipulacea* at 24.7 %). The lowest yield (0.610 %) was obtained with freeze-dried acetonic extract of *Halodule uninervis*.

Total phenolic content (tpc)

Total phenolic content (TPC) was measured in milligrams of gallic acid equivalent (GAE) per gram of extract, ranging from 0.447 to 60.1 mg GAE/g (*Thalassodendron ciliatum* highest). Overall, oven drying resulted in higher TPC than freeze drying for all species. Acetonic extracts consistently showed over 30 % higher TPC compared to methanolic extracts (Fig. 3).

Antioxidant activity

DPPH radical scavenging activity

Percentage inhibition of DPPH was analyzed through ANOVA. The percentage inhibition ranged from 18.0 % to 69.3 % (freeze dried *Thalassodendron ciliatum* methanolic extract and oven dried *T.ciliatum* acetonic extract, respectively). All extracts demonstrated significant

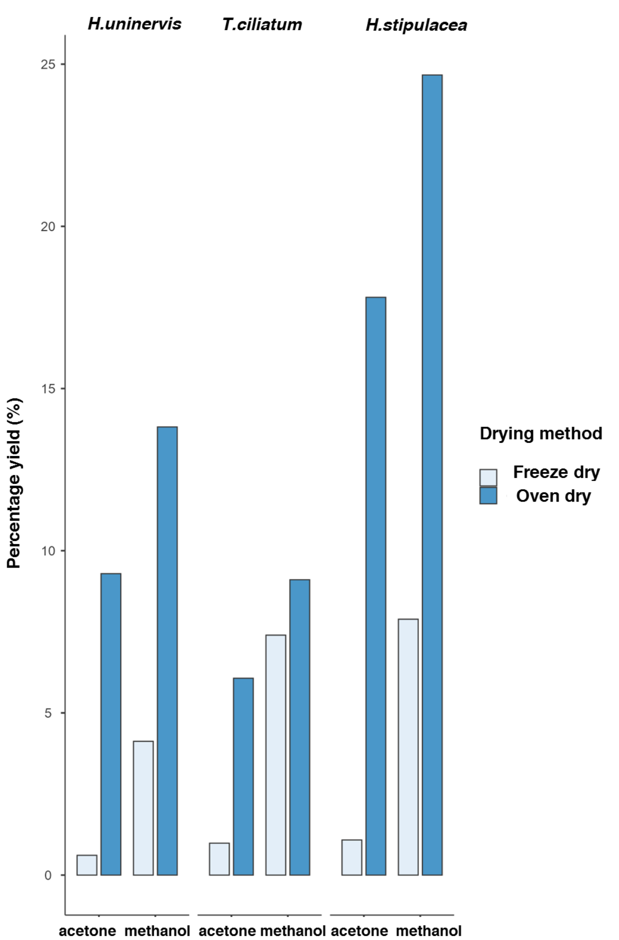

Figure 2. Dry extract percentage yield.

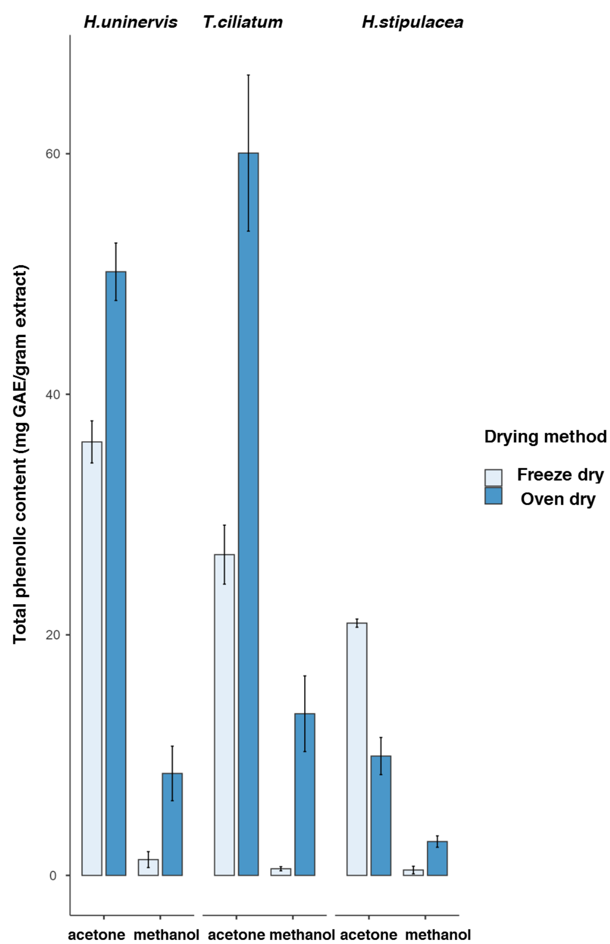

Figure 3. Total Phenolic Content of seagrass extract.

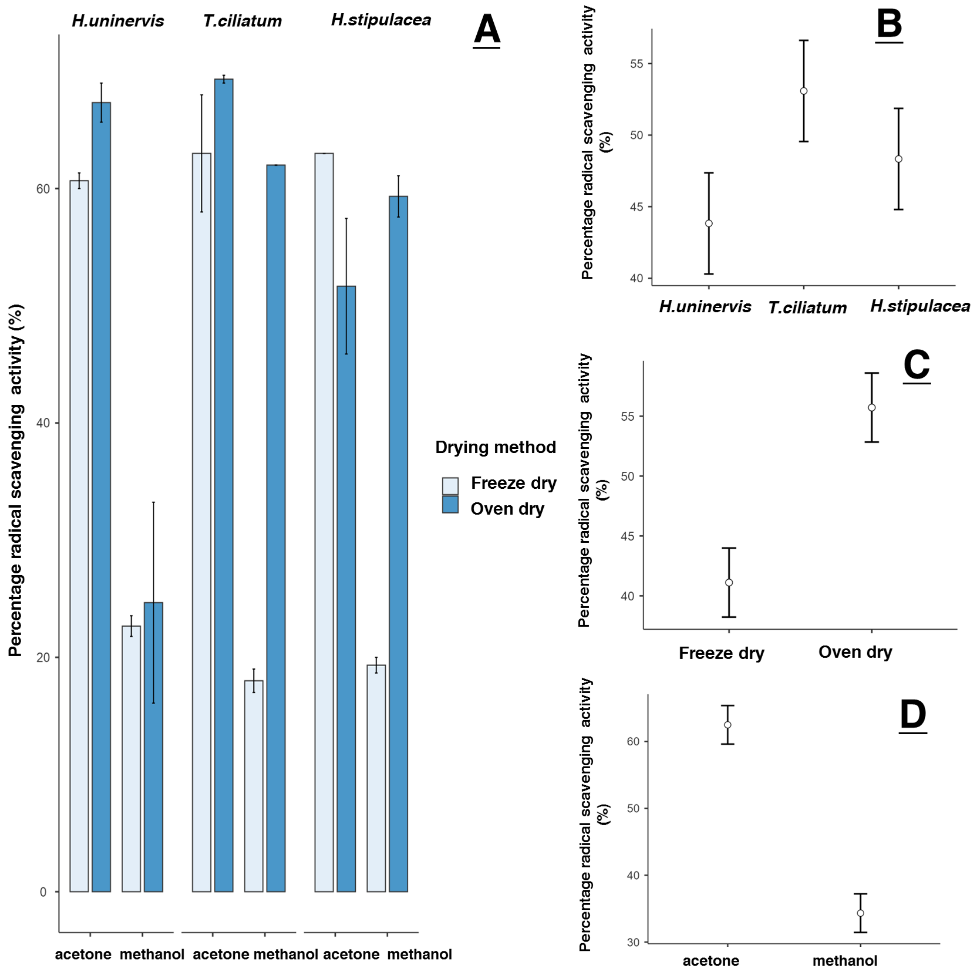

Figure 4. DPPH assay result (i). A: DPPH radical scavenging activity; B: Estimated marginal means of species; C: Estimated marginal means of drying method; D: Estimated marginal means for extraction solvent.

DPPH radical scavenging ($p < 0.05$). Main effects of the factors revealed significant distinct trends: *T.ciliatum* > *H.stipulacea* > *H.uninervis* for species, oven-drying > freeze-drying for drying method, and acetonic extracts > methanolic extracts for solvents.

The results showed clear evidence of significant interactions between the main factors ($p < 0.05$) (Fig. 4 and 5):

- **Species and drying method:** *T.ciliatum* had the highest marginal mean when oven-dried (around 65 %) and the lowest when freeze-dried (approx. 40 %). Notably, a reverse pattern emerged with freeze-drying.

- **Species and solvent:** *H.stipulacea* in methanol showed a 40 % significantly higher marginal mean inhibition than *H.uninervis*. Conversely, with acetone, *H.uninervis* exhibited higher inhibition (approx. 63 %).

- **Solvent and drying method:** In methanol, significantly higher marginal mean inhibition was seen in both freeze-drying (40 %) and oven-drying (30 %).

- **Solvent, drying method, and species:** Percentage marginal mean inhibition was relatively similar between drying methods when macerated in acetone, varying by approx. 2 %. But were significantly different when macerated in methanol.

ABTS radical scavenging activity

In the ABTS assay, *T.ciliatum* (acetone, oven-dried) exhibited the highest inhibitory activity at 29.33 %. All extracts displayed statistically significant ABTS radical scavenging activity ($p < 0.05$). Marginal plots revealed the main effects of the factors resulting in the following trends in percentage inhibition:

- Species: *T.ciliatum* > *H.stipulacea* > *H.uninervis*
- Drying Method: Freeze-drying > oven-drying
- Solvent: Acetonic extracts > methanolic extracts.

The ANOVA revealed significant interactions between the various main factors ($p < 0.05$) (Fig. 6 and 7):

- **Solvent and species**: The highest mean % inhibition was observed with *T.ciliatum* in both solvents, with a lower value in methanol (approx. 10 %) compared to acetone (approx. 25 %).
- **Drying method and species**: All species exhibited a higher mean % inhibition (approx. 2 %) when freeze-dried compared to oven-dried, except for *T.ciliatum*.
- **Solvent, drying method, and species**: When freeze-dried, *T.ciliatum* showed the highest mean activity (approx. 12 %) when macerated in methanol. Conversely, when oven-dried, its acetonic extract exhibited the highest activity (29 %).

Antimicrobial assay

The antimicrobial activity of the seagrass extracts was assessed in terms of zones of inhibition, as outlined in Table I. Notably, the extracts displayed pronounced effectiveness against *Pseudomonas aeruginosa*, particularly the freeze-dried samples, with larger inhibition zones (21.27 ± 3.12 mm and 27.3 ± 11mm for Acetonic Freeze-dried *T.ciliatum* and *H.stipulacea* respectively). In the case of *Staphylococcus aureus*, the highest zone of inhibition observed was 8.33 ± 7.89 mm. However, no activity was detected against *Escherichia coli* and *Bacillus cereus*. Concerning fungal strains, *T.ciliatum* extracts exhibited notable inhibition zones against *Candida albicans* (24.7 ± 4.06 mm).

A 2-fold broth microdilution assay was carried out for the freeze dried extracts to determine their MIC against *P.aeruginosa*. A minimum concentration of 1.25 mg/ml acetonic *T.ciliatum* extracts was needed for inhibition, while for acetonic *H.stipulacea*, a 5 mg/ml extract was required.(Table II)

The zones of inhibition produced by some of the seagrass extracts were subsequently compared with those of other plants, including terrestrial plants, as depicted in Table III.

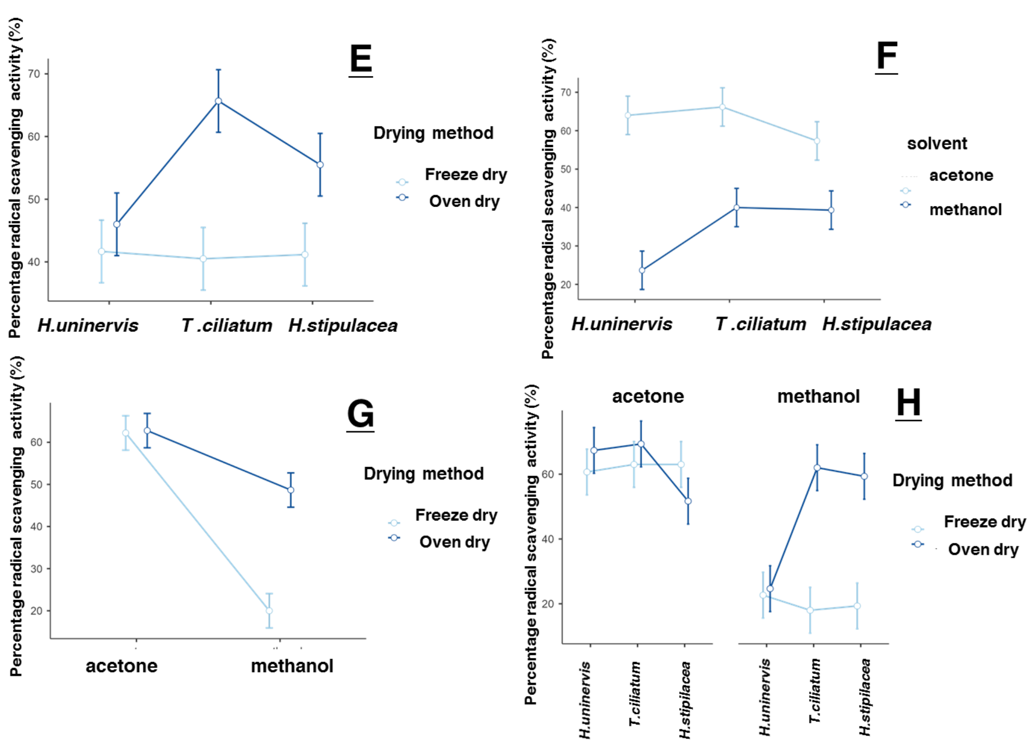

Figure 5. DPPH assay result (ii). E: Estimated marginal means Species ∗ drying method; F: Estimated Marginal Means species ∗ extraction solvent; G: Estimated Marginal Means extraction solvent ∗ Drying Method; H: Estimated Marginal Means species ∗ drying method.

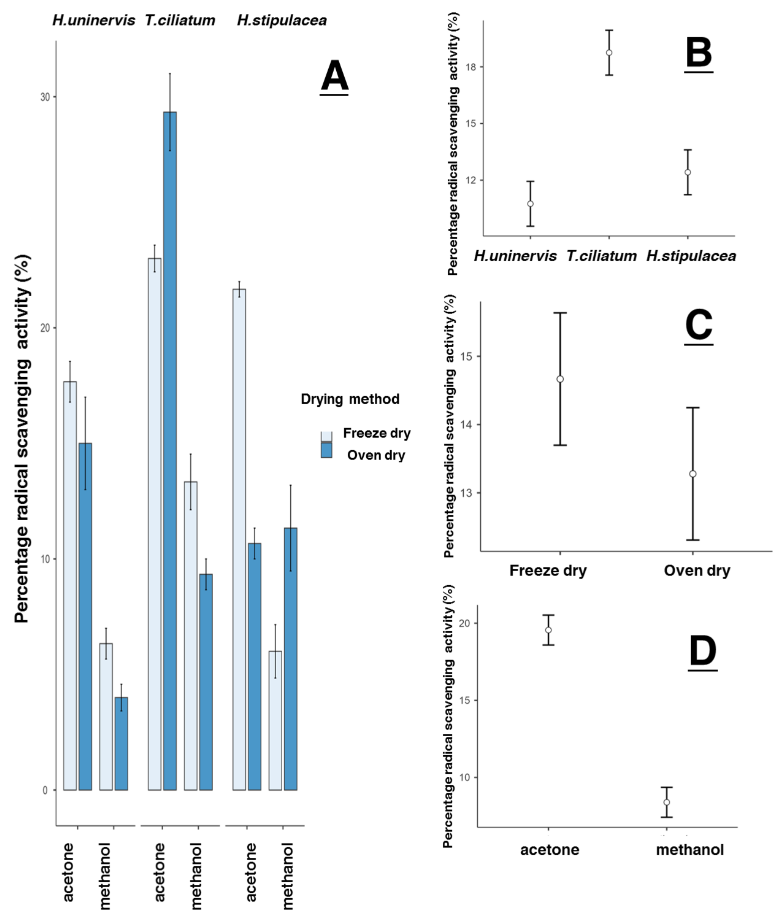

Figure 6. ABTS ASSAY RESULT (i). A: ABTS radical scavenging activity recorded(i). B: Estimated marginal means of species; C: Estimated marginal means of Drying method; D: Estimated marginal means of Extraction solvent.

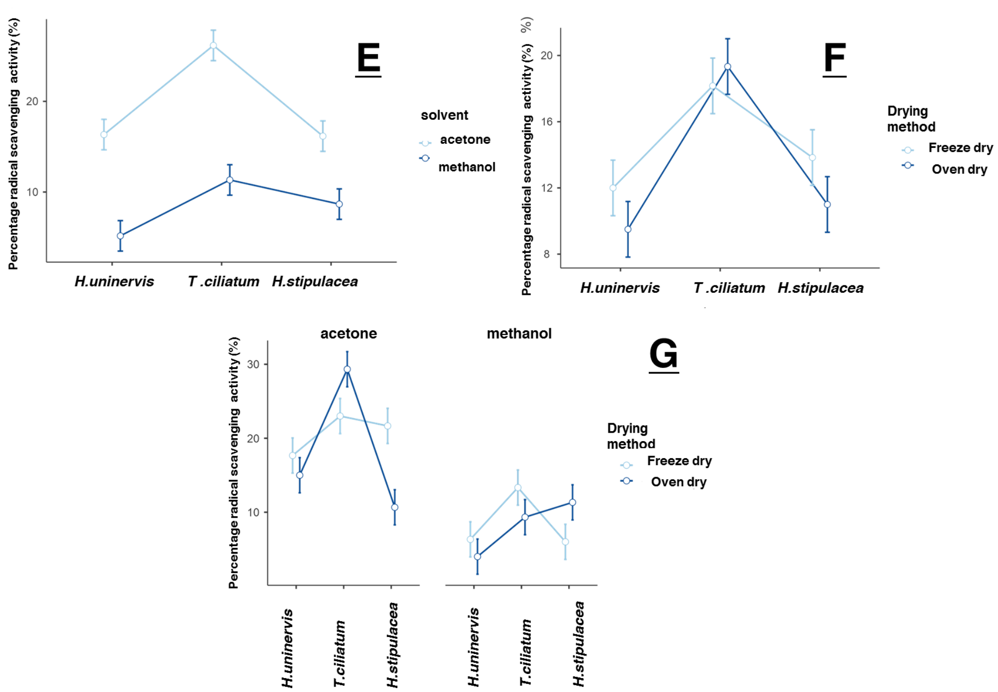

Figure 7. ABTS ASSAY RESULT (ii). E: Estimated Marginal Means species * extraction solvent; F: Estimated Marginal Means species * drying method; G: Estimated Marginal Means species * extraction solvent * drying method.

Table 1. Inhibition zones for disk diffusion assay.

Extracts			Zone of inhibition(mm)				
			Bacteria				Fungus
Species	Drying method	extraction solvent	*P. aeruginosa*	*E.coli*	*S. aureus*	*B. cereus*	*C. albicans*
H. stipulacea	Oven dry	methanol	-	-	5.17 ± 8.95	-	-
H. stipulacea	Oven dry	acetone	-	-	-	-	-
H. stipulacea	Freeze dry	methanol	10.77 ± 0.85	-	-	-	-
H. stipulacea	Freeze dry	acetone	27.3 ± 11.0	-	-	-	-
H. uninervis	Oven dry	methanol	11.63 ± 0.40	-	-	-	6.67
H. uninervis	Oven dry	acetone	8.03 ± 2.10	-	2.4 ± 4.16	-	7 ± 5.86
H. uninervis	Freeze dry	methanol	13.63 ± 2.18	-	4.37 ± 7.56	-	3.57± 6.18
H. uninervis	Freeze dry	acetone	16.6 ± 0.6	-	-	-	-
T. ciliatum	Oven dry	methanol	-	-	-	-	-
T. ciliatum	Oven dry	acetone	11.87 ± 3.88	-	8.33 ± 7.89	-	-
T. ciliatum	Freeze dry	methanol	12.13 ± 2.00	-	-	-	-
T. ciliatum	Freeze dry	acetone	21.27 ± 3.12	-	4.6 ± 7.97	-	24.7 ± 4.06
Positive control (Ampicillin for bacteria and Nystatin for fungus)			-	29.03 ± 1.33	18.1 ± 2.25	31.17 ± 9.76	-

Table 2. Minimum Inhibitory Concentration of freeze dried seagrass samples against *P. aeruginosa*.

species	extraction solvent	MIC(mg/ml)
H. stipulacea	methanol	>2.5
H. stipulacea	acetone	>5
H. uninervis	methanol	>2.5
H. uninervis	acetone	>2.5
T ciliatum	methanol	>2.5
T ciliatum	acetone	> 1.25
Chloramphenicol (positive control)	None	>0.938
Methanol		-
Nutrient broth		-

Table 3. Comparison of the antimicrobial properties of seagrass extracts with some terrestrial plants (ND: No data).

Plant		Extract	Microbe (Zone of inhibition in mm)				References
			S. aureus	P. aeruginosa	E. coli	B. cereus	
Seagrass	*T. ciliatum*	Acetonic (freeze-dried)	4.6 ± 7.97	21.27 ± 3.12	-	-	
	H. stipulacea	Acetonic (freeze-dried)	-	27.3 ± 11.0	-	-	
	H. uninervis	Acetonic (freeze-dried)	-	16.6 ± 0.6	-	-	
Mangrove	*Suaeda nudiflora*	Acetonic	5.76 ± 0.25	3 ± 0.2	ND	ND	(Eswaraiah *et al.*, 2020)
	Lumnitzera racemosa	Acetonic	7.3 ± 0.2	5.33 ± 0.15	ND	ND	
	Ipomoea tuba	Acetonic	5.83 ± 0.15	4.8 ± 0.2	ND	ND	
	Avicennia alba	Acetonic	6.83 ± 0.15	5.83 ± 0.15	ND	ND	
Aloe vera		Acetonic	12 ± 0.45	19 ± 0.57	14 ± 0.38	ND	(Nejatzadeh-Barandozi, 2013)
Licorice	*Glycyrrhiza glabra*	Methanolic	10.04 ±1.34	-	6 ±1.22	7 ±1	(Jafari-Sales and Bolouri, 2018)

Discussion

The ability of seagrass to produce secondary metabolites as a defense mechanism has drawn attention to their phytochemical properties and pharmacological potential (Kalaivani *et al.*, 2019). While numerous studies (Wisespongpand *et al.,* 2022; Lahay and Amiin, 2023; Punginelli *et al.*, 2023) on seagrass bioactivity have been conducted globally, research on Mauritian seagrasses remains limited. Only one study (Ramah *et al.*, 2014) has investigated their antioxidant potential, and none has explored their antimicrobial properties. This study, therefore, examines the phytochemical characteristics of three Mauritian seagrass species: *T. ciliatum*, *H. stipulacea*, and *H. uninervis*.

An essential aspect of this study involved optimizing the extraction process to obtain maximum phytochemical yield and bioactivity. In line with this, two drying methods (freeze-drying and oven- drying) and two solvents (Acetone and Methanol) were tested for phytochemical extraction. Oven drying yielded higher extract yield compared to freeze drying which was consistent with the results obtained by Lee *et al.*(2022) when they compared several drying methods. This difference may be ascribed to the greater porosity of the freeze-dried samples, potentially causing rapid moisture reabsorption (Benjamin *et al.*, 2022). Methanol extraction yielded higher phytochemical levels compared to acetone. The polarity of the solvent plays a crucial role in this difference, whereby methanol is more polar than acetone. In this case, it is suggested that the seagrasses possessed more polar compounds than non-polar. Ozbil *et al.* (2024), in a similar context, compared the effect of different polar and non-polar solvents including methanol and acetone, on seagrass *Posidonia oceanica* and methanol resulted in greater yield as compared to acetone in both the leaves and roots.

Phenolic compounds are renowned for their capacity to scavenge free radicals and reactive species that pose potential harm to cellular structures (Pratyusha, 2022). Throughout this study, the three seagrass species possessed different levels of phenolic compounds within their respective samples. Notably, *T. ciliatum* demonstrated the highest total phenolic content (TPC) among the three seagrasses. This discovery aligns with the study of Ramah *et al.* (2014), who also identified *T. ciliatum* as having the highest TPC among five seagrass species in Mauritius. Nevertheless, the TPC values obtained in this study were lower than those reported by Ramah *et al.*, and this difference could stem from their direct use of fresh seagrass samples, which contrasts with the dried seagrass utilized in this study. Remarkably, higher phenolic content resulted from oven drying, rather than freeze drying, deviating from some studies (Ningsih *et al.*, 2022; Wan *et al.*, 2021). Although, methanol is a commonly utilized solvent for phenolic extraction as phenolic compounds are more soluble in more polar solvents (Bharathi *et al.*, 2019), the current study revealed that acetonic extracts displayed a higher TPC than methanolic extracts..

DPPH and ABTS assays are two commonly used antioxidant assays. All the seagrass extracts in this study demonstrated significant DPPH and ABTS radical scavenging activities. Species-specific variations ($p=0.003$ for DPPH and $p<0.001$ for ABTS) were observed, with *T. ciliatum* showing the highest activity, agreeing again with the DPPH results of Ramah *et al.* (2014) in Mauritius. The obtained DPPH value for *T. ciliatum* was approximately similar with the results obtained by Hamdy *et al.* (2012) in Egypt. Hamdy *et al.* attributed this efficacy of *T. ciliatum* to the presence of flavonoids such as quercetin 3-O-β-d-xylopyranoside, asebotin, 3-hydroxyasebotin, rutin, and racemic catechin. Additionally, marked dissimilarity was observed between the ABTS and DPPH assay results, with oven drying yielding better results with the DPPH assay and freeze-drying yielding better results with the ABTS assay. The heightened antioxidant capacity observed with the DPPH assay may stem from its capacity to react with weaker antioxidants, as noted by Christodoulou *et al.* (2022). Moreover, as mentioned above, antioxidant activity arises due to the phytochemical components present. Therefore, the impact of the drying method on the targeted phytochemical compounds and their solubility is another aspect to consider (Sun *et al.*, 2015). The highest total phenolic content was observed in the oven-dried, acetone-macerated extracts, which also exhibited the strongest DPPH activity. This demonstrates that the phenolic compounds contribute significantly to antioxidant activity. This relationship of the antioxidant capacity with the TPC clarifies the significantly varying marginal means among species, drying methods, and solvents ($p<0.05$).

Heightened consumer concern about synthetic compounds has spurred research on natural antimicrobial compounds. This study explored the antimicrobial potential of three Mauritian seagrasses. Many studies (Bharathi *et al.*, 2019; Kavitha *et al.*, 2022; Amiin and Lahay, 2023) on seagrasses have shown that they possess this ability due to their phytochemical components, including phenols, flavonoids, and alkaloids (Amiin and Lahay, 2023). However, in this study, none of the seagrass extracts showed antimicrobial activity against the gram-negative *Escherichia coli*. *H. stipulacea* and other *Halophila* spp. showed effective antimicrobial activity against *E. coli* in other studies (Gumgumjee *et al.*, 2018; Yuvaraj *et al.*, 2012). Discrepancies in geography could account for this observation. Terrestrial plants like *Aloe vera* and *Glycyrrhiza glabra* could exhibit potential activity against *E. coli*, highlighting a distinction in bioactive components between marine and terrestrial plant species (Jafari-Sales and Bolouri, 2018; Nejatzadeh-Barandozi, 2013; Table III).

As for the other gram negative bacteria used in this study, varying positive results were observed against *Pseudomonas aeruginosa.* The freeze-dried acetonic extracts of *T. ciliatum* and *H. stipulacea* displayed large inhibition zones. Yet, in another study in Egypt, the same seagrass species were unable to inhibit *P. aeruginosa* (Ahmed *et al.*, 2023*)*. Conversely, oven-dried and methanolic extracts exhibited little to no activity against the bacteria, indicating a potential loss of antimicrobial components attributed to high-temperature drying and solubility of phytochemicals on maceration solvent. The MIC determination of the extracts against *P. aeruginosa* provided a more detailed insight. Species-wise, *T. ciliatum* exhibits the highest antimicrobial potential, which could be linked to *T. ciliatum*'s elevated phenolic content observed in this study. Interestingly, *H. uninervis* demonstrated a higher activity than *T. ciliatum* against the gram-negative *Salmonella typhi* in Tanzania (Hamisi *et al*., 2023). Significantly, the seagrass extracts were more effective against *P. aeruginosa* than several terrestrial plants, including the medicinal plant *Aloe vera.* (Table III).

Regarding gram-positive bacteria, *Staphylococcus aureus* and *Bacillus cereus* were non-susceptible to the extracts. In contrary, in the WIO region, Mabrouk *et al.*, (2024) reported *H. stipulacea* as more effective against gram-positive bacteria (including *S. aureus*) than against gram-negative ones (including *P. aeruginosa*). On a similar note, *S. aureus* and *B. cereus* could also resist several seagrass leaf extracts of *Cymodocea rotundata* and *Cymodocea serrulata* (Wisespongpand *et al.,* 2022). Gram-positive bacteria have thicker cell walls containing teichoic acids, which are absent in gram-negative bacteria, which may explain this non-susceptibility (Jubeh *et al.,* 2020). Conversely, studies on mangroves and other terrestrial plants demonstrated significant inhibition of *S. aureus* growth, and *Glycyrrhiza glabra* extract effectively inhibited *B. cereus* (Table III). As for fungus, *Candida albicans* was most susceptible to the freeze-dried acetonic extract of *T. ciliatum,* while the other species exhibited minimal to no activity. Although anti-candidal studies of these species is absent in the WIO region, outside the WIO, *H. stipulacea* and other seagrasses were found to act against *C. albicans* (Mabrouk *et al.*, 2024; Punginelli *et al.*, 2023) .

Conclusions

In conclusion, to the knowledge of the authors, this is the first study in the WIO region assessing the combined effect of drying method and maceration solvent on the bioactivity of the three seagrasses, *H. stipulacea, H. uninervis, and T. ciliatum.* This study allowed identification of the optimum maceration solvent, drying method and species to use to obtain a high antimicrobial and antioxidant activity. Specifically, oven-drying and acetone proved more effective in extracting phenolic compounds. Notably, among the examined species, *T. ciliatum* demonstrated the highest antioxidant potential when subjected to oven drying and macerated in acetone, while also possessing the highest phenolic compounds. This study has also, for the first time, determined the antimicrobial activity of seagrasses in Mauritius. *T. ciliatum* displayed remarkable antimicrobial efficacy against *P. aeruginosa* and *Candida albicans*, particularly when freeze-dried and macerated in acetone. It is therefore recommended that more studies are carried out on the use of seagrass extracts, especially, *T. ciliatum*, in the pharmaceutical industry.

Acknowledgments

Authors are grateful to all the staff in University of Mauritius whose assistance was a milestone in the preparation and completion of this study.

References

Ahmed F, Mahmoud A-B, EL-Swaify Z, Salah El-Din R (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: 169-182 [doi:10.21608/ijtar.2023.220991.1070]

Amiin MK, Lahay AF (2023) Anti-bacterial effectiveness of *Cymodocea rotundata* extract and assay for primary bioactive composition. Journal of Aquatropica Asia 8: 6-12 [doi: 10.33019/joaa.v8i1.3920]

Amone-Mabuto M, Mubai M, Bandeira S, Shalli MS, Adams JB, Lugendo BR, Hollander J (2023) Coastal community's perceptions on the role of seagrass ecosystems for coastal protection and implications for management. Ocean & Coastal Management 244: 106811 [doi: 10.1016/j.ocecoaman.2023.106811]

Astudillo-Pascual M, Domínguez I, Aguilera PA, Garrido Frenich A (2021) New phenolic compounds in *Posidonia oceanica* seagrass: a comprehensive array using high resolution mass spectrometry. Plants 10: 864 [doi: 10.3390/plants10050864]

Bedulli C, Lavery PS, Harvey M, Duarte CM, Serrano O (2020) Contribution of seagrass blue carbon toward carbon neutral policies in a touristic and environmentally-friendly island. Frontiers in Marine Science 7: 1 [doi: 10.3389/fmars.2020.00001]

Benjamin MAZ, Ng SY, Saikim FH, Rusdi NA (2022) The effects of drying techniques on phytochemical contents and biological activities on selected bamboo leaves. Molecules 27 (19): 6458 [doi: 10.3390/molecules27196458]

Bharathi NP, Jayalakshmi M, Amudha P, Vanitha V (2019) Phytochemical screening and in vitro antioxidant activity of the seagrass *Cymodocea serrulata*. Indian Journal of Geo Marine Sciences 48 (08): 1216-1221

Bijak AL, Reynolds LK, Smyth AR (2023) Seagrass meadow stability and composition influence carbon storage. Landscape Ecology: 4419-4437 [doi: 10.1007/s10980-023-01700-3]

Christodoulou MC, Orellana Palacios JC, Hesami G, Jafarzadeh S, Lorenzo JM, Domínguez R, Moreno A, Hadidi M (2022) Spectrophotometric methods for measurement of antioxidant Activity in food and pharmaceuticals. Antioxidants 11: 2213 [doi:10.3390/antiox11112213]

CLSI (2012) Performance standards for antimicrobial disk susceptibility tests; Approved s tandard—Eleventh Edition. CLSI document M02-A11. Wayne, PA, Clinical and Laboratory Standards Institute, Vol. 32. 76 pp

Divyashri S, Arivarasu L, Sivaperumal P, Thangavelu L (2021) Antioxidant activity from *Cymodocea serrulata* seagrass crude extract. Journal of Pharmaceutical Research International 33 (61B): 309-317 [doi:10.9734/jpri/2021/v33i61B35603]

Eloff JN (1998) A sensitive and quick microplate method to determine the minimal inhibitory concentration of plant extracts for bacteria. Planta Medica 64 (8): 711-713 [doi:10.1055/s-2006-957563]

Eswaraiah G, Peele KA, Krupanidhi S, Kumar RB, Venkateswarulu TC (2020) Studies on phytochemical, antioxidant, antimicrobial analysis and separation of bioactive leads of leaf extract from the selected mangroves. Journal of King Saud University - Science 32 (1): 842-847 [doi:10.1016/j.jksus.2019.03.002]

Gono CMP, Ahmadi P, Hertiani T, Septiana E, Putra MY, Chianese G (2022) A comprehensive update on the bioactive compounds from seagrasses. Marine Drugs 20 (7): 406 [doi:10.3390/md20070406]

Gumgumjee NM, Bukhari DA, Alshehri WA, Hajar AS (2018) Antibacterial activity of *Halodule uninervis* leaves extracts against some bacterial pathogens strains. Pharmacophore 9 (2): 52-59

Halliwell B (2024) Understanding mechanisms of antioxidant action in health and disease. Nature Reviews Molecular Cell Biology 25: 13-33 [doi: 10.1038/s41580-023-00645-4]

Hamdy AH, Mettwally WSA, Fotouh MAE, Rodriguez B, El-Dewany AI, El-Toumy SAA, Hussein AA (2012) Bioactive phenolic compounds from the Egyptian Red Sea seagrass *Thalassodendron ciliatum*. Zeitschrift für Naturforschung C 67 (5-6): 291-296 [doi:10.1515/znc-2012-5-608]

Hamisi MI, Mbusi LD, Lyimo TJ (2023) Antibacterial activity against *Salmonella typhi* and phytochemical screening of seven seagrass species from the coast of Tanzania. Western Indian Ocean Journal of Marine Science 22: 83-93

Jafari-Sales A, Bolouri P (2018) Evaluation of the antimicrobial effects of *Glycyrrhiza glabra* on some gram positive and gram negative pathogenic bacteria in laboratory conditions. Jorjani Biomedicine Journal 6: 78-84 [doi:10.29252/jorjanibiomedj.6.4.78]

Jubeh B, Breijyeh Z, Karaman R (2020) Resistance of gram-positive bacteria to current antibacterial agents and overcoming approaches. Molecules 25: 2888 [doi:10.3390/molecules25122888]

Kalaivani P, Kavitha D, Vanitha V (2019) A review on phytochemical and pharmacological activities of *Syringodium isoetifolium*. International Journal of Research in Pharmaceutical Sciences 11: 207-214 [doi: 10.26452/ijrps.v11i1.1808]

Kavitha D, Padmini R, Chandravadivelu G, Magharla DD (2022) Phytoconstituents screening and antioxidant activity of *Syringodium isoetifolium* leaf extracts. Indian Journal of Pharmaceutical Sciences 84(5): 1309-1322 [doi:10.36468/pharmaceutical-sciences.1028]

Lahay AF, Amiin MK (2023) Antibacterial potential of seagrass *Cymodocea rotundata* (Alismatales: *Cymodoceaceae*) extract on the pathogenic bacteria *Staphylococcus aureus*. Jurnal Biologi Tropis 23: 355-360 [doi:10.29303/jbt.v23i2.4884]

Lee SY, Ferdinand V, Siow LF (2022) Effect of drying methods on yield, physicochemical properties, and total polyphenol content of chamomile extract powder. Frontiers in Pharmacology. 13: 1003209 [doi: 10.3389/fphar.2022.1003209]

Lin J, Liu X, Lai T, He B, Du J, Zheng X (2021) Trophic importance of the seagrass *Halophila ovalis* in the food web of a Hepu seagrass bed and adjacent waters, Beihai, China. Ecological Indicators 125: 107607 [doi:10.1016/j.ecolind.2021.107607]

Mabrouk SB, Grami B, Ayache SB, Kacem A (2024) Antioxidant, antimicrobial and analgesic activities of the invasive seagrass *Halophila stipulacea* leaf and stem extracts. Authorea Preprints [doi:10.22541/au.171231882.23748315/v1]

Madi Moussa R, Bertucci F, Jorissen H, Gache C, Waqalevu VP, Parravicini V, Lecchini D, Galzin R (2020) Importance of intertidal seagrass beds as nursery area for coral reef fish juveniles (Mayotte, Indian Ocean). Regional Studies in Marine Science 33: 100965 [doi:10.1016/j.rsma.2019.100965]

Manso T, Lores M, de Miguel T (2021) Antimicrobial activity of polyphenols and natural polyphenolic extracts on clinical isolates. Antibiotics 11: 46 [doi:10.3390/antibiotics11010046]

Marine Pharmacology (2023) Marine Pharmacology. Mysite. [https://www.marinepharmacology.org]

Montaggioni L, Faure G (1980) Les Récifs coralliens des Mascareignes (Océan Indien). Université française de l'Océan indien, Centre universitaire de la Réunion. 151 pp

Nejatzadeh-Barandozi F (2013) Antibacterial activities and antioxidant capacity of *Aloe vera*. Organic and Medicinal Chemistry Letters 3 (1): 1-8 [doi:10.1186/2191-2858-3-5]

Ningsih AW, Syahrani A, Wahyuni KI (2022) Study of drying methods and extraction methods on phenolic content. In: International Conference on Green Energy and Materials Technology, Vol.1(1), Bandung City

Nopi NS, Anwar E, Nurhayati T (2018) Optimization of extraction condition to obtain antioxidant activity and total phenolic content of seagrass *Thalassia hemprichii* (Ehrenb.) Asch from Indonesia. Pharmacognosy Journal 10 (5): 958-962 [doi:10.5530/pj.2018.5.162]

Ozbil E, Ilktac M, Ogmen S, Isbilen O, Ramirez JMD, Gomez J, Walker JN, Volkan E (2024) In vitro antibacterial, antibiofilm activities, and phytochemical properties of *Posidonia oceanica* (L.) Delile: An endemic Mediterranean seagrass. Heliyon 10: e35592 [doi: 10.1016/j.heliyon.2024.e35592]

Pratyusha S (2022) Phenolic compounds in the plant development and defense: an overview. In: Plant stress physiology - perspectives in agriculture. IntechOpen 11: 125-140 [doi:10.5772/intechopen.102873]

Punginelli D, Catania V, Abruscato G, Luparello C, Vazzana M, Mauro M, Cunsolo V, Saletti R, Di Francesco A, Arizza V, Schillaci D (2023) New bioactive peptides from the Mediterranean seagrass *Posidonia oceanica* (L.) Delile and their impact on antimicrobial activity and apoptosis of human cancer cells. International Journal of Molecular Sciences 24: 5650 [doi:10.3390/ijms24065650]

Ramah S, Etwarysing L, Auckloo N, Gopeechund A, Bhagooli R, Bahorun T (2014) Prophylactic antioxidants and phenolics of seagrass and seaweed species: a seasonal variation study in a southern Indian Ocean island, Mauritius. Internet Journal of Medical Update-EJOURNAL 9 (1): 27-37

Re R, Pellegrini N, Proteggente A, Pannala A, Yang M, Rice-Evans C (1999) Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free Radical Biology and Medicine 26: 1231-1237 [doi:10.1016/S0891-5849(98)00315-3]

Richmond M (2011) A field guide to the seashores of Eastern Africa and the Western Indian Ocean Islands. 3rd edition, Swedish International Development Agency (SIDA), Stockholm, WIOMSA. 464 pp

Sansone C, Galasso C, Lo Martire M, Fernández TV, Musco L, Dell'Anno A, Bruno A, Noonan DM, Albini A, Brunet C (2021) In vitro evaluation of antioxidant potential of the invasive seagrass *Halophila stipulacea*. Marine Drugs 19 (1): 37 [doi:10.3390/md19010037]

Sun Y, Shen Y, Liu D, Ye X (2015) Effects of drying methods on phytochemical compounds and antioxidant activity of physiologically dropped un-matured citrus fruits. LWT - Food Science and Technology 60: 1269–1275 [doi:10.1016/j.lwt.2014.09.001]

Susilo B, Setyawan HY, Prianti DD, Handayani MLW, Rohim A (2023) Extraction of bioactive components on Indonesian seagrass (*Syringodium isoetifolium*) using green emerging technology. Food Science and Technology 43: e086722 [doi:10.1590/fst.086722]

Tandrayen-Ragoobur V, Tengur ND, Fauzel S (2022) COVID-19 and Mauritius' tourism industry: an island perspective. Journal of Policy Research in Tourism, Leisure and Events: 1-17 [doi:10.1080/19407963.2022.2028159]

Jamovi (2024) The Jamovi Project (Version 2.5) [Computer Software]. [https://www.jamovi.org]

Wan Nasir WNH, Ibrahim NNA, Woon KH, Abu Bakar Sajak A, Sofian-Seng N-S, Wan Mustapha WA, Abdul Rahman H (2021) Effects of different drying methods and solvents on biological activities of *Curcuma aeruginosa* leaves extract. Sains Malaysiana 50 (8): 2207-2218

Wisespongpand P, Khantavong A, Phothong P, Wanghom W (2022) Antimicrobial, antioxidant, and antifouling activity from extracts of aboveground and belowground parts of seagrasses *Cymodocea rotundata* and *Cymodocea serrulata*. Letters in Drug Design & Discovery 46: 123-129

Yuvaraj N, Kanmani P, Satishkumar R, Paari A, Pattukumar V, Arul V (2012) Seagrass as a potential source of natural antioxidant and anti-inflammatory agents. Pharmaceutical Biology. 50 (4) 458-467 [doi:10.3109/13880209.2011.611948]