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Research Article

Antifungal activity of extracts from *Ulva*, *Sargassum*, and *Gracilaria* against three fungal pathogens and GC-MS analysis of the most effective extracts

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Abstract: Coastal marine macroalgae are highly diverse and rich in bioactive compounds, though only a few studies have explored their antifungal potential against plant pathogens in Sri Lanka. This study investigated the antifungal activity of *Ulva* sp., *Gracilaria* sp., and *Sargassum* sp. from Thalpe Reef, Galle, Sri Lanka, against the fungal pathogens *Lasiodiplodia theobromae, Pseudopestalotiopsis theae,* and *Diaporthe eugeniae*. These pathogens cause leaf necrosis, leaf chlorosis, and leaf blight, respectively, in *Solanum melongena* plants. To evaluate the antifungal activity of each species, sequential crude extraction was performed using ethyl acetate and methanol. The poisoned food technique was used to screen the antifungal activity and extracts showing the highest antifungal activity were further analyzed using Gas Chromatography-Mass spectrometry (GC-MS). The best inhibition against *D. eugeniae* and *P. theae* was exhibited by *Ulva*-ethyl acetate (UE) at 2000 ppm with inhibition percentages of 79.29% and 56.68%, respectively. *Ulva*-methanol (UM) at 2000 ppm showed the highest inhibition against *L. theobromae*, with an inhibition percentage of 43.09%. These results revealed that UE and UM extracts effectively controlled tested fungal pathogens. GC-MS analysis revealed the presence of three compounds in UE, nine in UM, and seven in *Gracilaria*-ethyl acetate (GE) extracts. Notably, the most abundant compounds with potential antifungal activity included Dihydroactinidiolide (30.02%), 4-Hydroxy-2-butanone (37.37%), and 6,10,14-Trimethylpentadecan-2-one (58.86%).

1. INTRODUCTION

Marine macroalgae, or seaweeds, are multicellular, eukaryotic, photosynthetic organisms (Makkar *et al*., 2016). They are classified into three divisions based on pigmentation: Chlorophyta (green algae), Phaeophyta (brown algae), and Rhodophyta (red algae) (Biris-Dorhoi *et al*., 2020). By 2009, 125 macroalgal taxa including 44 Chlorophyceans, 10 Phaeophyceans, and 71 Rhodophyceans, had been identified along the Sri Lankan coast. The Thalpe Reef in Sri Lanka features an extensive coastline with a diverse population of marine macroalgae, where *Ulva*, *Sargassum*, and *Gracilaria* are the most abundant genera for their respective divisions (Coppejans *et al*., 2009).

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Marine algae are utilized as food sources for both humans and animals, as fertilizers, biofuels, and raw materials for industrial products such as agar, carrageenan, and alginate (Jayasinghe *et al.*, 2018). This is due to their high content of complex organic compounds, alongside primary and secondary metabolites with diverse biological activities. The bioactive chemicals in macroalgae include carbohydrates, peptides, lipids, enzymes, vitamins, phytopigments (xanthophylls and carotenoids), phenolic compounds, tannins, and terpenoids (Biris-Dorhoi *et al.*, 2020). These compounds exhibit various pharmacological activities, such as anticancer, antioxidant, antimicrobial, antifungal, antiviral, and anti-inflammatory effects (Pérez *et al*., 2016). The extent of their antifungal and antioxidant effect is generally attributed to their phenolic compositions (Jayaprakasha *et al*., 2003).

Plant diseases significantly impact crop yield, with over US$ 220 billion spent annually on disease management practices (FAO, 2022). These diseases can be caused by various agents, including bacteria, viruses, nematodes, parasitic plants, and especially fungi. Currently, farmers heavily rely on chemical pesticides and fertilizers to boost crop production. According to Padmajani *et al*. (2014), herbicides are the most commonly used pesticides in Sri Lanka followed by insecticides. The vegetable sector heavily relies on insecticides, with fungicides being the second most prevalent (Nagenthirarajah & Thiruchelvam, 2008). In 2011, Sri Lanka imported 8902.87 metric tons of pesticides, a 49% increase from 2006 (Padmajani *et al*., 2014). Further, significant concentrations of organo-chlorine and organophosphate pesticides have been detected in the Walawe and Nilwala rivers (De Silva, 2003). Also, it is estimated that more than 50% of pesticides do not reach their target and instead contaminate the soil (Padmajani *et al*., 2014). The improper use of pesticides leads to immediate health effects as well as long-term health risks such as cancer, kidney ailments, and reproductive problems. Furthermore, in Sri Lanka, farmers who apply pesticides are at a higher risk of developing chronic renal failure (Wanigasuriya *et al*., 2007). Consumer attitudes toward pesticide use in agriculture have shifted due to these health impacts and environmental damage, increasing demand for safer and more efficient alternatives (Aktar *et al*., 2009). Marine resources, including algal species, offer a vast reservoir of unique, biologically active compounds. These species thrive under extreme climatic and environmental stresses such as high salinity, intense light, and high temperatures, making them potential sources for discovering novel and effective compounds for plant disease management (Maldeniya *et al.,* 2020).

Few studies have investigated the functional properties of Sri Lankan macroalgal extracts, particularly their antifungal properties against plant pathogens. Therefore, this study aims to determine the antifungal potential of ethyl acetate and methanol extracts from *Ulva* sp., *Sargassum* sp., and *Gracilaria* sp. against the fungal pathogens *Diaporthe eugeniae*, *Pseudopestalotiopsis theae* and *Lasiodiplodia theobromae*, which affect *Solanum melongena*. The poisoned food technique was used to assess antifungal activity, and Gas Chromatography-Mass Spectrometry (GC-MS) analysis was employed to identify the potential antifungal compounds present in the algal extracts.

2. MATERIAL and METHODS

2.1. Sample Collection and Preparation

2.1.1 *Collection of macroalgae samples*

Samples of *Ulva* sp., *Sargassum* sp., and *Gracilaria* sp. were collected from Thalpe Reef (6.00^{o} N, 80.29^{o} E) in Galle, Southern Province of Sri Lanka, based on their abundance in September 2022 (Figure 1). The algal samples were hand-picked and placed in zip-lock bags half-filled with seawater for transport to the laboratory at the University of Kelaniya, Sri Lanka. Initially, the samples were washed with seawater to remove sand particles, invertebrates, and epiphytes, followed by a rinse with tap water to eliminate salt. The algal samples were identified based on macroscopic and microscopic morphological characteristics as described by Durairatnam (1961) and Coppejans *et al*. (2009). The algal samples were then air-dried for approximately ten days and ground into a fine powder using an electric blender. The powdered samples were stored in sterilized glass bottles and algal extracts preparation was started on the same day.

Figure 1. Thalpe Reef, Sri Lanka.

2.1.2 *Preparation of macroalgal extracts*

Bioactive compounds in 7.0 g of dried powder from each macroalgal sample were sequentially extracted using 150 mL of ethyl acetate in a Soxhlet apparatus (Electrothermal, Canada) for four hours, followed by extraction with an equal amount of methanol (Martins *et al*., 2018). The heating mantle temperature of the Soxhlet apparatus was kept below the boiling points of each solvent (Radhika & Mohideen, 2015). Organic solvents were evaporated under reduced pressure using a rotary evaporator (BIOBASE, RE-201D, China) at 35 rpm and 40 ^{o}C to obtain the crude extracts, which were then stored in a refrigerator at 4 ^{o}C until further use. The percentage yield (% yield) of the crude product was determined using the equation provided by Agbaje-Daniels *et al*. (2020).

$$\% \text{ yield } = \frac{\text{Weight of the crude (g)}}{\text{Weight of the dried algae powder used for extraction (g)}} \text{ x } 100$$

2.1.3 *Preparation of fungal cultures*

Fungal cultures of *Diaporthe eugeniae* U11 (MT990529), *Pseudopestalotiopsis theae* U10 (MT990526) and *Lasiodiplodia theobromae* H32A (MT990527), isolated from *Solanum melongena* (brinjal) leaves showing symptoms of leaf blight, leaf yellowing and leaf necrosis, respectively, were obtained from the Department of Plant and Molecular Biology at the University of Kelaniya. Mycelial discs (5 mm) were cut using a cork borer and aseptically transferred onto petri plates containing potato dextrose agar (PDA). Petri plates were incubated at room temperature (30±2 ^{o}C).

2.2 Screening of Macroalgal Extracts for Antifungal Activity

The poisoned food technique was performed as described by Abhishek *et al*. (2021). Four concentrations of algal extract (250 ppm, 500 ppm, 1000 ppm, and 2000 ppm), were incorporated into PDA plates. The required weight of crude algal extract for each concentration was measured and dissolved in 200 µL of dimethyl sulfoxide (DMSO). The dissolved crude was then mixed into melted PDA medium (40 ^{o}C) in a conical flask. Fifteen mL of the agar-crude extract mixture was poured into petri plates (poisoned plates) and allowed to solidify.

Agar disks containing the fungus (5 mm diameter) were cut using a cork borer from the peripheral regions of seven-day-old cultures of *D. eugeniae, P. theae* and *L. theobromae,* and transferred to the center of the poisoned agar plates. Five replicates were prepared for each experiment. Additionally, three negative controls were prepared by adding equal amounts of 0.03% (v/v) DMSO without algal extracts, and three positive controls were prepared with Captan (a commercial fungicide) at a concentration of 1000 ppm. Plates with *D. eugeniae* and *P. theae* were incubated at room temperature (30±2 ^{o}C) for seven days, while plates with *L. theobromae* were incubated at the same temperature for 24 hours. After the incubation period, the radial growth of the fungal colony (diameter in mm) was measured using a ruler along two perpendicular axes and the average diameter was calculated. The inhibition percentage of

fungus was calculated using the following equation based on the average radial growth (Ammar *et al.*, 2017).

$$I\ \% = (C-T)/C \times 100$$

Where, I % = inhibition percentage, C = radial growth in control DMSO plates, T = radial growth in plates with each concentration of crude extract

2.3 Statistical Analysis

All the data were presented as mean values ± standard error. The Kruskal-Wallis test and Dunn's test were used for the statistical analysis of percentage inhibition data using R software (version 4.3.3).

2.4 GC-MS Profiling of Selected Macroalgal Extracts

The *Ulva*-ethyl acetate (UE), *Ulva*-methanol (UM), and *Gracilaria*-ethyl acetate (GE) extracts subjected to Gas Chromatography-Mass Spectrometry (GC-MS) analysis as described by Kamal *et al*. (2011) at the Residue Analysis Laboratory, Industrial Technology Institute (ITI), Colombo 7, Sri Lanka, to identify compounds potentially possessing antifungal properties. The samples were analyzed using a GC system (Agilent, 6890 series) equipped with an HP-5 MS column (0.25 mm x 30 m x 0.25 µm), with Helium serving as the carrier gas at a flow rate of 1.0 mL/min. The injector volume was 0.2 µL, with injector and detector temperatures set at 250 ^{o}C and 300 ^{o}C, respectively. The oven temperature was initially maintained at 40 ^{o}C for 5 minutes, then increased to 240 ^{o}C at a rate of 15 ^{o}C/min, followed by a further increase to 280 ^{o}C for 2 minutes at a rate of 10 ^{o}C/min.

Peak identification was conducted by comparing the obtained mass spectrum with the National Institute of Standards and Technology (NIST) mass spectral library (Shobier *et al.*, 2016). To assess the antifungal potential, the identified compounds were analyzed using the PASS (Prediction of Activity Spectra for Substances) online Program using Way2Drug informational-computational platform (version 2.0) (Chy *et al*., 2019; Druzhilovskiy *et al.*, 2017).

3. RESULTS

3.1. Yield of Macroalgal Crude Extracts Using Methanol and Ethyl Acetate

The choice of organic solvent in the extraction process significantly influenced the extract yield (p=0.033). Methanol produced the highest yield across all algal species, with yields of 2.01%, 2.61%, and 3.21% of the total weight for *Ulva* sp., *Sargassum* sp., and *Gracillaria* sp., respectively (Figure 2).

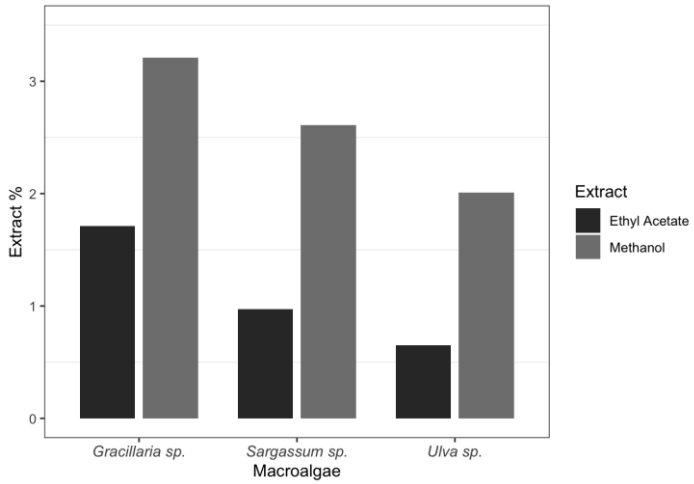

Figure 2. Percentage yield of crude extracts obtained from *Ulva* sp., *Sargassum* sp., and *Gracillaria* sp. with methanol and ethyl acetate.

3.2 Screening macroalgal extracts for antifungal activity

The inhibition percentage of *D. eugeniae* (p=2.61e^{-14}), *L. theobromae* (p=1.88 e^{-13}), and *P. theae* (p=6.42e^{-14}) by the three macroalgae were statistically significant at 0.05 confidence level.

The highest inhibitory percentage against *D. eugeniae* was demonstrated by the UE extract at 2000 ppm, reaching up to 79.29% (Table 1, Figure 3). The lowest inhibition was shown by the *Sargassum*-methanol extract (SM) at 1000 ppm, with only 0.81%. SE extracts at 250 ppm and 500 ppm were ineffective in inhibiting *D. eugeniae* growth. The UE extract exhibited the best antifungal properties against *D. eugeniae*. The GE extract, showing more than 50% inhibition at its lowest concentration (250 ppm), can also be considered an effective antifungal extract. The highest inhibition percentage against *L. theobromae* was shown by the UM extract at 2000 ppm (43.09%), followed by the *Gracilaria*-methanol (GM) extract at 2000 ppm (41.18%), and 1000 ppm (40.83%) (Table 1, Figure 3). The lowest inhibition was shown by the GE extract at 500 ppm (01.53%). None of the SM extracts exhibited any antifungal activity against *L. theobromae*.

For *P. theae*, the inhibitory percentages of each algal extract and concentration were significantly different (Table 1, Figure 3). The maximum inhibitory percentage against *P. theae* was recorded with UE extract at 2000 ppm (56.68%), although there was no significant difference compared to the UE extract at 1000 ppm (53.16%) and at 500 ppm extract (50.08%). The minimum inhibition was observed with the GM extract at 2000 ppm (05.20%).

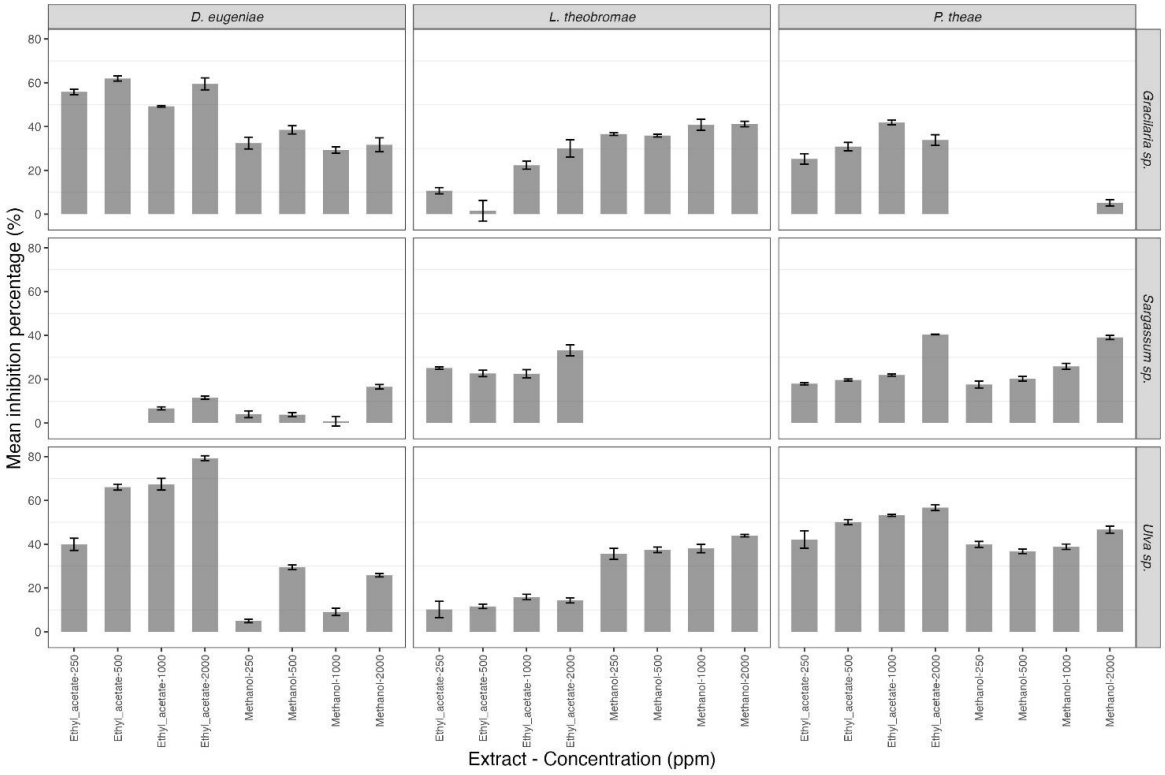

Figure 3. The graph of inhibition percentages of *Ulva* sp., *Sargassum* sp., and *Gracilaria* sp. ethyl acetate and methanol extracts against *D. eugeniae*, *L. theobromae* and *P. theae*

3.3 GC-MS profiling of selected macroalgal extracts

The most effective antifungal extracts were subjected to GC-MS analysis to identify the chemical compounds with antifungal potential. In the GE extract seven different peaks were identified, indicating the presence of seven chemical compounds. Similarly, three potential chemical compounds were identified in the UE extract, while nine were found in the UM extract. These compounds underwent evaluation for antifungal activity using previous literature (Abbassy *et al*., 2014; Johnson *et al.*, 2014; Shobier *et al.*, 2016; Ragunathan *et al*., 2019) and the PASS online program (Table 2). According to PASS predictions, few identified chemical compounds demonstrate promising antifungal activity. Notably, the most abundant compounds with antifungal activity in the GE, UM, and UE extracts were 6,10,14-Trimethylpentadecan-2-one (58.86%), 4-Hydroxy-2-butanone (37.37%), and Dihydroactinidiolide (30.02%), respectively.

Table 1. Inhibition percentages of *Ulva* sp., *Sargassum* sp., and *Gracilaria* sp. methanol and ethyl acetate extracts against *D. eugeniae*, *L. theobromae*, and *P. theae*.

Algae	Solvent	Concentration (ppm)	Inhibition percentage (%) ± Standard Error		
			D. eugeniae	*L. theobromae*	*P. theae*
Ulva sp.	Ethyl acetate	250	39.94^{abcde} ± 2.76	10.19^{abc} ± 3.71	42.17^{abcd} ± 3.96
		500	66.03^{cd} ± 1.35	11.61^{abc} ± 1.02	50.08^{bcd} ± 1.15
		1000	67.38^{cd} ± 2.67	15.87^{abc} ± 1.25	53.16^{bd} ± 0.46
		2000	**79.29^{d} ± 1.10**	14.34^{abc} ± 1.19	56.68^{d} ± 1.35
	Methanol	250	04.95^{abe} ± 0.80	35.62^{abc} ± 2.49	**39.90^{abcde} ± 1.35**
		500	29.49^{abcde} ± 1.04	37.42^{ac} ± 1.26	36.76^{abcde} ± 1.01
		1000	09.09^{abcde} ± 1.68	38.03^{ac} ± 1.93	38.84^{abcde} ± 1.24
		2000	25.8^{abcde} ± 0.78	**43.09^{c} ± 0.55**	46.69^{bcd} ± 1.62
Sargassum sp.	Ethyl acetate	250	-	25.11^{abc} ± 0.54	17.92^{acd} ± 0.51
		500	-	22.62^{abc} ± 1.46	19.62^{abce} ± 0.46
		1000	06.68^{abce} ± 0.68	22.50^{abc} ± 1.85	21.89^{abcde} ± 0.48
		2000	11.52^{abcde} ± 0.73	33.20^{abc} ± 2.48	40.44^{abcde} ± 0.11
	Methanol	250	03.97^{abe} ± 1.55	-	17.57^{ace} ± 1.58
		500	03.78^{abe} ± 0.94	-	20.19^{abcde} ±1.08
		1000	00.81^{be} ± 2.21	-	25.86^{abcde} ± 1.37
		2000	16.53^{abcde} ± 1.01	-	39.06^{abcde} ± 0.97
Gracilaria sp.	Ethyl acetate	250	55.83^{abcd} ± 1.25	10.73^{abc} ± 1.43	25.22 ^{abcde} ± 2.38
		500	61.94^{acd} ± 1.16	01.53^{ab} ± 4.74	30.86 ^{abcde} ± 1.90
		1000	49.25^{abcde} ± 0.35	22.35^{abc} ± 1.82	41.85^{abcd} ± 1.06
		2000	59.51^{acd} ± 2.72	30.06^{abc} ± 3.98	33.84^{abcde} ± 2.44
	Methanol	250	32.47^{abcde} ± 2.68	36.57^{abc} ± 0.61	-
		500	38.51^{abcde} ± 1.87	35.88^{abc} ± 0.65	-
		1000	29.30^{abcde} ± 1.48	40.83^{ac} ± 2.51	-
		2000	31.69^{abcde} ± 3.20	41.18^{c} ± 1.25	05.20^{ae} ± 1.41
Positive control		1000	94.30 ± 0.80	91.17 ± 0.76	86.66 ± 0.92

Note: Means with different letters within a column are significantly different (p=0.05); Negative control data were incorporated into calculations according to formula in Method 2.2.1.

Table 2. Chemical compounds exhibiting antifungal properties from *Ulva*-ethyl acetate, *Ulva*-methanol and *Gracilaria*-ethyl acetate extracts identified through GC-MS analysis and their probabilities of antifungal activity determined using PASS WAY2DRUG online software.

Algal extract	Compound	Compound CID	Molecular formula	Retention time Ttime (min)	% of total	Chemical group	Pa	Pi
Ulva-ethyl acetate	Dihydroactinidiolide	27209	$C_{11}H_{16}O_2$	14.94	30.02	Terpene	0.28	0.09
	Heptadecene	5364555	$C_{17}H_{34}$	16.94	12.28	Alkene	0.47	0.04
Ulva-methanol	Phenylephrine 3-Methoxyamphetamine	6041 152234	$C_9H_{13}NO_2$ $C_{10}H_{15}NO$	01.10	00.12	Phenol	0.26	0.10
	4-Hydroxy-2-butanone	111509	$C_4H_8O_2$	01.15	37.37	Ketone	-	-
	17-Octadecenal Bicyclo[3.1.1]heptane,2,6,6 trimethyl-, (1alpha,2beta,5alpha)-	41922 12314300	$C_{18}H_{34}O$ $C_{10}H_{18}$	02.33	01.75	Aldehyde	0.32	0.08
	Palmitic acid	985	$C_{16}H_{32}O_2$	16.80	00.57	Fatty acid	0.59	0.02
Gracilaria-ethyl acetate	6,10,14-Trimethylpentadecan-2-one	10408	$C_{18}H_{36}O$	16.00	58.86	Sesquiterpenoid	0.38	0.06
	Dihydroactinidiolide	27209	$C_{11}H_{16}O_2$	25.68	00.03	Terpene	0.28	0.09
	(E)-5-Octadecene	5364598	$C_{18}H_{36}$	07.00	04.25	Alkene	0.30	0.08
	Heptadecane	12398	$C_{17}H_{36}$	14.94	02.09	Alkene	0.47	0.04

Note: Pa = probability to be active, Pi = probability to be inactive; Pa higher than Pi are considered to possess antifungal potential

4. DISCUSSION

The majority of algae species produce unique secondary metabolites with various biological capabilities, such as antifungal, antibacterial, antiviral, antioxidant, anticancer, and anti-inflammatory effects (Omar *et al.*, 2018). Due to exposure to challenging environmental conditions like salt, light, temperature, and marine chemical composition, most algal species generate distinct secondary metabolites (Mickymaray & Alturaiki, 2018). In Sri Lanka, only a limited number of studies have explored the biological activities of marine macroalgae, with few focusing on the antifungal potential of marine macroalgae against plant-pathogenic fungi (Fernando *et al.*, 2017; Lakmal *et al*., 2014). Therefore, this study investigates the antifungal potential of *Ulva* sp., *Sargassum* sp., and *Gracilaria* sp. found on the Thalpe Reef in Sri Lanka against selected pathogenic fungi of *S. melongena.* The potential antifungal compounds were identified through GC-MS analysis and PASS online server. The chemical components were extracted sequentially using the Soxhlet apparatus with two solvents of increasing polarity; ethyl acetate and methanol. The number and quantity of bioactive compounds dissolved in a solvent mainly depend on its polarity (Ullah *et al.*, 2019).

In *D. eugeniae, Ulva* sp. exhibited a higher inhibitory percentage with ethyl acetate-2000 ppm extract (79.29%), followed by ethyl acetate-1000 ppm extract (67.38%). Methanol-2000 ppm extract resulted in 25.8% inhibition. In *L. theobromae, Ulva* sp. showed a higher inhibitory percentage with methanol-2000 ppm extract (43.09%). For *P. theae, Ulva* sp. demonstrated a higher inhibitory percentage with ethyl acetate-2000 ppm extract (56.68%), followed by methanol-2000 ppm extract (46.69%). Similarly, Bahammou *et al*. (2021) reported that a 2 mg mL^{-1} methanol extract of *Ulva lactuca* exhibited the highest antifungal activity against plant-pathogenic fungi *Botrytis cinerea*, with an inhibition diameter of 9.5±0.07 mm and *Penicillium digitatum*, with an inhibition diameter of 10.1±0.13 mm. Further, Chanthini *et al*. (2012) documented an antifungal effect of 5% ethyl acetate extract of *U. lactuca* against *Alternaria solani* with an inhibition percentage of approximately 35% using the disk diffusion method. Moreover, Mostafa *et al*. (2021) reported antifungal activity of *Ulva fasciata* extracts against the pathogenic fungus *Fusarium solani*, with methanol and ethyl acetate extracts showing inhibition percentages of 4% and 26.8%, respectively. Additionally, Supriya & Haritha (2022) found that the ethyl acetate extract of *U. lactuca* demonstrated antifungal activity against *Aspergillus oryzae* (69.16%), *Rhizopus artocarpi* (37.73%), and *Fusarium oxysporum* (53.65%). Their study also revealed that the methanol extract showed even higher antifungal activity, with inhibition rates of 74.91% against *A. oryzae*, 61.92% against *R. artocarpi*, and 67.68% against *F. oxysporum*.

The findings of this study indicate that, in many cases, ethyl acetate extracts and occasionally methanol extracts exhibit the highest antifungal potential against the studied plant-pathogenic fungi. However, previous studies have often reported that methanol extracts demonstrate the highest antifungal activity. This deviation could be attributed to differences in the secondary metabolites of marine macroalgae, influenced by variations in geographical locations, environmental factors, and maturity stage of the macroalgal specimens. Additionally, in the present study, the algal extractions were conducted sequentially, beginning with ethyl acetate followed by methanol, a greater proportion of bioactive compounds may have been extracted into ethyl acetate solvent. Further, antifungal activity can differ depending on the fungal species or strain being tested. Some fungi may be more sensitive to compounds extracted with methanol, while others may exhibit better responses to compounds extracted with ethyl acetate. Therefore, the differences in fungal strains used in different studies could contribute to the variation in reported results.

The mechanism of action of antifungal compounds derived from macroalgae remains incompletely understood, with several proposed mechanisms. Typically, compounds present in various algal extracts can target fungi by affecting the cell wall or membrane, as well as intracellular organelles such as the nucleus and mitochondria. Upon penetration of the fungal

cell, antifungal agents may disrupt protein synthesis, and interfere with the mitochondrial respiratory chain, thereby disturbing the cell's homeostasis and stability, ultimately reducing its lifespan (Lopes *et al.*, 2013). Fatty acids identified in macroalgae have exhibited antifungal properties by integrating into the fungal membrane, increasing its fluidity and permeability, and inducing changes in its organization, leading to cell death (Avis & Bélanger, 2001). This mechanism has been observed against fungal species such as *Cladosporium cucumerinum*, *B. cinerea*, and *Fusarium oxysporum* f.sp. *radices-lycopersici* (Hajlaou *et al.*, 1994).

In this study, GC-MS analysis was conducted on the most effective algal extracts against the tested plant-pathogenic fungi. The extracts from *Ulva* sp. (UE and UM) and *Gracilaria* sp. (GE) were subjected to GC-MS analysis, which revealed a variety of diverse compounds. *Ulva* extracts displayed a total of three peaks for ethyl acetate and nine peaks for methanolic extracts. The ethyl acetate extract of *Gracilaria* exhibited seven peaks. To identify their bioactivity, the compounds were compared with previously isolated substances and predicted using the PASS WAY2DRUG online server. It predicts a compound's activity spectrum as probable activity (Pa) and probable inactivity (Pi), with values ranging from 0.000 to 1.000. A compound is considered experimentally active if Pa>Pi (Chy *et al.*, 2019).

Results indicated that three phytocompounds were identified in the UE extract, with Dihydroactinidiolide (retention time RT= 14.94 min) and 8-Heptadecene (RT=16.94 min) being the main chemical constituents with potential antifungal activity, as shown in Table 2. For the methanolic extract of *Ulva*, nine compounds were identified. Among these, 17-Octadecenal (RT=2.33 min), Palmitic acid (RT= 16.80 min), 1,2-Benzisothiazol-3-amine (RT= 25.77 min), and Phenylephrine (RT=01.10 min) have antifungal potential according to PASS predictions and previous studies (Abbassy *et al.*, 2014; Shobier *et al.*, 2016). Interestingly, this is the first report of the compound 17-Octadecenal (RT=2.33 min) in the UM extract with potential antifungal activity.

Previous phytochemical investigations have identified different chemical compounds, including those reported in this study, in various extracts of *Ulva* sp. For instance, an ethyl acetate extract of *Ulva* collected from the Alexandria coast, Egypt found to contain Dichloroacetic acid, heptadecyl ester, (9Z)-9,17-Octadecadienal, and 8-Heptadecene (Shobier *et al.*, 2016). Also, Johnson *et al*. (2014) found that an ethanolic extract of *U. lactuca* from the south coast of India contains seventeen different chemical constituents, including 7-Hexadecene, 8-Heptadecene, Hexadecanoic acid, and 6,9,12,15-octadecatetraenoate. Moreover, Abbassy *et al*. (2014) reported that *Ulva*-methanol extract contains 42 components, with the main five being 1,2-benzene dicarboxylic acid, bis(2-ethylhexyl) ester, palmitic acid, benzene,1,2,4-trimethyl, 8-octadecanoic acid methyl ester and benzene,1-ethyl-2-methyl.

Seven phytocompounds were identified in the GE extract. Among these Dihydroactinidiolide (RT= 25.68 min), (E)-5-Octadecene (RT= 07.00 min), Heptadecane (RT=14.94 min) and 6,10,14-Trimethylpentadecan-2-one (RT= 16.00 min) are notable constituents with potential antifungal activity according to PASS predictions. Ragunathan *et al*. (2019) identified n-Hexadecanoic acid, Heptadecane, Pentadecanoic acid, Oleic acid, and N-(5-chloro-2-hydroxyphenyl)dodecanamide as the most abundant compounds in GE extracts. This suggests that the antifungal activity of these extracts may result from the collective effect of multiple compounds, rather than a single component.

5. CONCLUSION

GC-MS analysis revealed the presence of potential antifungal compounds in UE extract which exhibited the highest inhibition against *Diaporthe eugeniae* and *Pseudopestalotiopsis theae*, and UM extract which had the highest antifungal activity against *Lasiodiplodia theobromae.* Furthermore, these extracts have demonstrated antifungal potential, indicating their possible future applications in sustainable agriculture and the development of novel fungicides to protect crops from fungal pathogens.

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Declaration of Conflicting Interests and Ethics

The authors declare no conflict of interest. This research study complies with research and publishing ethics. The scientific and legal responsibility for manuscripts published in IJSM belongs to the authors.

Authorship Contribution Statement

Conceptualization, supervision, fund acquisition, reviewing, and editing were done by HMH, PE, and RPW. BKDMR, AHDA, and BMCMB contributed to designing the study, material preparation, data collection, and data analysis. The initial draft of the manuscript was written by BKDMR, AHDA, and BMCMB, with input from all authors on earlier versions. All authors read and approved the final version of the manuscript.

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