Assessment of Antioxidant and Antifungal Capacity of Selected Drifted Brown Seaweeds.

K. Bhanumathi[1], Ganeshan Petchidurai[2]*, S. Tamilselvi[1], S. Wincy[2], R. Vana Padmavathi[2], M. Sasirekhamani[3]

[1]Head, Department of Zoology, Kamaraj College (Autonomous), Thoothukudi-628003, Tamil Nadu, India - 628 003, Affiliated to Manonmaniam Sundaranar University, Tirunelveli, 627012, Tamil Nadu, India, Email: banumadhu1978@gmail.com

[2]*PG and Research Department of Zoology, Kamaraj College (Autonomous), Thoothukudi, Tamil Nadu, India - 628 003, Affiliated to Manonmaniam Sundaranar University, Tirunelveli, 627012, Tamil Nadu, Telephone number, Email: durai.s.m.m@gmail.com

[3]Assistant Professor, PG Department of Food Science and Nutrition Department, Holy Cross Home Science College, Thoothukudi, Affiliated to Manonmaniam Sundaranar University, Tirunelveli-627012, Tamil Nadu, India, Email: sasirekhamani@gmail.com

***Corresponding Author:** Ganeshan Petchidurai
*PG and Research Department of Zoology, Kamaraj College (Autonomous), Thoothukudi, Tamil Nadu, India - 628 003, Affiliated to Manonmaniam Sundaranar University, Tirunelveli, 627012, Tamil Nadu, Telephone number, durai.s.m.m@gmail.com

Abstract
Seaweeds, also known as marine macroalgae, are renewable biological resources found worldwide and possess various secondary metabolites. This study evaluated the phytochemicals, antifungal, and antioxidant activity of locally, and mass number of available seaweeds. Selected seaweeds were extracted using 70% methanol using heat methods. The GC-MS analysis of tested samples displayed, *S. wightii* and *S. marginatum* extracts present 13 and 4 bioactive complexes. In *S. wightii* the alkaloid compound 2,3-Dicyano-5,6-diphenylpyrazine was found to be high, and Silicic acid or dihydrogen tetroxide disilicate riches in *S. marginatum*. Tested both samples presented high potential to antifungal activity with range from 9 to 74 % and 6 to 74% for *F. oxysporum* and *A. niger* respectively. Moreover, *F. oxysporum* is highly sensitive ($\bar{x}$ = 74.66%, $F_{25,4}$ = 561.219, p = 0.004) to drifted *S. marginatum.* Minimum inhibitory concentration obeyed *S. wightii* 395.75 (IC_{50}), sufficient to inhibit 50% of *F. oxysporum.* In the DPPH method,, higher concentration of *S. marginatum* ($\bar{x}$ = 70.95±1.12, $F_{25,4}$ = 449.853, p –0.005) and *S. wightii* ($\bar{x}$ = 63.96 ± 1.12, $F_{25,4}$ = 397.90, p – 0.005) showed the significantly highest DPPH scavenging inhibition activity. In the Phosphomolybdenum method, *S. marginatum* extract noted significantly top topmost inhibition activity ($\bar{x}$ = 92.71±1.17, $F_{25,4}$ = 244.197, p – 0.003) while *S. wightii* exhibited slightly lower inhibition activity observed at ($\bar{x}$ = 80.54±2.35, $F_{25,4}$ = 159.80, p – 0.003). IC_{50} value points to a higher antioxidant activity, IC_{50} (287.64) value of *S. wightii* confirms that *S. wightii* has high antioxidant potential activity by total antioxidant assay methods when compared to *S. marginatum*. The present findings suggested that drifted seaweeds can be considered as a biological waste and used as a pharmacological activity.

Keywords: Drifted seaweeds; antifungal; antioxidant; *F. oxysporum*; *A. niger*

INTRODUCTION

The coastal marine areas contain some of the world's most diverse and productive biological systems. India has an extensive coastal of 8129 km with Exclusive Economic Zone (EEZ) (Nammalwar *et al*., 2009). The Tamil Nadu and Puducherry coastline is around 1076 km in length and constitutes around 15% of India's total coastline. Shoreline of Tamil Nadu encompasses dissimilar natures of vegetation, like mangroves and their associates—scrub jungles, aquatic vegetation, and coastal dune vegetation (Nisha Thomas *et al*., 2023). This vegetation has an enormous kind and numbers of animals, insects, coral reefs, seagrass and seaweeds, which play a critical role in maintaining the marine ecosystem, as we as human beings and seashore terrestrial animals.

Seaweeds are simple, primitive plants without a root and shoot system, popularly termed as Sea vegetables and widely distributed in the oceans from the tidal level to considerable depths, and floating freely with attached to rocky substrate with a holdfast (Petchidurai *et al*., 2019). Based on their pigments, seaweeds are broadly categorized into 3 types, viz. Brown algae (Phaeophyta), Green algae (Chlorophyta), and Red algae (Rhodophyta). Seaweeds are rich in a lot of macro and micro nutrients, carbohydrates, proteins, minerals, and vitamins (Kirtankumar *et al*., 2016). Browen seaweeds are abundantly available in our country, followed by green and red seaweeds (Bagavan Reddy *et al*., 2023). The Gulf of Mannar, located in the southern part of Tamil Nadu, has a rich diversity of all three seaweed groups. Intertidal and subtidal rocks extend up to 1 m deep, and they support abundant growth of *Sargassum*, *Acanthophora*, and *Hypnea* species (Jha *et al*., 2009).

Seaweed is a staple food in Japan and China since time ages. The green seaweeds *Enteromorpha, Ulva, Caulerpa,* and *Codium* are utilized exclusively as the source of food. These are often eaten as fresh salads or cooked as vegetables along with rice. *Porphyra* (Nori), *Laminaria* (Kombu), and *Undaria* (Wakame) are used for making fish and meat dishes as well as soups and accompaniments. Seaweeds contain more than 60 trace elements; their concentration is greatly higher than in terrestrial plants, and they also encompass vitamins, proteins, essential amino acids, iodine, bromine, antibiotics, and several bioactive substances. They are also used as feed for livestock, poultry, fish, and prawns, and as manure for many plantation crops. Agar-agar, agarose, and carrageenan are commercially valuable substances extracted from red seaweeds and find extensive use in many industries like food, confectionery, pharmaceutical, biomedical, dairy, textile, paper, and paint industries as gelling, stabilizing, and thickening agents (Janet Rani *et al*., 2013; Meenakshisundaram Ganesan *et al*., 2019; Manickavasagam *et al*., 2019).

Aspergillus is a common filamentous environmental fungus; there are approximately 200 species, less than 20 of which are pathogenic to humans. Aspergillosis is an opportunistic mycosis, causing the disease under immunocompromised situations of the host or when humans and animals are exposed to an overwhelming number of infectious spores of the fungus. Certain occupational groups, such as poultry farmers, gardeners, and agricultural workers, etc seem to be more susceptible to aspergillosis (Mahendra Pal, 2020). *A. niger* has been associated with otomycosis (Araiza *et al*., 2006) caused to cutaneous infections (Loudon *et al*., 1996) and pulmonary disease.

Fusarium oxysporum (F. oxysporum) is a pathogenic soil-borne ascomycete fungus affecting many plants in the world by causing fusarium wilt, which is a lethal vascular syndrome in plants (Flood Julie, 2006). There are over 100 host-specific species of *F. oxysporum* of which are widely distributed around the world. Commercially, *F. oxysporum* strains infect, cause disease symptoms, and kill a wide range of plants, for instance, the Solanaceae group comprising tomatoes, potatoes, peppers, and eggplants. The fungal pathogen affects the plants by producing macro and micro conidia, finally entering to human being (Chung Gait Fee, 2018).

In humans, *Fusarium* spp. Causes a variety of infections, which are highly dependent upon the portal of entry and the immune status of the host (Nucci *et al*., 2015), in immunocompetent people, it is the most common etiological agent of superficial infections such as keratitis and onychomycosis, but it can appear in other organs causing infections such as peritonitis in patients receiving dialysis (Gaur *et al.*, 2010), thrombophlebitis, arthritis (Gradon *et al*., 1990), osteomyelitis (Sierra-Hoffman *et al*., 2005), endophthalmitis (Ahearn *et al*., 2008; Proença-Pina *et al*., 2010), fungemia (Dananché *et al*., 2015), sinusitiss (Macêdo *et al*., 2008) and pneumonia (Poignon *et al.,* 2020) in severely immunocompromised patients, locally invasive or disseminated infections are more frequent and are usually associated with positive blood cultures (Nucci and Anaissie, 2007). The species that are most commonly involved in human infection are *F. solani*, followed by *F. oxysporum*, *F. verticillioides*, and *F. moniliforme* (Cighir *et al*., 2023).

Nowadays, microbes are increasingly developing resistance against antibiotics and fungicides in use. Therefore, a large library of novel compounds is required to combat these drug-resistant microbes. Since natural products from seaweeds offer a rich source of bioactive molecules, the present work was intended to evaluate the antifungal efficiency of organic solvent extracts of the most dominant drifted marine algal species. Seaweeds have been widely used as food (Bonotto, 2016) as they are the chief source of vitamins and minerals (Hoppe, 2016). The extracts and their products are effective nutritional supplements (Gross, 2016). Apart from the nutritional support, it has also been used against various biological diseases like antimicrobial, antiviral, antifungal, antiallergic, anticoagulant, anticancer, antifouling, and antioxidant activities (Pooja, 2014). The marine brown alga, i.e. *Sargassum wightii*, has anti-tumor, anti-inflammatory, antioxidant, and antibacterial activities (Cotas, 2020; Farghali, 2023).

Antioxidants are inhibitors of the process of oxidation, even at relatively small concentrations, and thus have diverse physiological roles in the body. Antioxidant constituents of the plant material act as radical scavengers and help in converting the radicals to less reactive species. A variety of free radical scavenging antioxidants are found in dietary sources like fruits, vegetables, and tea etc (Sulekha Mandal *et al.*, 2009). Antioxidant compounds play an important role against various diseases (e.g., chronic inflammation, atherosclerosis, cancer, and cardiovascular disorders) and ageing processes (Kohen and Nyska, 2002), which explains their considerable commercial potential in medicine, food production, and the cosmetic industry. Moreover, interest in employing antioxidants from natural sources is considerably enhanced by consumer preference for natural products and concern about the potential toxic effects of synthetic antioxidants (Safer and al-Nughamish, 1999).

In the past, several investigators have found that seaweeds have antioxidant, antifungal, antimicrobial, antiviral, insecticidal activity, repellent activity, etc. Nevertheless, Petchidurai *et al*. (2023) testified drifted brown seaweeds tannin hold insecticidal acidity. To date, no one has initiated research on drifted seaweeds that possess antioxidant and antifungal potential. When compared to the other two types of marine algae, brown algae are the rich source of secondary metabolites, abundance numbers, availability, drifting ability of seaweeds. Based on these motives, I have selected drifted brown seaweeds *Stoechospermum marginatum* (*S. marginatum*) for my research to test antifungal activity against *Aspergillus niger* (*A. niger*) and *Fusarium oxysporum* (*F. oxysporum*) and antioxidant activity.

MATERIALS AND METHODS

Seaweed collection and preparation

Seaweed species, *S. wightii* and *S. marginatum* were collected from Manapadu in November 2023 (8.3765° N, 78.0563°E), Tuticorin district of Tamil Nadu. The collected seaweeds were washed twice in seawater, tap water, and distilled water to

remove debris and sand. They were dried in the shade for two weeks and then partially powdered with a domestic blender (Preethi, XL7, ATK product, India) and stored for further processing.

Seaweed extraction (Heat method)

Drifted seaweeds were extracted from *S. wightii* and *S. marginatum* by the heat methods (water bath) according to the methodology of Vanimakhal and Balasubramanian (2016) with little modifications. In the former, partially powdered seaweeds (100 g) were suspended in 250 ml of 70% Methanol containing 0.01% ascorbic acid in an airtight conical flask and kept in a water bath at 50 °C (Technico) for 24 hours. The samples were filtered and air dried in a laminar airflow chamber. After that, the crude extract was placed in a hot air oven (Technico) at 50 °C for 1 hour.

GC-MS analysis

Crude extract is GC-MS analysis Using a Perkin-Elmer GC System 7820A, MSD 5977E and gas chromatograph interfaced to a mass spectrometer (GC-MS) equipped with an Elite-I, fused silica capillary column (30mm X 0.25mm DB-5, composed of 100% Dimethyl poly siloxane), drifted brown algal crude extracts were subjected to GC-MS analysis. An electron ionization device with an ionizing energy of 70 eV was employed for GC-MS detection. With an injection volume of 2 µl and a split ratio of 10:1, helium gas (99.999%) was utilized as the carrier gas. The injector temperature was 100 ºC, while the ion source temperature was 270 ºC. With pieces ranging in size from 45 to 450 Da, mass spectra were recorded at a scan rate of 70 eV and 0.5 seconds. The GC ran for 36 minutes in total. Software called TurboMass was used to handle mass spectra and chromatograms, and it was used to calculate the relative percent amount of each component by comparing its average peak area to the total areas (Monrroy *et al*., 2020).

The components of the test materials' names, molecular weights, and structures were determined using the National Institute of Standards and Technology's (NIST's) database.

Anti-fungal activity

The Agar Well Diffusion Method (Alvarez Benito, 1990) was used to measure the antifungal action. For the growth inhibition investigations, sterile Petri plates (9 cm in diameter and 1 cm in height) containing 20 ml each of sterile potato dextrose agar (PDA) were prepared. *F. oxysporum* and *A. niger* conidia were added to the prepared PDA medium and poured in Petri dish and inoculated at normal room temperature (27 ± 2 ºC). After the solidification, a sterile cork borer was used to create wells (5 mm) under aseptic circumstances. Different amounts of the tested drifting brown algae *S. wightii* and *S. marginatum* (100, 200, 400, and 800 mg), and a positive control was fluconazole, were added to the corresponding wells. As a negative control, sterile double-distilled water. For each concentration, three replications were kept. The culture was kept in the BOD incubator at 27 ± 2 °C (Kemi, India). Using an antibiotic susceptibility ruler, a definite zone of inhibition was observed after 72 hours of incubation. The following formula was used to calculate the test extract's relative percentage inhibition against the positive control (Yousif *et al*., 2015)

Percentage inhibition = (C-T) x 100/C

Were,

C = colony diameter (mm) of the control.

T = colony diameter (mm) of the test plate.

Antioxidant activity

DPPH Radical Scavenging Capacity

Inspected the capability of drifted seaweeds extracts to scavenge the DPPH free radical (1, 1-diphenyl-2-picrylhydrazyl) technique, method described by Yen and Chen (1995). Two mL of the test drifted seaweeds *S. wightii* and *S. marginatum* extract from every concentration (100, 200, 400, and 800 mg) were mixed with 2 mL of a 0.16 mM DPPH methanol solution. The absorbances were measured at 517 nm after the liquid was vortexed and kept in the dark for 30 min. The antioxidant activity was expressed as an inhibition percentage of DPPH radical (% inhibition).

Total antioxidant assay (Phosphomolybdenum method)

The total antioxidant activity was evaluated by the phosphomolybdenum method described by Prieto *et al.* (1999). 1.0 ml of the drifted seaweeds *S. wightii* and *S. marginatum* extract from every concentration (100, 200, 400, and 800 mg) was mixed with 1.0 ml of the standard reagent solution (0.6M sulphuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate) in a test tube. The capped tubes are incubated in a thermal block at 95°C for 90 min. After cooling to room temperature, the absorbance was measured at 695 nm against a reagent blank.

Percentage of antioxidant activity calculated using the formula

% Antioxidant activity = Sample Absorbance/Standard Absorbance × 100

Statistical analysis

Data are presented as the mean of six replicates ± Standard Error (SE). The attained data were examined statistically using one-way analysis of variance (ANOVA). Probability levels for both antifungal and antioxidant activity were expressed at $p \leq 0.05$ levels of significance.

RESULT

GC-MS

GC-MS investigation of drifted seaweeds *S. wightti* 70 % carbinol crude extract, existing 13 bioactive complexes, whereas *S. marginatum* has 4 biochemical complexes as shown in Figs 1 and 2. The names of the biochemicals present in the drifted crude extract and their molecular weight, and molecular formula of also given in Table 1.

Table 1. List of biochemicals identified using GC-MS for a methanolic extract of drifted brown seaweeds.

S. n	Compound name	RT	RA	RT %	Molecular formula	Molecular weight
S. wightii						
1	9-Octadecenamide (z),	14.114	24956	6.89	$C_{18}H_{35}NO$	531.9
2	2-(Acetoxymethyl)-3-(methoxycarbonyl)biphenylene	14.199	42961	11.87	$C_{17}H_{14}O_4$	282.29
3	Phenol, 2,6-dichloro-4-nitro	14.360	24161	6.67	$C_6H_3Cl_2NO_3$	208.00
4	2(1H)-Pyrimidinone, 5-chloro-4,6-diphenyl	14.435	31731	8.77	$C_{16}H_{11}ClN_2O$	282.72
5	1,3,5-Triazine, 2-chloro-4,6-bis(methylthio)	14.492	14611	4.04	$C_7H_6ClN_3S_2$	207.7
6	2-Methyl-6-(5-methyl-2-thiazolin-2-ylamino) pyridine	14.540	21344	5.90	$C_{10}H_{13}N_3S$	101.17
7	2,3-Dicyano-5,6-diphenylpyrazine	14.681	55425	15.31	$C_{18}H_{10}N_4$	282.3
8	Phenol, 2,4-dichloro-6-nitro	14.861	34642	9.57	$C_6H_3Cl_2NO_3$	208.00
9	Phenol, 4-[2-(5-nitro-2-benzoxazolyl) ethenyl]	15.041	39171	10.82	$C_{19}H_{12}N_2O_5$	240.21
10	Quinoline, 2-chloro-6-methoxy-4-methyl	15.390	17571	4.85	$C_{11}H_{10}ClNO$	207.65
11	Cyclopentanecarboxylic acid, 3-methylene-2,2-dimethyl-5-[(E)-1-propenyl]-, methyl ester	15.438	21725	6.00	$C_{18}H_{28}O_2$	154.21
12	4-[N-Methylpiperazino]-5-nitro veratrole	15.532	17520	4.84	$C_{14}H_{20}N_2O_4$	309.06
13	Purine-2,6-dione, 8-(3-ethoxypropylamino)-1,3-dimethyl-3,9-dihydro	15.599	16181	4.47	$C_{21}H_{29}N_5O_3$	399.5
S. marginatum						
1	Silicic acid or dihydrogen tetroxide disilicate	17.825	229901	36.5	$H_8O_8Si_2$	192.23
2	Tris(tert-butyldimethylsilyloxy)	19.464	204058	32.4	$C_{18}H_{45}AsO_3Si_3$	871.302
3	2-Ethylacridine	19.753	172192	27.3	$C_{15}H_{13}N$	207.27
4	2-Methyl-7-phenylindole	20.534	23436	3.7	$C_{15}H_{13}N$	2178587

In retraction percentage of biochemical range between 4.47 % to 15.31% and 3.7% to 36.5% at *S. wightii* and *S. marginatum,* respectively. In *S. wightii*, the alkaloid compound 2,3-Dicyano-5,6-diphenylpyrazine was found to be high with values of 15.31 %. After that, 2-(Acetoxymethyl)-3-(methoxycarbonyl) biphenylene was found to be existing in high quantity (Fig. 1 and Table 1). Alternatively, in *S. marginatum* are majorly presented in Silicic acid (36.5%), Tris (tert-butyldimethylsilyloxy) (32.4%). Before the help of GC-MS and HPLC, Petchidurai *et al*. (2024) reported drifted brown algae crude extract have an active chemical complex, Moreover, El-Sheekh *et al*. (2020) recognized several phytochemicals and fatty acids in brown algae *C. myrica*, *S. cinereum*, and *P. boergesenii*.

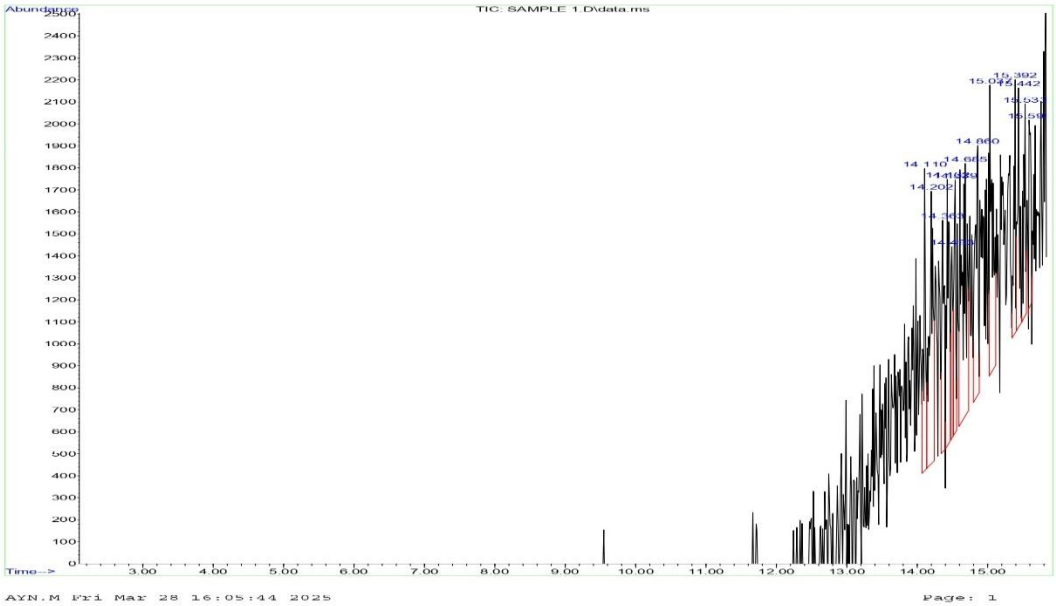

Fig. 1. GG-MS analysis of methanolic extract of drifted seaweeds *S. wightii*

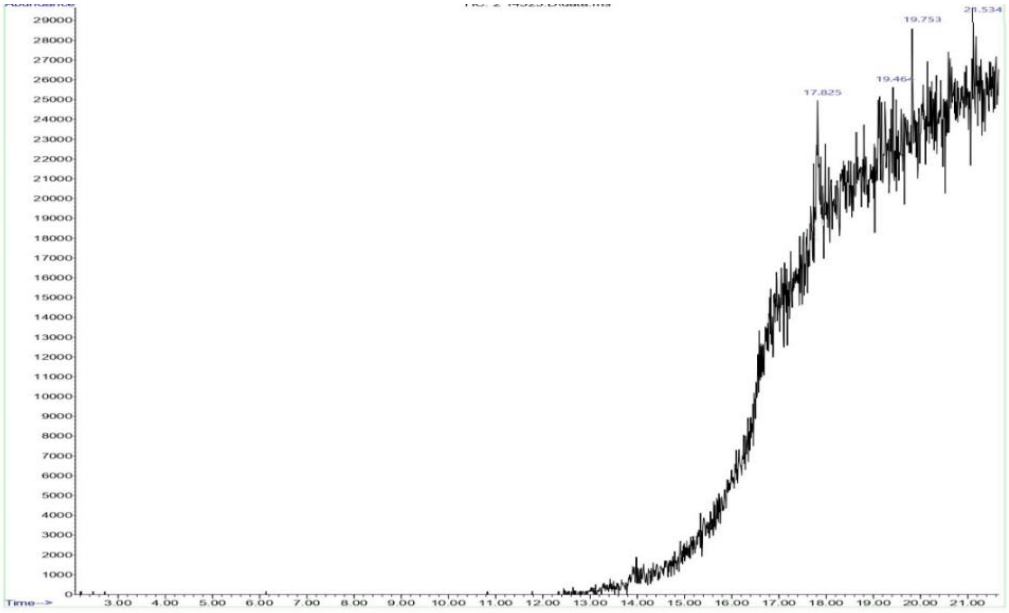

Fig. 2. GG-MS analysis of the methanolic extract of drifted seaweeds *S. marginatum*

In nature, terrestrial plants and seaweeds contain silicic acid to enhance crop yield, quality, plant growth, root growth, promote resilience to abiotic stresses like heat and drought, work against pests, reduce insect pest populations, and reduce plant accumulation of toxic heavy metals (Rao *et al*., 2023). Generally, Marine algae particularly need Si for such as diatoms, hence silica uptake (silicic acid) in place of the surrounding seawater by plasma membrane (transporters) transporting system of their cells (Martin-Jézéquel, 2000) mainly in case of green algae *Cladophora glomerata* (Moore and Traquair, 1976) and the brown algae *Padina gymnospora* cell wall (Salgado *et al*., 2005; Hiroyuki *et al*., 2021). This indicates that the occurrence of drifted brown algae *S. marginatum* may contribute to an increased level of silicic acid in the extract because of its natural content. As a result, this feature may increase the potential uses of the extract in, for instance, agriculture or environmental science.

Current GC-MS investigation displayed that the quality and quantity of bioactive complex are not the same in two seaweeds, because of the factors such as modifications in geographical setting, season, and salinity, temperature, extraction methods, time, extraction technique, solvent concentration, and solvent polarity (Nawaz *et al*., 2020).

Antifungal activity

70% methanolic extract of drifted *S. wightii* and *S. marginatum* crude extract confirmed antagonistic to pathogenic fungus by agar well diffusion techniques, , pathogenicity ability was evaluated with various concentrations (100, 200, 400, 800 mg) of extracts. The results suggest that tested samples have high fungicidal action than the positive control (Fluconazole) against both *A. niger* and *F. oxysporum.* In addition, the pathogenicity action is potentially increased when the tested two sample doses are increased (Table 2). Tested both samples presented high potential to antifungal activity with range from 9 to 74 % and 6 to 74% for *F. oxysporum* and *A. niger* respectively. Moreover *F. oxysporum* being highly sensitive ($\bar{x}$ = 74.66%, $F_{25,4}$ = 561.219, p = 0.004) to drifted *S. marginatum* when compared *S. marginatum* treated *A. niger* ($\bar{x}$ = 68.66%, $F_{25,4}$ = 138.591, p = 0.005) as well as *S. wightii* crude treated *F. oxysporum* ($\bar{x}$ = 70.50±2.60; $F_{25,4}$ =144. 85; p = 0.005) and *A. niger* ($\bar{x}$ = 70.16±2.40; $F_{25,4}$ = 272.52; p = 0.004) at concentration of 800. Whereas the positive control Fluconazole showed 15% and 17% antifungal activity for *F. oxysporum* and *A. niger* respectively (Table 2). When compared to two tested materials and two pathogen, Output of Minimum inhibitory concentration obeyed *S. wightii* 395.75 (IC_{50}) sufficient to 50% inhibitory of *F. oxysporum* when compared to *A. niger* (Fig. 3 and Table 3).

Table 2. Antifungal efficacy of methanolic extract of selected drifted brown seaweeds against the pathogen.

Seaweeds	Con	$\bar{x}$ ± S. E	DF	F	Sig	$\bar{x}$ ± S. E	DF	F	Sig
	F. oxysporum					***A. niger***			
S. marginatum	100	$09.00±0.90^{gF}$	25,4	561.219	0.093	$09.50±0.76^{gF}$	25,4	138.591	0.56
	200	$28.50±1.54^{eD}$			0.069	$20.33±1.83^{eE}$			0.011
	400	$45.50±1.72^{dC}$			0.013	$44.66±1.50^{cC}$			0.007
	800	$74.66±1.33^{aA}$			0.004	$68.66±4.56^{bB}$			0.005
Fluconazole		15.00±1.35				17.00±073			
S. wightii	100	$13.83±1.10^{fF}$	25,4	144.85	0.103	$06.83±0.90^{gG}$	25,4	272.52	0.256
	200	$28.16±1.70^{eD}$			0.083	$18.50±1.05^{efE}$			0.026
	400	$51.00±2.51^{cB}$			0.043	$39.00±1.89^{dC}$			0.005
	800	$70.50±2.60^{bA}$			0.005	$70.16±2.40^{aA}$			0.004
Fluconazole		13.83±1.10				17.00±073			

Same (^{abcd}) lowercase letter in a column shows significance at 0.05% among the algae, and upper case (^{ABCD}) latter shows the significance at 0.05% between pathogens.

Table 3. Probit analytical data, chi-square, and significance) against pathogen growth by well diffusion assay.

Pathogen	R^2	Y	Chi-Square	Sig
S. marginatum				
F. oxysporum	0.990	Y= -5.6+23.15x X	1.190	0.552
A. niger	0.992	Y= -5.35+1.99x X	0.727	0.695
S. wightii				
F. oxysporum	0.992	y = 19.285x - 7.34	6.877162	0.009
A. niger	0.958	y = 21.049x – 19	4.763	0.029

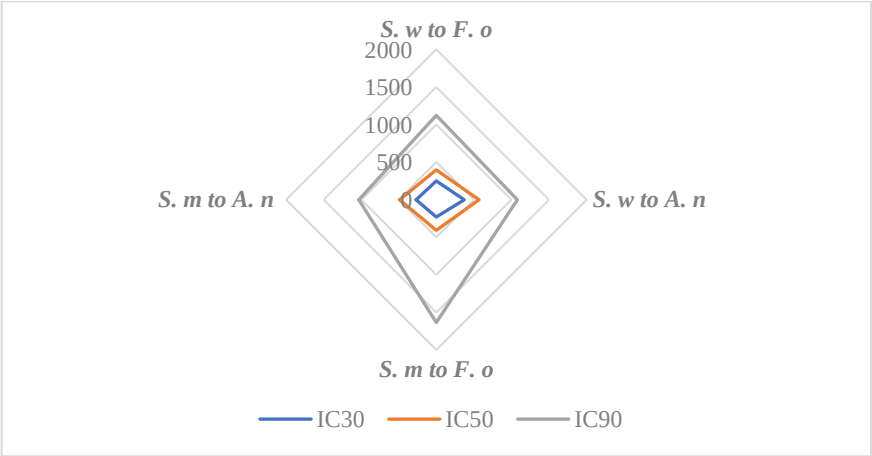

Fig. 3. LC data for drifted seaweeds extract against pathogen growth.

Antioxidant activity

DPPH method

Chemicals that undergo reduced oxidation reactions are named for their antioxidant activity, the activity expressed as a percentage of inhibition. Antioxidant activity of drifted seaweeds (different concentrations 100, 200, 400, and 800 mg) was determined by the DPPH method, as the outcome showed that antioxidant reduction activity was noticeably increased when the extract dose was increased, as given in Table 4. Higher concentration of *S. marginatum* ($\bar{x}$ = 70.95±1.12, $F_{25,4}$ = 449.853, p –0.005) and *S. wightii* ($\bar{x}$ = 63.96 ± 1.12, $F_{25,4}$ = 397.90 p, < – 0.005) showed significantly highest DPPH scavenging inhibition activity. IC_{50} is shown in Table 5, The IC_{50} value indicates *S. marginatum* has highly reduced antioxidant activity compared to *S. wihtii* and needs 372.42 mg *S. marginatum* crude extract for 50% inhibition activity by the DPPH method (Fig. 4).

Phosphomolybdenum method

In the same way, the antioxidant inhibition percentage was verified by the Phosphomolybdenum method. Similarly to DPPH, Phosphomolybdenum correspondingly exhibited dose depended antioxidant activity inhiation percentage as showed in Tabe 4 and fig. 4. 800 mg drifted seaweeds *S. marginatum* extract noted significantly top most inhibition activity ($\bar{x}$ = 92.71±1.17, $F_{25,4}$ = 244.197, p – 0.003) while *S. wightii* exhibited slightly low inhibition activity observed at ($\bar{x}$ = 80.54±2.35, $F_{25,4}$ = 159.80, p – 0.003). IC_{50} value showed in the table, IC_{50} points to *S. marginatum* 489.33 mg needed for 50% inhibition activity, while *S. wightii* 287.64 mg offered for 50% reduction activity (Table 5). When compared to both antioxidant methods and both tested materials, *S. marginatum* showed high potential antioxidant radical scavenging activity at both methods.

Table 4. Antioxidant potential of selected drifted brown seaweeds' crude extract.

Methods	Con	X	DF	F	Sig	X	DF	F	Sig
		S. marginatum				***S. wightii***			
DPPH	100	$14.69±0.80^{dG}$	25,4	449.853	0.073	$11.85±1.06^{dH}$	25,4	397.90	0.730
	200	$29.93±1.90^{cE}$			0.005	$21.18±1.16^{cF}$			0.050
	400	$55.66±1.10^{bC}$			0.005	$42.09±1.28^{bD}$			0.005
	800	$70.95±1.12^{aA}$			0.005	$63.96±1.12^{aB}$			0.005
Phospho molybdenum	100	$41.43±0.40^{dH}$	25,4	244.197	0.008	$32.23±1.03^{dI}$	25,4	159.80	0.077
	200	$55.05±1.00^{cE}$			0.005	$44.30±1.65^{cG}$			0.055
	400	$78.93±0.57^{bC}$			0.005	$63.60±1.40^{bD}$			0.005
	800	$92.71±1.17^{aA}$			0.003	$80.54±2.35^{aB}$			0.003

Same ([abcd]) lowercase letter in a column shows significance at 0.05% among the methods and uppercase case ([ABCD]) letter shows the significance at 0.05% between algae.

Table 5. Probit analytical data (chi-square and significance) of drifted seaweeds extract to antioxidant activity.

Pathogen	R^2	Y	Chi-Sqsuare	Sig
S. marginatum				
DPPH	0.940	Y = -4.68+1.82x X	0.698	0.705
Phosphomolybdenum	0.997	Y= -4.09+1.89x X	1.708	0.426
S. wightii				
DPPH	0.972	Y = 17.724x - 9.54	4.281	0.039
Phosphomolybdenum	0.992	Y = 16.423x 14.11	2.072713	0.150

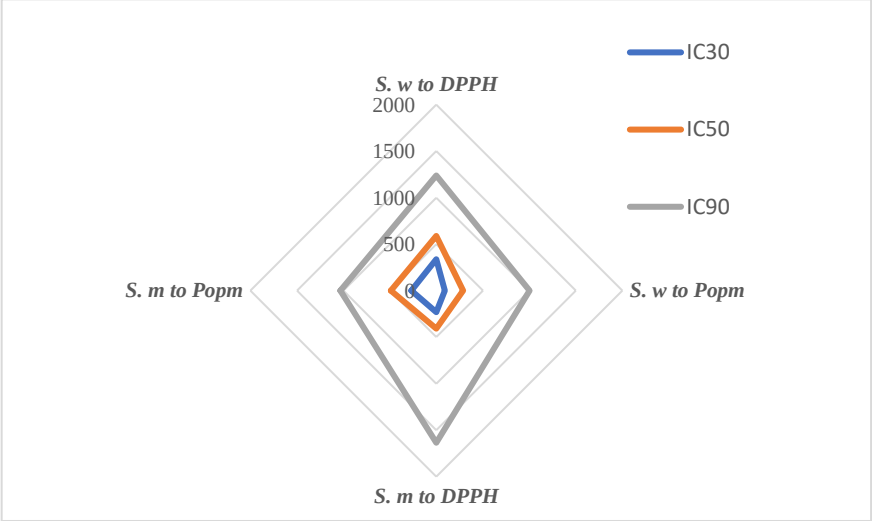

Fig. 4. LC data for drifted seaweeds extract for the antioxidant assay.

DISCUSSION

Prior, Manivannan *et al*. (2011) noticed that brown seaweeds *Turbinaria conoides, Padina gymnospora,* and *Sargassum tenerrimum* have significant antifungal effects. Alike, Sujatha *et al.* (2014) stated that *Sargassum myricocystum* significantly reduced the growth by 61.44% for *Rhizoctonia solani.* GC-MS identification exhibited that the *S. wightii* and *S. marginatum* extracts have more content of phenolic compounds, terpenoids, alkaloids, flavonoids, etc. The GC-MS identification of *S. wihtii* conformed that 2,3-Dicyano-5,6-diphenylpyrazine and 2-(acetoxymethyl)-3-(methoxycarbonyl)biphenylene are predominantly existing at verified samples, that compound may responsible for antifungal activity to *F. oxysporum* and *A. niger*. Rozaini Mohd Zohdi *et al.* (2025) report confirmed that the 2-(acetoxymethyl)-3-(methoxycarbonyl) biphenylene, and hexamethylcyclotrisiloxane form *Heterotrigona itama* can reduce the growth of *Bacillus subtilis, Enterococcus faecalis, Enterococcus faecium, Micrococcus luteus, Staphylococcus aureus, Streptococcus mutans*. The biological, chemical, and physical factors like habitat, extraction time, stages of seaweeds, extraction method, metabolites which is present, extracting solvent, etc, may be influenced by the antifungal action of seaweeds (Vimala & Poonghuzhali, 2017; Nofal *et al*., 2022).

In *S. marginatum,* the antifungal activity may be responsible to Silicic Acid or Tris (tert-butyldimethylsilyloxy), 2-Ethylacridine, because of more content (based on GC-MS result) present in drifted seaweed 70% methanol crude extract. Study of Sharma *et al*, (2021) support this finding, according to Sharma finding silicic acid inhibited 50 % growth of *Alternaria alternata, Alternaria macrospora, Alternaria solani, Cercospora arachidicola, Colletotrichum gloeosporioides, Corynespora cassiicola, Curvularia lunata, Bipolaris sorokiniana*, *Mycosphaerella fragariae, Mycopshaerella musicola, Phoma obscurans, Phyllosticta arachidis-hypogaeae* and *Pyricularia oryzae.*

More amount of flavonoid, phenolic compounds may switch the antifungal action of seaweeds as compared to red and green algae (Ambika and Sujatha, 2014; Ambika and Sujatha, 2015). These findings may agree with that opinion. Other than flavonoid and phenolic compounds, phytochemical compounds that consist of steroids, alkaloids, terpenoids, glycosides, amino acids, and oils in brown algae may contribute to the antimicrobial efficacy against human pathogens (Bansemir *et al*., 2006; Rupapara *et al*., 2015; Sheikh *et al*., 2018). Seaweed extracts not only inhibit antifungal action, which also improves plant resistance to pests, nematodes, pest and fungal disease, improve the plant growth, yield, and quality crop plant (Pardee *et al*., 2004; Jayaraj *et al*., 2008; Sujatha *et al*., 2014; Negara *et al*., 2021).

Fungal pathogen causes a broad spectrum of human disease, including mycotoxicosis, (Dignani and Anaissie, 2004), immunocompromised (Fourie *et al*., 2011), sinusitis, otitis, kerati-tis, endophthalmitis, pericarditis, endocarditis, os-teomyelitis, stomatitis, sinus granuloma (fungusball) besides central nervous system infection, skin infection, and wound infection (Alrajhi *et al*., 2001; Myoken *et al*., 2003) and affected numbers of economical important crop like tomato, eggplant and pepper, cotton root or stem rots, cankers, vascular wilts, fruit or seed rots, and leaf diseases.

(Ekwomadu *et al*., 2023). This finding helps to accredit that drifted brown seaweeds *S. wightii* and *S. marginatum* have strong antifungal activity and suggests using antifungal agents.

Reactive oxygen species (ROS) are produced inside the cells of living organisms during metabolism (Sharifi-Rad *et al*., 2020), and oxidation leads to some health disorders like cardiovascular diseases, diabetes, neurodegenerative diseases, cancer, structural deformities of mitochondrial DNA level, functional modification of enzymes, and cellular constructions (Sharifi-Rad *et al*., 2020). Seaweeds have outstanding antioxidant capacity and have been tested with various in vitro antioxidant systems (Arif Nisha Syad *et al*., 2013). In the same maner, this study was designed with drifted seaweeds brown *S. marginatum* and *S. wightii* extract tested their antioxidant inhibition activity by two methods.

Chemically, antioxidant compounds are documented as electron donors, electron-compounds, but in the case of biological, antioxidants are ingredients that can offset or reduce harmful oxidants through transferring electrons to a target oxidant compound (Nazirah *et al*., 2023). In the world wides several synthetic antioxidants are used, and they have exhibited some toxic and mutagenic effects, hence, we need naturally occurring antioxidants. Seaweeds offered many naturally occurring antioxidant substances (Devi *et al*., 2008). Prior findings of Angelina Lee Mei Ling *et al.* (2013), Ashour *et al*. (2021), Farghl *et al.* (2021) also obeyed brown seaweed extract notably (60.9, 70, and 72.48%) inhibited antioxidant activity (DPPH assay) respectively for the reason of the high amount of phenolic complex present in brown seaweeds.

This outcome showed that drifted seaweed *S. marginatum* and *S. wightii* have noteworthy antioxidant activity in both DPPH and Phosphomolybdenum methods, activity inhibited by biological substances present in the tested samples. Compared to the drifted *S. marginatum* and *S. wightii* extracts to normal seaweeds extract, drifted *S. marginatum* extract inhibition activity (70.95%) is slightly higher (66.2%) inhibition activity than normal *S. marginatum* extract (Palanivel *et al*., 2017). Both tested drifted seaweeds hold many antioxidant compounds similar to fresh ones, but in this GC-MS analysis of drifted *S. marginatum*, drifted seaweeds are rich in silicic acid content and other biochemical compounds. Silicic acid and other biochemical may be in authority to antioxidant inhibition activity in both assays for the reason that of hydrogen donating ability of seaweeds extract (Conforti *et al*., 2005) by method of increasing significant level of antioxidant defence enzymes like SOD, APX, POD, GR, and GSH (Rao *et al*., 2023). In earlier Petchidurai *et al.* (2019) confirmed that drifted seaweeds have more content of phenolic compounds. In addition, some investigators (Cahyana *et al*., 1992; Yan *et al*., 1996; Yan *et al*., 1999) found that seaweeds' phenolic compounds displayed potential antioxidant activity. That biochemical alteration reactive oxygen species (ROS) to non-toxic compounds, avoiding their harmful effect like ischemia, diabetes, cardiovascular disease, cancer **(**Farghl *et al.*, 2021**),** tumours, blood vessel narrowing, premature ageing (Rani, 2017).

As the outcome showed, the tested methanolic extract materials *S. wightii* hold potential antioxidant activity in a dose-dependent manner due to the presence of active ingredients. The antioxidant inhibition activity was expressed as percentage. Concentration of tested materials required to cause 50% of the DPPH radical and total antioxidant activity is known as IC_{50} (El- shafay *et al*., 2021). A lower IC_{50} value points to a higher antioxidant activity. In this study, the IC50-287.64 value of *S. wihtii* is best activity by total antioxidant assay methods. In a prior study by Kang *et al*. (2014), it was reported that the methanolic extract of brown seaweeds has higher antioxidant reduction activity when compared to the methanolic extract of red seaweeds; these findings also supported this investigation. This study outcome accords with that of El-Manawy *et al*. (2019) and Ali *et al*. (2024), who stated that the lowest activity IC_{50} with high antioxidant activity was recorded in the brown algae, then green and red algae.

Antioxidant activity of the tested *S. wightii* is triggered by secondary metabolites that are predominantly present in brown algae. GC-MS investigation proved, Pyrazine derivatives and phenolic active ingredients are rich in tested seaweeds in various amounts. Furthermore, solvent polarity considerably alters the extract yield, phenol solubility, and antioxidant activity of phenolic compounds in seaweeds (Nawaz *et al*., 2020). Antioxidant activity is not only responsible for phenolic compounds, which may also be caused due to other non-phenolic compounds of *P. tetrastromatica* (Souza *et al.*, 2012; Sobuj *et al*., 2021). Pyrazine derivatives retain numerous significant pharmacological properties, antimycobacterial (Riccardi *et al*., 2009), antibacterial, antifungal, antidiabetic, diuretic, anticancer (Abdul-Malik *et al.*, 2018), antiviral (Rusinov *et al.*, 2012), analgesic, and anti-inflammatory (Abdel-Mohsen, *et al*., 2016 and Tambat *et al*., 2022). *S. wightii* crude extract rich in pyrazine derivatives of 2,3-Dicyano-5,6-diphenylpyrazine and phenolic compound of 2-(acetoxymethyl)-3-(methoxycarbonyl)biphenylene, that biochemical may in authority for antioxidant inhibition activity in both methods. Mohammed and Al-Maliki (2014) and Brintha *et al*. (2021) find due to more amounts (2,3-Dicyano-5,6-diphenylpyrazine) and 2-(Acetoxymethyl)-3 (methoxycarbonyl) biphenylene from *Brassica oleracea* and *Manilkara zapota* caused antidiabetic and Antioxidant activity, respectively, in dose dose-dependent manner, that findings also support this study. Antioxidant inhibition activity of methanolic extract of *S. wightii* is caused by an H-donating property, allowing it to stop the oxidation process by transforming free radicals into stable compounds.

CONCLUSION

The antifungal and antioxidant capacity of the brown seaweeds, *S. marginatum* and *S. wightii*, were investigated. The present study reveals that tested drifted seaweeds potentially reduced the pathogenic fungal growth and inhibited antioxidant activity properties, due to which biochemical compounds are present. Similar to normal seaweeds, drifted seaweeds contain a huge number of secondary metabolites, enhancing plant growth and being environmentally safe. Drifted seaweeds can be considered as biological waste. Hence, this study recommended to use seaweeds for their pharmacological activity. Further, this kind of study should be undertaken to purify and identify the bioactive compounds involved in phytopathogenic and antioxidant activities.

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