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GC-MS PROFILING, CHEMICAL CHARACTERIZATION, ANTIOXIDANT, α-AMYLASE AND α -GLUCOSIDASE INHIBITION OF SELECTED SEAWEEDS FROM SOUTHEAST COAST OF INDIA: AN *IN VITRO* APPROACH

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ABSTRACT

The present study focuses on *in vitro* antioxidant and enzyme inhibitory activity of three different solvent extracts (Methanol, Ethyl acetate and Hexane) of 3 different seaweeds *viz*: *Sargassum wightii, Caulerpa racemosa,* and *Acanthophora spicifera.* The preliminary phytochemical analyses of the seaweed extracts have revealed the presence of various phytochemicals. The antioxidant activities of the seaweed extracts have shown the scavenging effect. Among the extracts, SWEA, SWME and SWHE have exerted effective antioxidant activity with the IC_{50} values (µg/mL) of: 32.86, 130.1 and 212.8, respectively. And similar trend of α-amylase and α-glucosidase activity/inhibitory property by seaweeds have been recorded. Hence, the ethyl acetate extract of *S. wightii* was subjected to gas chromatography. All the seaweed extracts were characterized through HPLC and FTIR analyses. The GC-MS analysis of ethyl acetate extract of *S. wightii* showed the presence of a bioactive compound, Heptadecanoic acid that might be have been the reason for the recorded inhibitory activity.

Keywords: Seaweeds, *Sargassum wightii*, DPPH, Column chromatography, GC-MS.

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INTRODUCTION

Diabetes mellitus (DM) is a chronic disease, and type 2 DM is characterized by disorder in carbohydrate, lipid and fat metabolism which severely affects the various organs of the body [1]. DM would cause various complications such as renal failure, cardiovascular disease, blindness or other liver disease [2]. In 2015, an estimated 1.6 million deaths were directly caused by diabetes. The recorded another 2.2 million deaths in 2012 has been related to the high blood glucose. Almost half of such deaths have been attributed to high blood glucose that occurs before the age of 70 years. WHO projects that diabetes will be the seventh leading cause of death in 2030 [3]. Type 2 diabetes is one of the primary threats to human health due to its increased prevalence, chronic course and disabling complications [4]. Hyperglycemic condition results in an uncontrolled production of free radicals, resulting in oxidative stress, which in turn leads to development and progression of diabetes and related complications [5]. Many diverse therapeutic strategies have been adopted for the treatment of Type 2 diabetes. The conventional available therapies for diabetes include stimulation of endogenous insulin secretion, enhancement of the action of insulin at

the target tissues, oral hypoglycemic agents, such as biguanids and sulfonylureas and the inhibition of degradation of dietary starch by glycosidases such as α-amylase and α -glucosidase. A number of anti-diabetic drugs including biguanides and thiazolidinedione have lost effectiveness against this disease, due to drug resistance and which leads to serious side effects, such as heart failure or liver disease, cardiovascular risk, hepatotoxicity, hypoglycemia, weight gain, lactic acidosis, acute pancreatitis and gastrointestinal adverse effects, and hence the need for searching safe antidiabetic medicines from natural plant sources seems to be promising [6]. During the recent decades, the bioactive compounds extracted from bio-resources like microorganisms, terrestrial and marine plants were tried against diabetes [7].

Seaweed has been traditionally consumed as a readily available whole food especially by coastal communities particularly in Asia. Seaweeds are rich in bioactive compounds in the form of polyphenols, carotenoids, vitamins, phycobilins, phycocyanins, and polysaccharides, among others, and many of these are known to possess beneficial applications in human health [8]. Polyphenolic compounds are known to form complexes upon interaction with numerous proteins and those derived from seaweeds exhibit various activities including anti-diabetes [9]. Some earlier researchers have reported that the seaweeds, *Caulerpa racemosa* [10], *Ecklonia stolonifera* [11], *Grateloupia elliptica* [12], *Sargassum ringgoldianum* [13], *Ulva rigida* [14] and *Sargassum patens* [15] possess potent inhibitory property of carbohydrate hydrolyzing enzymes. *Sargassum wightii* Greville ex J. Agardh 1848 is a brown alga belonging to the family, Sargassaceae and order Fucales and is widely distributed in tropical and temperate oceans and it is reported to be used as an animal food, food ingredient and fertilizer. A wide range of biological properties of this seaweed have been reported which includes antibacterial [16], antidiabetic [17], antioxidant [18], anti-inflammatory and antihypertensive [19]. *Acanthafora spicifera* (M.Vahl) Børgesen 1910 is a red alga belonging to the family, Rhodomelaceae and order Ceramiales. And some earlier investigation on this alga underlined its antibacterial [20], antifungal [21], antinociceptive and anti-inflammatory properties [22]. *Caulerpa racemosa* (Forsskål) J. Agardh 1873 is a green alga belonging to the family Caulerpaceae and order Bryopsidales. The bio-efficacy of this alga is antibacterial and larvicidal [23] and antioxidant & antiproliferative values [24]. In view of the above, the present study was to evaluate the *in vitro* enzyme inhibitory activity of above seaweeds against α-amylase and α-glucosidase.

MATERIALS AND METHODS

Sample collection

Fresh samples of *Sargassum wightii*, *Acanthafora spicifera* and *Caulerpa racemosa* were collected from the intertidal region of Mandapam, Ramanathapuram District, Tamil Nadu, Southeast coast of India (9° 22' N, 78° 52 ' E) (**Figure. 1**).

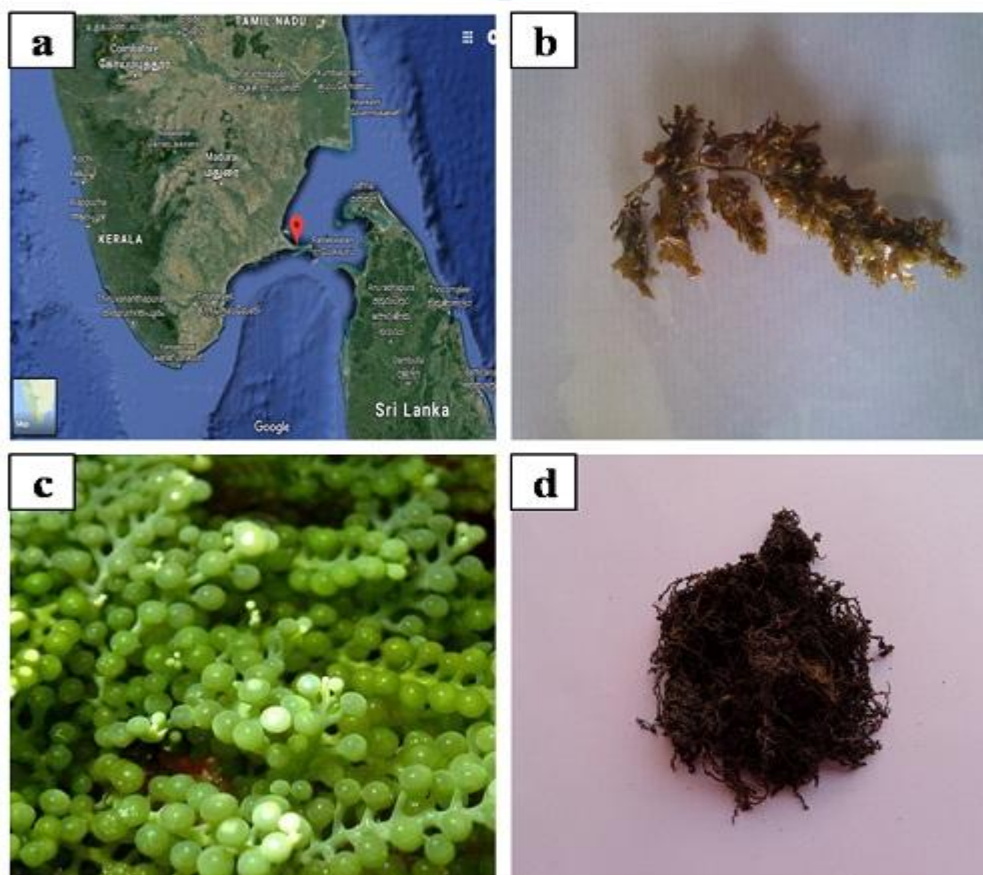

Figure 1: **a)** Map showing site of seaweeds collection **b)** *Sargassum wightii*, **c)** *Acanthafora spicifera* and d) *Caulerpa racemosa*

Processing of seaweed samples

The collected seaweed samples were washed with marine water and then transported to the laboratory in a plastic cover and then washed thoroughly with tap water followed by distilled water until the debris and associated biota were removed. Then they were shade-dried for 3-weeks. Then the dried materials of all the seaweeds were ground using the kitchen electric blender. The seaweeds were identified based on the morphological characteristics using the standard keys [25] and the identification was subsequently confirmed by Dr. N. Kaliaperumal, Principal Scientist (Retd.), Central Marine Fisheries Research Institute, Mandapam Camp, Ramanathapuram District (India). The reference specimens (PU/MBEG/V.022) have been kept in the Department of Biotechnology, Periyar University, Salem, Tamil Nadu (India). All the chemicals used in the study were of analytical grade with maximum purity.

Bioactive compound extraction

About 10 g of each powdered seaweed material was initially soaked into 150 mL of methanol, ethyl acetate and hexane for three days with mild shaking. Then the extract was filtered through Whatman filter paper and concentrated in Rotary evaporator. Further the concentrated methanol, ethyl acetate and hexane extracts of *Sargassum wightii* (SWME, SWEA and SWHE), *Acanthafora spicifera* (ASME, ASEA and ASHE) and *Caulerpa racemosa* (CRME, CREA and CRHE) were stored in refrigerator until use.

Phytochemical screening

The freshly prepared seaweed samples were subjected to preliminary phytochemical screening as per the standard protocol [26].

Characterization of seaweed extracts

Purity analysis by High Performance Liquid Chromatography (HPLC)

The HPLC analyses of seaweed extracts were carried out by following the standard protocol [27]. About 1 mg of each sample was dissolved in 1 mL of methanol and 20 µL was injected. Methanol: water (50:50) was used as mobile phase. The experiment was performed in Shimadzu LC solution 20 AD, Japan and SPD 20 A, an instrument equipped with a UV detector (254 nm) in order to determine the peak purity. LCGC C18 column was used for isocratic resolution using the mobile phase at a flow rate of 1.0 mL/min.

Fourier Transformer Infrared Spectrophotometer (FT-IR)

The FTIR analysis of seaweed extracts were performed using FTIR spectrometer coupled with TGS (Tri-glycine sulphate) detector (Bruker, D8, Germany model). In brief, 1 mg of each dried sample was mixed with 100 mg of potassium bromide (KBr) and then compressed to prepare salt-disc (3 mm dia). These discs were recorded in the mid-IR region 4000-400 cm^{-1} at room resolution 4 cm^{-1} [28].

DPPH radical scavenging assay

DPPH (1, 1-Diphenyl-2-picryl-hydrazyl) radical scavenging activity of seaweed extracts were performed according to the earlier method [29]. In brief, 2 mL(1 mg/mL) of samples and standard at different concentrations (3.125, 6.25, 12.50, 25, 50 and 100 µg/mL) were mixed with 1 mL of 0.1 mM DPPH in methanol. The mixture was instantly shaken and incubated for 30 min in dark condition. The absorbance of the samples and control was measured at 517 nm.

Ferric Ion Reducing Power (FRAP) assay

FRAP assay of To-HE and To-AE was performed according to Adedayo et al [30]. The FRAP stock reagent was prepared with 300 mM/L acetate buffer (pH-3.6), 10 mM/L TPTZ (2, 4, 6- tri [2-pyridyl]-s-triazine) and 20 mM/L ferric chloride hexahydrate solution. The working solution was prepared with 30mLof acetate buffer, 4 mL of TPTZ solution and 4mLof ferric chloride hexahydrate solution. In brief, 500 µL (1 mg/mL) of different concentration (3.125-100 µg/mL) of samples and standard were prepared and methanol was added to 2.5 mL of FRAP reagent and then incubated in dark for 30 min. The absorbance of the samples and control was measured at 593 nm. All the experiments were performed in triplicate. Ascorbic acid was used as a standard [30].

Statistical analysis

The *in vitro* antioxidant activities of the samples were calculated using the following formula:

$$\% \text{ Inhibition} = \frac{A_0 - A_1}{A_0} \times 100$$

Where, A_0 is absorbance of the control, A_1 is absorbance of the sample.

***In vitro* Antidiabetic activity**

α- amylase inhibitory activity

The α-amylase inhibitory activity of seaweed extracts was evaluated according to the standard procedure of Matsui et al. [31]. Two hundred micro liter of starch (0.4 mg/mL) and 100 µL of seaweed extracts (1 mg/mL), at varied concentration (3.125-100 µg/mL), were dissolved in phosphate buffer (20 mM. NaH_2PO_4 and 6.7 mM. NaCl, pH 6.9). Consequently, 50 µL of α-amylase solution (1 U/mL in phosphate buffer) was added to the sample mixture, after which phosphate buffer was added to obtain a final volume of 500 µL. Then, the reaction mixture was incubated at room temperature for 3 min to facilitate the enzymatic reaction to occur. Finally, the reaction was terminated by adding 1mLof 0.1% hydrochloric acid. Subsequently, iodine reagent was added to the mixture. The decrease in starch concentration due to the activity of α-amylase was measured at 660 nm using a multi-well ELISA plate reader (Tokyo, Japan).

α-glucosidase inhibitory activity

The α-glucosidase inhibitory activity of seaweed extracts was performed as per the modified method of Sheliya et al. [32]. In brief, 50 µL of seaweed extracts (1 mg/mL) at different concentrations (3.125-100 µg/mL)

and 50 µL of 0.1 M phosphate buffer (pH 6.8) containing α-glucosidase solution (0.1 µL/mL) were incubated at 37°C for 10 min. After, 50 µL of 2.5 mM p-nitrophenyl-α-D-glucopyranoside (PNPG) solution was added in the same buffer and incubated for 20 min. Then, the reaction was terminated by adding 100 µL of 0.2 M Na_2CO_3 and absorbance was recorded at 405 nm.

Gas Chromatography-Mass Spectrometry (GC-MS)

GC analysis of SWEA was performed by injecting 1µL of sample on a 5MS column of GC-MS model (Perkin Elmer, Massachusetts, USA) mobile phase: Helium. The qualitative and quantitative analysis of SWEA was carried out using a CP 3800 Saturn 2200 Gas Chromatography-Mass Spectrometer. The temperature program was 80°C to 350°C at the rate of 3°C/min increase. Ion temperature was 200°C and scan range was 20-500 AMU (Atomic Mass Unit). The identification of components was based on comparison of their mass spectra with those of Wiley library [33].

RESULTS

Phytochemical screening

The results of phytochemical analyses of all the seaweed extracts revealed the presence of various phyto-constituents (**Table.1**). SWEA revealed the presence of all the tested phytochemical whereas SWME has shown positive result to all the tested phytochemical except alkaloids. Similarly ACHE has indicated that except tannin all the other phytochemicals were found to be present.

Table 1: Phytochemical analyses of seaweed extracts

Phytochemical	SWME	SWEA	SWHE	ACME	ACEA	ACHE	CRME	CREA	CRHE
Flavonoids	+	+	-	+	-	+	+	+	-
Saponins	+	+	+	-	+	+	+	-	+
Tannin	+	+	-	+	+	-	-	-	+
Phenolics	+	+	+	+	+	+	+	+	+
Alkaloids	-	+	+	-	-	+	+	-	+
Steroids	+	+	-	+	-	+	-	+	-

[+] Present; [-] Absent

High Performance Liquid Chromatography (HPLC)

The HPLC analysis of seaweed extracts indicated the presence of various bioactive compounds as it shown many peaks (**Fig.2**). The major peaks found in the extracts of *S. wightii* with the retention time (R_T) are of 5.464 (SWME), 5.494 (SWEA) and 5.283 (SWHE), whereas the extracts of *A. spicifera* are: 2.836 (ASME), 2.851 (ASEA) and 2.565 (ASHE). Similarly, the extracts of *C. racemosa* have shown the following peaks: 2.959 (CRME), 2.856 (CREA) and 2.983 (CRHE).

Fourier Transformer Infrared Spectrophotometer (FT-IR)

The results of FTIR analysis of the three different solvent extracts of *Sargassum wightii*, *Acanthafora spicifera* and *Caulerpa racemosa* are shown in **Figs. 3-5** and their corresponding functional groups are depicted in **Tables 2-4**. The FTIR analysis of SWME revealed the presence of eight major peaks at 3408.87, 2925.22, 1736.31, 1651.14, 1541.00, 1169.89, 1037.00 and 721.58 cm^{-1}, and the SWEA displayed nine peaks at 3423.68, 2925.57, 2854.2, 1569.89, 1424.31, 1299.38, 1015.26, 879.35 and 650.84 cm^{-1}. And the SWHE have shown ten peaks at 3192.54, 2918.12, 2849.72, 2081.86, 1632.30, 1578.03, 1541.39, 1419.37, 1249.31 and 1077.04 cm^{-1}. The methanol extract of *A. spicifera* has revealed the presence of seven peaks at 3243.08, 2917.53, 2849.94, 2077.74, 1636.61, 1384.52 and 1045.60 cm^{-1}, and the ASEA revealed the presence of nine major peaks at 3406.02, 2919.57, 2852.00, 1725.12, 1561.34, 1420.80, 1272.20, 1078.49 and 718.43 cm^{-1}.And the ASHE have also displayed the presence of nine major peaks at 3408.97, 2922.06, 2851.41, 1651.52, 1457.03, 1229.06, 1037.56, 873.97 and 464.15 cm^{-1}.

The FTIR analysis of CRME revealed the following peak vibrations at 3297.52, 2954.59, 2856.92, 2398.64, 1694.29, 1579.39, 1443.58, 1323.34 and 1098.58 cm^{-1} and the CREA has shown the functional groups of 3383.98, 2920.83, 2852.38, 1687.31, 1563.13, 1418.65, 1264.60, 1057.23 and 731.36 cm^{-1}. Similarly, the CRHE has also revealed the 9-peak vibrations at 3447.72, 2922.37, 2852.05, 1765.32, 1636.57, 1541.45, 1465.34, 1242.31 and 1039.17 cm^{-1}.

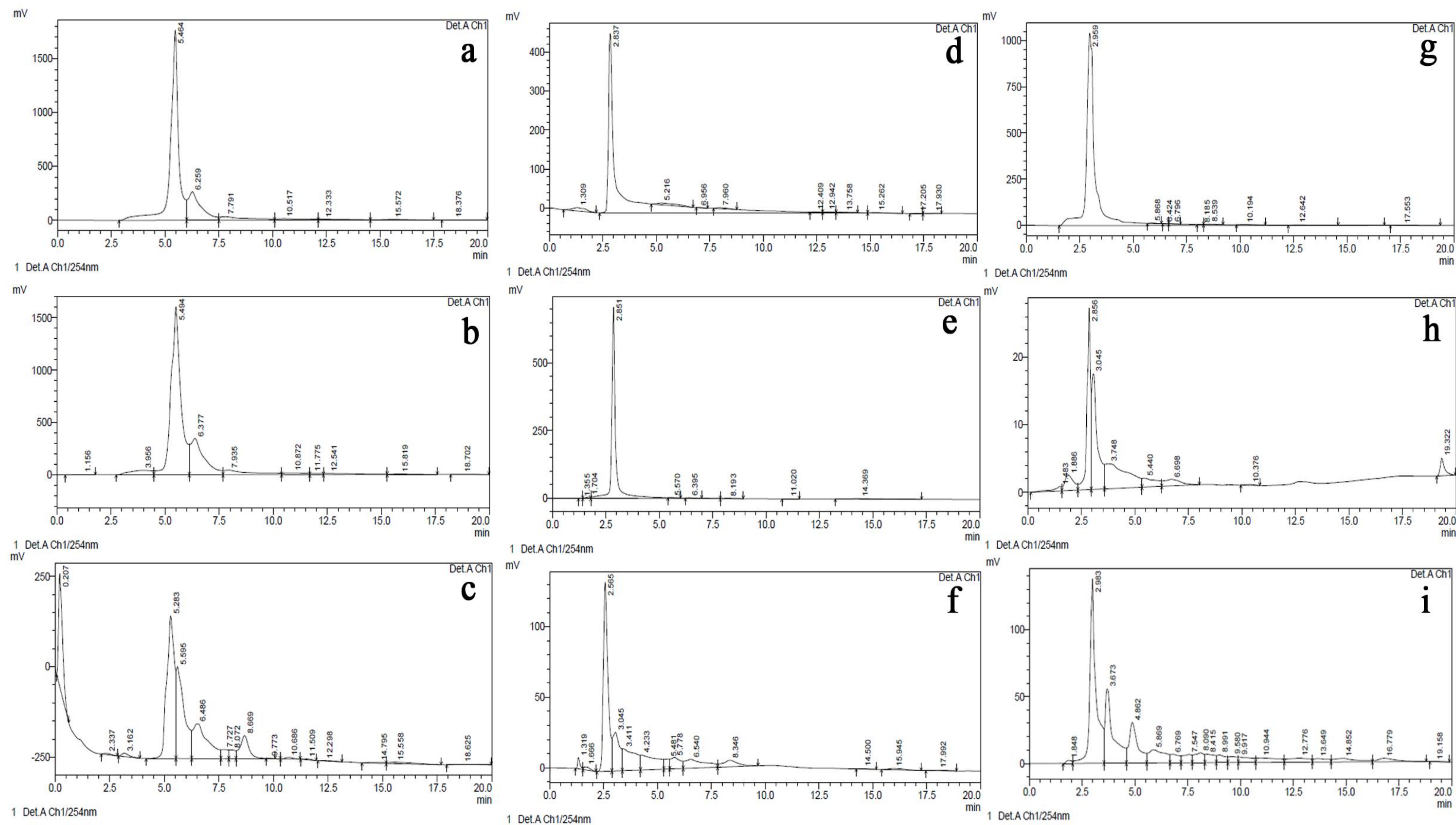

Figure 2: HPLC analyses of seaweed extracts of a) SWME, b) SWEA, c) SWHE, d) ASME, e) ASEA, f) ASHE, g) CRME, h) CREA and i) CRHE.

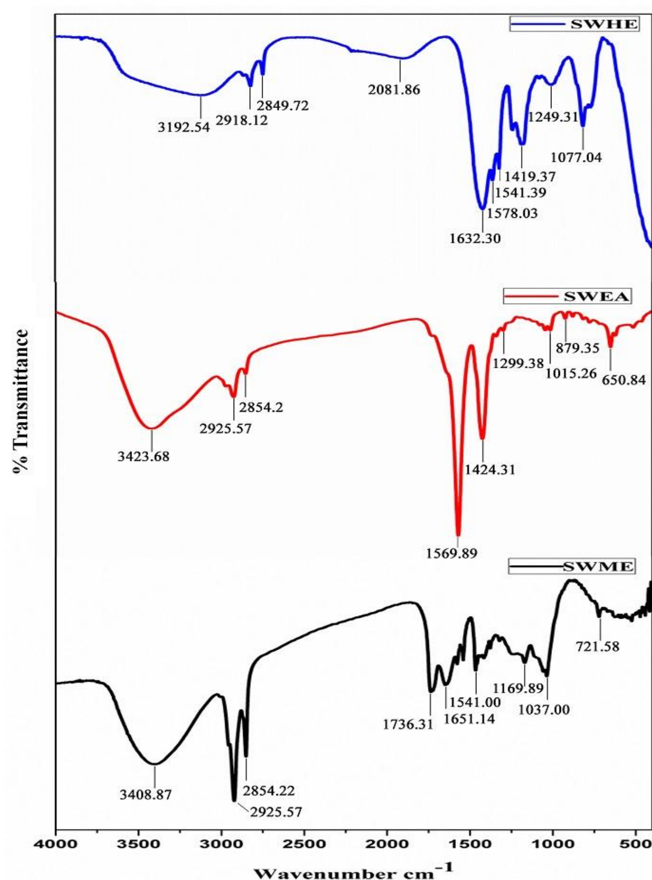

Figure 3: FTIR spectrum analyses of *Sargassum wightii*

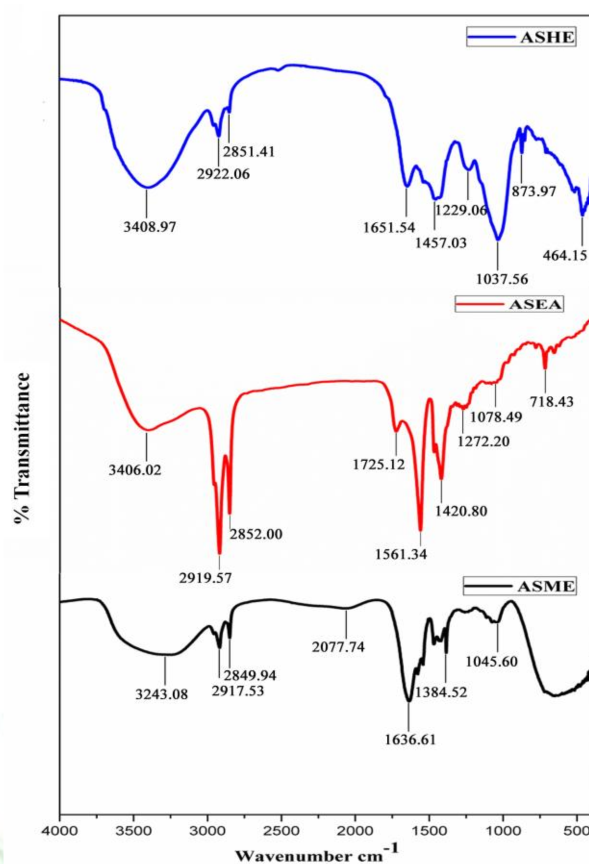

Figure 4: FTIR spectrum analyses of *Acanthafora spicifera*

Table 2: Functional group analyses of various solvent extracts of *S. wightii*

Sample name	Vibration	Stretch	Functional Group
	3408.87	CH	Alkanes
	2925.22	-CH2-	Alkanes
	1736.31	C=O	Esters
SWME	1651.14	C=C	Alkanes
	1541.00	NH	Amines
	1169.89	C-N	Amines
	1037.00	S=O	Miscellaneous
	721.58	Bending mode	Alkanes
	3423.68	CH	Alkanes
SWEA	2925.57	-CH2-	Alkanes
	2854.2	-CH2-	Alkanes
	1569.89	NH2	Amines
	1424.31	Ar C-C	Aromatics
	1299.38	C-F	Alkyl halides
	1015.26	P-OR	Miscellaneous
	879.35	C-H	Aromatics
	650.84	C-Br	Alkyl halides
	3192.54	Dimer OH	Carboxylic acids
	2918.12	C=H	Alkanes
	2849.72	C=O	Carboxylic acids
	2081.86	N=C in R-N=C=S	Miscellaneous
SWHE	1632.30	C=O	Amides
	1578.03	C-O	Carboxylic acids
	1541.39	NH	Amides
	1419.37	Ar C-C	Aromatics
	1249.31	C-Br	Alkyl halides
	1077.04	C-O	Ethers

Table 3: Functional group analyses of various solvent extracts of *A. spicifera*

Sample Name	Vibration	Stretch	Functional Group
ASME	3243.08	dimer OH	Carboxylic acids
	2917.53	CH	Alkanes
	2849.94	-CH2	Alkanes
	2077.74	N=C in R-N=C=S	Miscellaneous
	1636.61	C=N	Miscellaneous
	1384.52	S=O sulfate ester	Miscellaneous
	1045.60	C-N	Amines
ASEA	3406.02	CH	Alkanes
	2919.57	CH	Alkanes
	2852.00	CH	Alkanes
	1725.12	C=O	Esters
	1561.34	C=C	Alkenes
	1420.80	Ar C-C	Aromatics
	1272.20	C-F	Alkylhalides
	1078.49	C-N	Amines
	718.43	S-OR esters	Miscellaneous
ASHE	3408.97	CH	Alkanes
	2922.06	dimer OH	Carboxylic acids
	2851.41	-CH2-	Alkanes
	1651.52	C=O	Amides
	1457.03	Ar C-C	Aromatics
	1229.06	C-N	Amines
	1037.56	S=O	Miscellaneous
	873.97	S-OR esters	Miscellaneous
	464.15	CBr-	Alkyhalides

Table 4: Functional group analyses of various solvent extracts of *C. racemosa*

Sample Name	Vibration	Stretch	Functional Group
CRME	3297.52	dimer OH	Carboxylic acids
	2954.59	CH	Alkanes
	2856.92	CH	Alkanes
	2398.64	P-H	Miscellaneous
	1694.29	dimer C=O	Carboxylic acids
	1579.39	C-O	Carboxylic acids
	1443.58	S=O	Miscellaneous
	1323.34	C-F	Alkylhalides
	1098.58	dimer C=O	Carboxylic acids
CREA	3383.98	dimer OH	Carboxylic acids
	2920.83	CH	Alkanes
	2852.38	CH	Alkanes
	1687.31	dimer C=O	Carboxylic acids
	1563.13	N=O nitroso	Miscellaneous
	1418.65	S=O sulfate ester	Miscellaneous
	1264.60	dimer C=O	Carboxylic acids
	1057.23	S=O sulfoxide	Miscellaneous
	731.36	S-OR esters	Miscellaneous
CRHE	3447.72	CH	Alkanes
	2922.37	dimer OH	Carboxylic acids
	2852.05	CH	Alkanes
	1765.32	Monomer C=O	Carboxylic acids
	1636.57	C=O	Amides
	1541.45	NH	Amides
	1465.34	Ar C-C	Aromatics
	1242.31	C-F	Alkylhalides
	1039.17	P-H	Miscellaneous

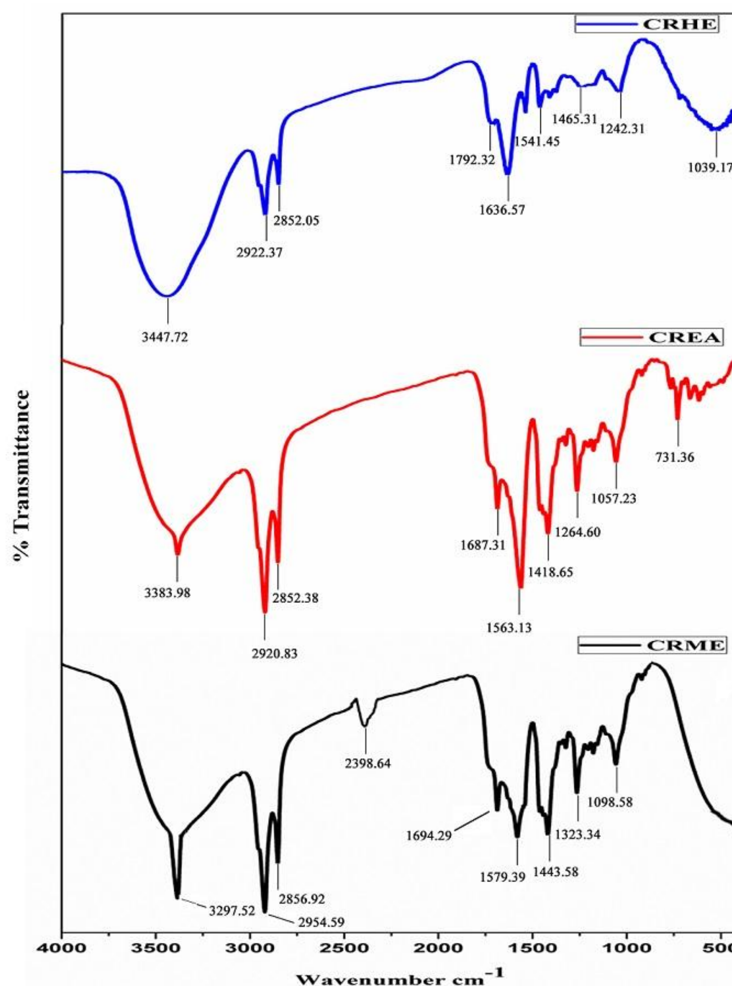

Figure 5: FTIR spectrum analyses of *Caulerpa racemosa*-extract

DPPH radical scavenging assay

The DPPH scavenging property of the seaweed extracts were compared with the positive control, ascorbic acid (**Fig. 6**). Among the different concentration of samples/control used in this study, 100 µg/mL of *S. wightii* extracts resulted in the highest scavenging effect of 41.40% (SWME), 68.01% (SWEA), 36.06% (SWHE) and 72.49% (ascorbic acid). The IC_{50} values (µg/mL) of SWME, SWEA, SWHE and ascorbic acid were: 130.1±1.44, 32.86±1.96, 212.8±0.77 and 30.32±1.92, respectively.

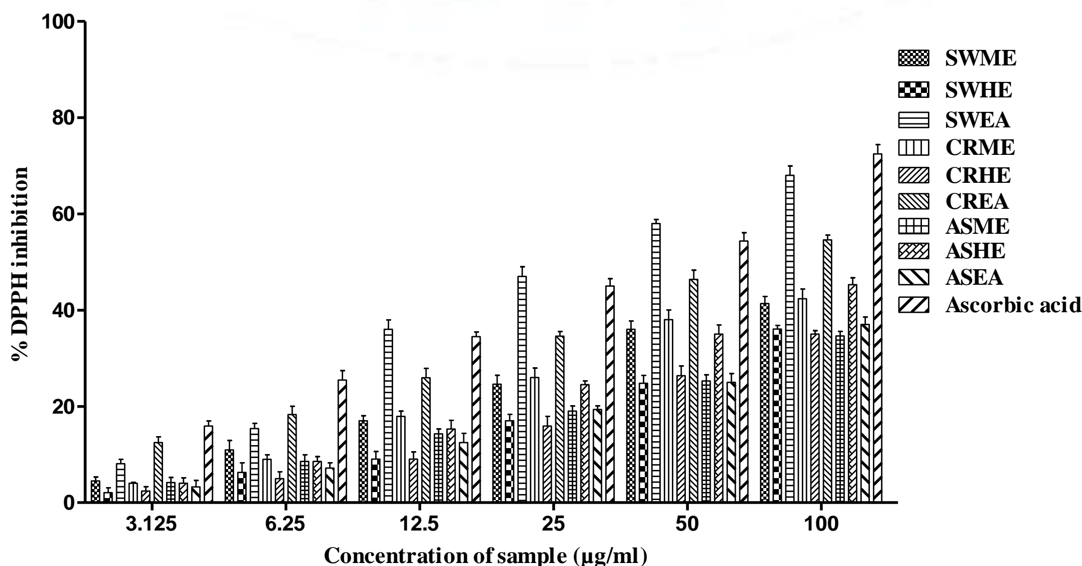

Figure 6: DPPH scavenging activities of seaweed extracts.

Ferric Ion Reducing Power (FRAP) assay

Among the various concentration of seaweed extracts, 100 μg/mL of *S. wightii* extract exerted the highest scavenging effect of 36.95 % (SWME), 68.09% (SWEA), 32.94% (SWHE) and 75.03% (ascorbic acid) (**Fig.7**). The IC_{50} values (μg/mL) of SWME, SWEA, SWHE and ascorbic acid were: 306.5±0.99, 32.56±1.39, 226.6±0.37 and 27.24±2.01, respectively.

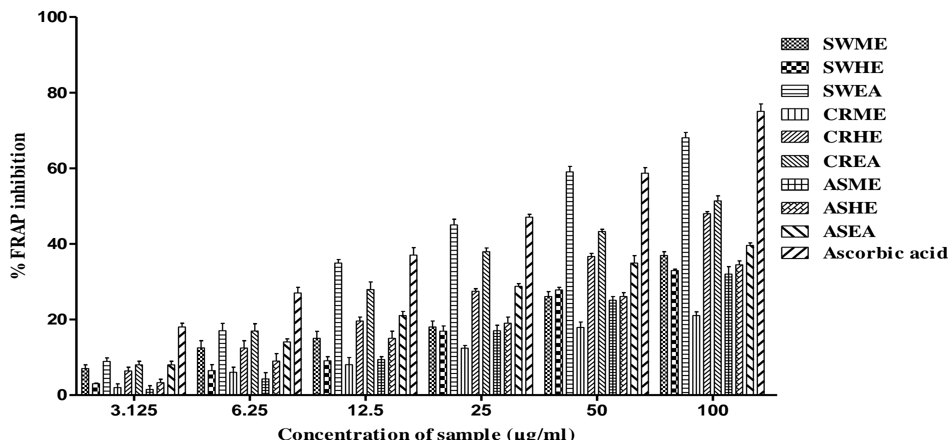

Figure 7: FRAP scavenging activities of seaweed extracts.

In vitro Antidiabetic activity

α- amylase inhibitory activity

The inhibitory percentage of α- amylase by the different seaweed extracts are shown in **Fig. 8.** The three different extracts of *S. wightii* have showed the highest inhibitory percentage of α- amylase when compared to the seaweed extracts of *A. spicifera* and *C. racemosa* with the IC_{50} values (μg/mL) of SWME, SWEA and SWHE being: 41.8 ±0.64, 13.15 ±1.17 and 122.5 ±1.95 respectively. The positive control (acarbose) has shown the potent α-amylase inhibitory activity with the IC_{50} value (μg/mL) of 8.946 ±1.21.

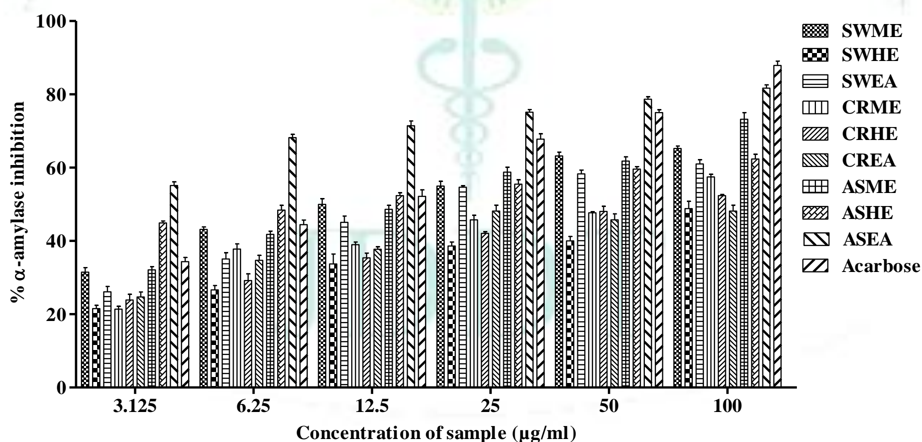

Figure 8: α-amylase inhibitory activity of seaweed extracts.

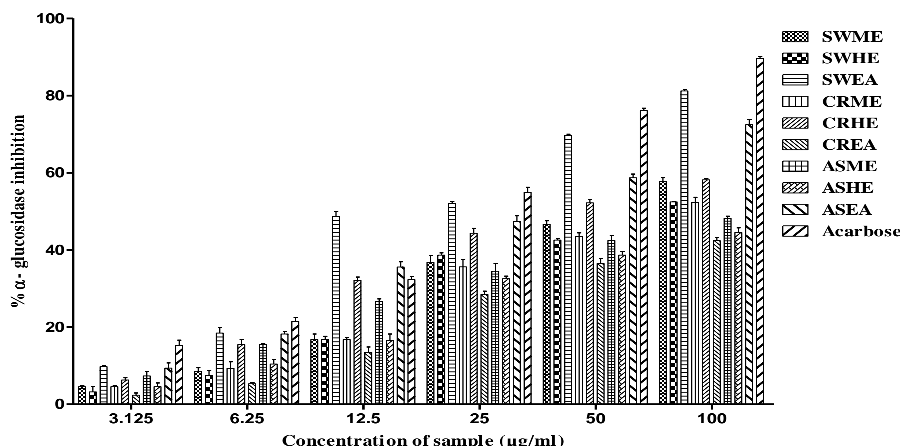

Figure 9: α-glucosidase inhibitory activity of seaweed extracts

α-glucosidase inhibitory activity

The inhibition of α-glucosidase by the different seaweed extracts are depicted in **Fig. 9.** The maximum inhibition of α-glucosidase was exerted by the extracts of *S. wightii* with the IC_{50} values (μg/mL) of SWME, SWEA and SWHE being: 60.12±0.92, 20.44±0.36 and 71.20±0.12 respectively. And the effective inhibitory property of α-glucosidase was revealed by the positive control (acarbose) with the IC_{50} value (μg/mL) of 20.10±0.53.

Gas Chromatography-Mass Spectrometry (GC-MS)

The GC-MS analysis of SWEA indicated the presence of 8 compounds (**Fig. 10 and Table.5**). The recorded phyco-constituents of the ethyl acetate extract of *S. wightii* are: Phloretin (MW: 273, MF:$C_{15}H_{13}O_5$, R_T:2.96), Heptadecanoic acid (MW: 270, MF: $C_{17}H_{34}O_2$, R_T:19.95), Oleic acid (MW: 282, MF: $C_{18}H_{34}O_2$, R_T:21.00), 5-p-coumaroylquinic acid (MW: 337, MF: $C_{16}H_{17}O_8$, R_T:24.09), Phloretinrhamnosyl(1→6) glucoside (MW: 581, MF: $C_{27}H_{33}O_{14}$, R_T:26.41), Quercetin-3-O- arabinpyranoside (MW: 433, MF: $C_{20}H_{17}O_{11}$, R_T:26.80), Caffeic acid hexoside (MW: 341, MF: $C_{15}H_{17}O_9$, R_T:28.47) and Squalene (MW: 410, MF: $C_{30}H_{50}$, R_T:29.97). The percentage of peak area and details of the biological properties of each compounds are represented in **Table 5**.

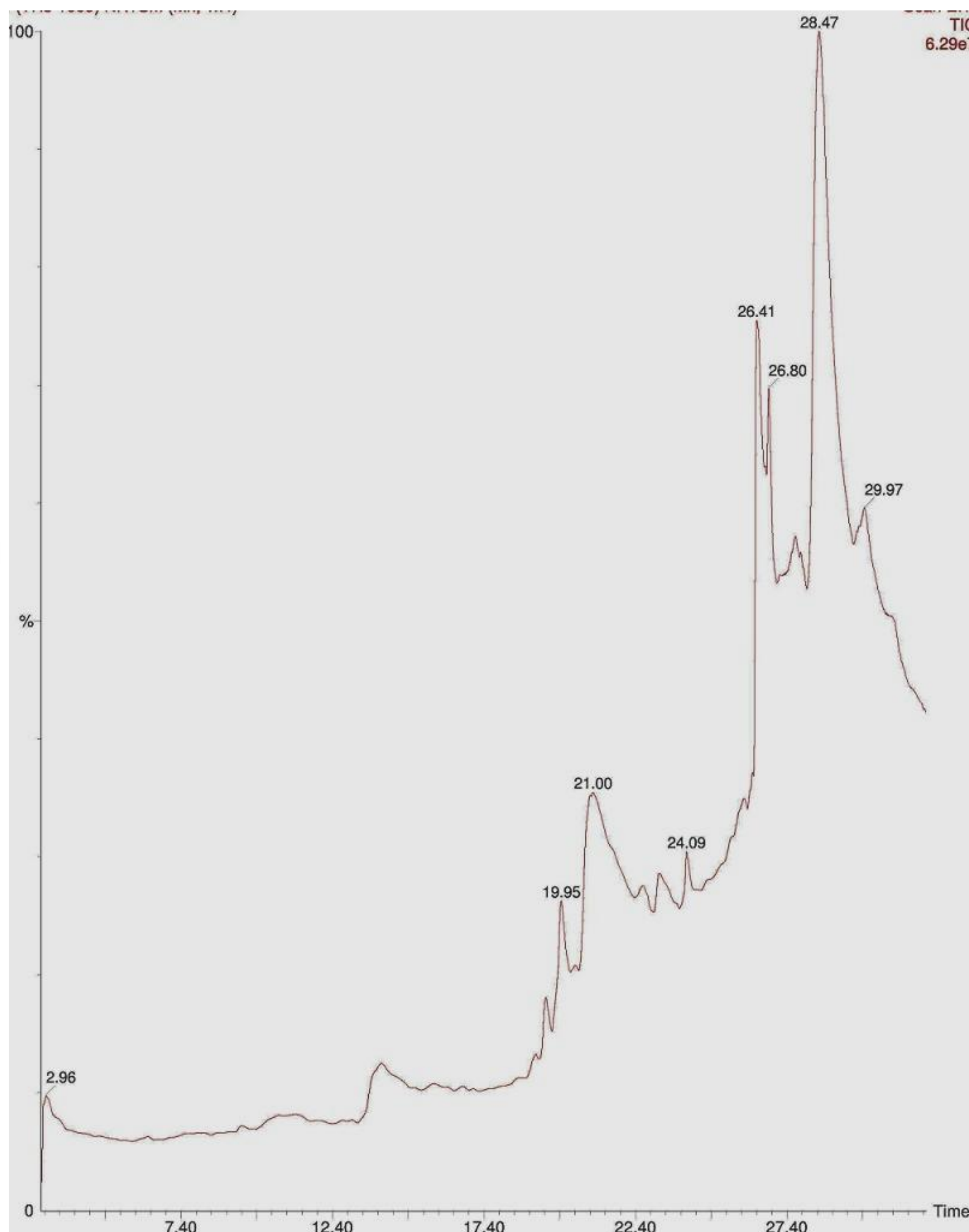

Figure 10: GC-MS analysis of SWEA

Table 5: Chemical constituents of SWEA analyzed by Gas Chromatography-Mass Spectrometry

R_T (min)	S. No	Compound Name	MW	Molecular formula	Peak area	Biological Properties	Reference
2.96	1	Phloretin	273	$C_{15}H_{13}O_5$	1.52	Antioxidant	Nithiya and Udayakumar, 2015
19.95	2	Heptadecanoic acid	270	$C_{17}H_{34}O_2$	66.38	Antioxidant and anti-proliferative	Elagbar et al., 2016
21.00	3	Oleic acid	282	$C_{18}H_{34}O_2$	1.50	Antioxidant, cell proliferation and prevents the Pro-apoptotic effect	Wei et al., 2016; Mancini et al., 2015
24.09	4	5-*p*-coumaroylquinic acid	337	$C_{16}H_{17}O_8$	4.37	Antioxidant	Karamać et al., 2012
26.41	5	Phloretinrhamnosyl(1→6) glucoside	581	$C_{27}H_{33}O_{14}$	6.45	No activity found	----
26.80	6	Quercetin-3-*O*-arabinpyranoside	433	$C_{20}H_{17}O_{11}$	8.57	Antibacterial, anti-inflammatory, antioxidant, and antiviral	David et al., 2016
28.47	7	Caffeic acid hexoside	341	$C_{15}H_{17}O_9$		Antioxidant and cytoprotection	Gaglione et al., 2013
29.97	8	Squalene	410	$C_{30}H_{50}$	11.10	Antioxidant, anticancer, anti-infectant	Kim and Karadeniz, 2012

DISCUSSION

Marine algae are known to be an important source for bioactive compounds of important pharmaceutical metabolites and biomedical potential. In particular, the brown algae have a variety of biological compounds such as pigments, fucoidans, phycocolloids, and phlorotannins. Among them, phlorotannins have been extensively studied for their potential health benefits and they have shown promising antidiabetic effects [34]. There are many reports that suggested that seaweeds contain biologically active compounds than the plants found in the terrestrial area [35]. Seaweeds are a natural source of bioactive molecules with a broad range of biological activities, such as antibiotics, antivirals, anti-tumorals, antioxidants and anti-inflammatories. The phytochemical constituents such as flavonoids, tannins, phenolics, sterols and terpenoids are secondary metabolites of seaweeds that serve as defense mechanism against different infectious diseases [36]. In the present study the phytochemical analysis of three different solvent (methanol, ethyl acetate and hexane) extracts of seaweeds, *S. wightii*, *A. spicifera* and *C. racemosa* revealed the presence of various phytochemicals at different concentrations of solvent polarity. The earlier report of Baleta et al. [36] have showed the phytochemicals present in the brown algae, *S. oligocystum* and *S. crassifolium* were found to vary according to the type of solvents used. The phyto-constituents such as, phenols and flavonoids were reported for enhancing the scavenging of free radicals, as we have found in the present study [37]. The biological activity of any bio-organic compound may be influenced by its functional groups. Hence, the FTIR analysis of the extract would shed light on the physicochemical properties of a compound. Presently, the FTIR analysis of seaweed extracts have shown the presence of various functional groups *viz*; carboxylic acid, alkanes, alkynes, aromatics and alkyl halides which are similar to the earlier study of Fernando et al. [38].

Natural antioxidants are not limited to terrestrial sources and reports have revealed seaweeds to be rich sources of natural antioxidant compounds. The presence of antioxidant substances in seaweeds is considered to be an endogenous defense mechanism as a protection against oxidative stress due to extreme level of environmental conditions [39]. During the earlier study it was found reported that the antioxidant activity of brown seaweed extracts correlated with their polyphenol content and this might have been the cause of the presently recorded inhibitory effects [40]. Devi et al. [41] reported the maximum inhibition of DPPH scavenging by red alga, *Gelidiella acerosa* with the IC_{50} value (µg/mL) of: 72.5 ± 2.78. Indu and Seenivasan, [42] have reported that maximum scavenging effect of DPPH was shown by the ethanol extract of *S. wightii* (79.1±0.23 %) and also its acetone extract (78.8±0.18 %) that are comparable to the present findings. The methanolic extracts of the brown seaweeds were able to reduce Fe^{3+} to Fe^{2+} in a concentration-dependent manner as a function of reducing power as reported earlier [43].

The most common therapeutic approach to treat diabetes is reduction of postprandial hyperglycemia. This can be achieved by inhibition of enzymes involved in the release of glucose from foods and this approach is used in the management of type 2-diabetes, with the main target being α-amylase and α-glucosidase. α -amylase is an enzyme responsible for the breakdown of complex carbohydrate like starch to more simple sugars like glucose. Thus, the inhibition of this enzyme can delay the carbohydrate digestion and reduce the rate of glucose absorption. Natural α-amylase inhibitor offers an attractive therapeutic approach in the treatment of postprandial hyperglycemia, through decreased glucose release from starch. Marine seaweeds that can reduce

postprandial hyperglycemia by inhibiting enzymes such as α -amylase and α -glucosidase are found to be an effective strategy for the management of diabetes [44]. In the present study, ethyl acetate extract of *S. wightii* have shown the maximum inhibition of α-amylase (13.15 ±1.17). Similarly, Payghami et al. [45] have reported that the brown alga, *Sargassum glaucescens* exerted maximum inhibitory activity on α-amylase with has the minimum IC_{50} value of 8.9 ± 2.4 mg/mL. Presently, the GC-MS analysis of SWEA revealed the presence of 8 active compounds *viz;* Phloretin, Heptadecanoic acid, Oleic acid, 5-p-coumaroylquinic acid, Phloretinrhamnosyl(1→6) glucoside, Quercetin-3-O-arabinpyranoside, Caffeic acid hexoside and Squalene. Among them, Phloretin was found to have antioxidant activity as reported earlier [46]. The previous research on GC-MS analysis of *Grateloupia elliptica* revealed the presence of squalene that has been reported to have antioxidant, anticancer and anti-infectant effects. The presently isolated bioactive compound, Heptadecanoic acid of SWEA, has been known to have both antioxidant and anti-proloferative properties [47]. And the presently recorded enzyme inhibitory property of *S. wightii* could be due to its bioactive compound, Heptadecanoic acid.

CONCLUSION

The present study revealed that the ethyl acetate extract of *S. wightii* (SWEA) was more effective than the other seaweed extracts due to the presence of Heptadecanoic acid. In addition, the SWEA has shown maximum inhibition percentage with minimal inhibitory concentration (IC_{50}) during the antioxidant and enzyme inhibitory studies. Therefore, the findings of the present study could form a basis for further studies towards the development of new pharmaceutical drug formulation for the treatment of Diabetes mellitus.

Conflicts of interest statement

The authors have no conflict of interest to declare.

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