Antioxidant and DPP-IV inhibitory activities of *Sargassum cristaefolium* extract: A therapeutic potential agent for type-2 diabetes

Agung Giri Samudra[1,2], *Agung* Endro Nugroho[3*], *Retno* Murwanti[3,4]

[1]Faculty of Pharmacy, Universitas Gadjah Mada, Yogyakarta 55281, Indonesia
[2]Department of Pharmacy, Faculty of Mathematics and Natural Sciences, Bengkulu University. Bengkulu 38371, Indonesia
[3]Department of Pharmacology and Clinical Pharmacy, Faculty of Pharmacy, Universitas Gadjah Mada, Yogyakarta 55281, Indonesia
[4]Medicinal Plants and Natural Products Research Center, Faculty of Pharmacy, Universitas Gadjah Mada, 55281 Yogyakarta, Indonesia

Abstract. Diabetes mellitus (DM) is a metabolic disorder characterized by elevated blood glucose levels due to impaired insulin function. Dipeptidyl peptidase 4 (DPP-IV) inhibitors are potential agents for managing type-2 DM. This study evaluates the total phenolic and flavonoid content, antioxidant activity, and DPP-IV inhibitory potential of the ethanolic extract and ethyl acetate fraction of *Sargassum cristaefolium*. Antioxidant activity was assessed using the DPPH assay, while DPP-IV inhibition was compared with sitagliptin. Gas Chromatography-Mass Spectrometry (GC-MS) was used to identify active compounds. The total phenolic content in the ethanol extract and ethyl acetate fraction was 1.04% and 7.33% GAE, respectively, while total flavonoid content was 1111.70 and 3529.01 µgQE/g. Antioxidant activity increased in a concentration-dependent manner, with IC_{50} values of 1.38 mg/mL (ethanol extract) and 0.71 mg/mL (ethyl acetate fraction). The ethanolic extract (500 ppm) and ethyl acetate fraction (62.5 ppm) exhibited DPP-IV inhibitory activity of 92.07% and 95.87%, respectively. GC-MS identified hexadecenoic acid, methyl ester, and trans-13-octadecenoic acid, methyl ester as dominant compounds. *S. cristaefolium* exhibits promising antioxidant and DPP-IV inhibitory activity, supporting its potential role in type-2 DM management.

1 Introduction

According to the International Diabetes Federation (IDF) Diabetes Atlas, it is projected that by 2030, approximately 643 million adults aged 20 to 79 years, representing an estimated prevalence of 11.3%, will live with diabetes. By 2045, this number is predicted to increase to approximately 783 million adults, with a projected prevalence of 12.2% [1]. Diabetes mellitus refers to a variety of metabolic illnesses that are generally defined as persistently elevated blood sugar levels [2]. This syndrome is caused by a decrease in the production of

* Corresponding author: nugroho_ae@ugm.ac.id

insulin, a decrease in the effectiveness of insulin, or a combination of both mechanisms [3]. Elevated blood glucose levels in diabetes lead to increased production of harmful free radicals, contributing to tissue damage and complications, such as nephropathy, neuropathy, retinopathy, and cognitive impairment [4].

Oxidative stress is recognized as a major contributor to the development and progression of diabetes [5]. It occurs because of an imbalance between the excessive production of reactive oxygen species (ROS) and deficiencies in the antioxidant defense system. This imbalance is strongly associated with the onset of various diseases, including diabetes and its complications [6, 7]. Bioactive compounds, such as alkaloids, phenolic acids, flavonoids, steroids, saponins, and glycosides, have shown potential in diabetes therapy because of their dual function as DPP-IV inhibitors and antioxidants. DPP-IV inhibitors alleviate hyperglycemic conditions by extending the half-life of incretin hormones including glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP). These incretins play critical roles in enhancing insulin secretion and maintaining β-cell mass. Concurrently, antioxidants combat oxidative stress, a key factor in diabetes pathogenesis, by scavenging free radicals, thereby protecting pancreatic β-cells from oxidative damage [8, 9].

The exploration of marine plants provides an alternative approach to drug discovery. Accordingly, research on drug development and discovery uses materials from extraction processes, fractions, sub-fractions, and the isolation of active compounds from plants, either separately or in combination [10, 11]. Marine macroalgae, which are abundant producers of biologically active compounds in marine environments, exhibit significant potential for medical applications [12]. *Sargassum*, a genus of brown algae belonging to the family Sargassaceae, encompasses approximately 400 species worldwide. Throughout generations, *Sargassum* species, including *S.fusiforme*, have been integral to traditional diets and folk health practices in Korea and China [13]. Known for its pharmacological properties, such as hypoglycemic, anticancer, immunomodulatory, anti-obesity, and anti-inflammatory activities [14], *S. cristaefolium* was selected for this study to analyze its bioactive components, specifically phenolics and flavonoids, using gas chromatography-mass spectrophotometry (GC-MS). These bioactive compounds are of particular interest because of their potential roles in diabetes therapy, including their ability to inhibit the DPP-IV enzyme, which prolongs incretin activity and enhances insulin secretion, and their antioxidant properties, which mitigate oxidative stress and protect the pancreas. Additionally, this study aimed to evaluate the antioxidant and DPP-IV inhibitory activities of the ethanol extract and ethyl acetate fraction of *S.cristaefolium.*

2 Research Method

2.1 Collection of brown marine algae

Brown marine algae (*S. cristaefolium*) were collected from the coastal area of Gunung Kidul Beach in Yogyakarta, Indonesia (8°08'11.2″ S, 110°33'52.0″ E). Marine algae were collected for sample identification at the Department of Pharmaceutical Biology, Faculty of Pharmacy, Universitas Gadjah Mada, Indonesia.

2.2 Preparation of marine algae extracts

S. cristaefolium was washed with running water to remove dirt, coral, and sand. It was then cut into small pieces, dried in the sun, and covered with a black cloth for three days. Dried *S.cristaefolium* was ground to obtain a dry powder. *S. cristaefolium* extract was obtained by maceration by soaking 100 g of dry powder in 400mL of ethanol solvent for one week at

37°C. It was then sieved through filter paper and concentrated in a rotary vacuum evaporator to yield a concentrated ethanol extract [15]. The fractionation was performed using the liquid-liquid method with an ethyl acetate (semi-polar) solvent. Fractionation was performed using the separating funnel method (liquid-liquid extraction) by suspending the extract in 100 mL of distilled water, followed by the addition of 100 mL of ethyl acetate, shaking, and leaving it until homogeneous and separated into two phases. The ethyl acetate phase was separated from the insoluble ethyl acetate phase and collected in different containers. The mixture was then evaporated using a rotary vacuum evaporator. The concentrated ethyl acetate fraction was stored in an airtight bottle at ± 4°C [16].

2.3 Determination of total phenolics

Total phenolic content was determined using a UV-Vis spectrophotometer (Model UV-1800, Shimadzu). Twenty milligrams of the sample was weighed carefully, and 1 mL of distilled water was added. Folin-Ciocalteu reagent (0.5 mL) was added to the water phase, followed by the addition of 5 mL of aquabidest. The solution was allowed to stand for 10 min at ambient temperature, after which sodium carbonate solution (1.5 mL) at a concentration of 20% was added. Aquabidest was added to a volume of 10 ml and the concentration was adjusted as required. It was then transferred to a cuvette for spectrophotometric analysis, with absorbance maintained at a wavelength of 760 nm [17].

2.4 Determination of total flavonoids

Total flavonoid content was determined using a UV-Vis spectrophotometer (Model UV-1800, Shimadzu). Twenty milligrams of each sample were weighed and placed in a test tube. Then, 0.3 mL of 5% sodium nitrite was added and the mixture was allowed to react for 5 min. Then, 0.6 mL of 10% aluminium chloride was added and left for 5 min. Next, 2 mL of 1 M NaOH was added to the mixture. The total volume was adjusted to 10 mL using distilled water in a measuring flask. The solution was then diluted, as required. The prepared solution was transferred to a cuvette and absorbance was measured at a wavelength of 510 nm [17].

2.5 DPPH radical scavenging activity

The DPPH radical scavenging activity was determined using a UV-Vis spectrophotometer (Model UV-1800, Shimadzu). A total of 50 µL of test sample at varying concentrations, corresponding to IC_{50} values (the concentrations of extract or fraction yielding 50% antioxidant activity relative to the control as determined by linear regression), was combined with 1.0 mL of 0.4 mM DPPH and 3.950 mL of ethanol. The mixture was then completely blended and left undisturbed for 30 min. After incubation, the absorbance of the solution was measured at 517 nm. A blank solution, consisting of 50 µL of the extract and 4.950 mL of ethanol was used as a reference. Absorbance measurements were conducted on a control solution consisting of 1.0 mL and ethanol (4.0 mL) [17].

2.6 GC-MS chemical profiling

Chemical profiling determination via GC-MS was conducted using a Thermo Scientific Trace 1310 Gas Chromatograph, coupled with a Thermo Scientific ISQ LT Single Quadrupole Mass Spectrometer. The system was equipped with a TriPlus RSH auto-injector for precise sample handling and was controlled using the Chromeleon software for data acquisition and analysis. A sample solution of 1µL was injected into a GC-MS system

equipped with a capillary column measuring 30 mm in length, 0.25 mm in diameter, and 0.25 μm in thickness. Helium was used as the carrier gas at a rate of 1 mL/min, with a split ratio of 1:50. The initial oven temperature was set to 150°C and held isothermally for five minutes. Subsequently, the temperature was increased at a rate of 10°C/min until it reached 250°C, which was maintained for an additional five minutes [18].

2.7 DPP-IV assay

An Elabscience® DPP-IV Inhibitor Screening Assay Kit (Catalog No: E-BC-D007) was used for measurements using a fluorescence microplate reader. For blank wells, 20 μL of buffer solution was added. The control wells received 20 μL of enzyme working solution, and the sample wells received 20 μL of enzyme working solution. Next, 30 μL of sample solvent was added to both the blank and control wells, and 30 μL of the sample was added to the sample wells. The contents were thoroughly mixed using a microplate reader for three seconds and then incubated at 37 °C for 10 min. Following this, 170 μL of the reaction working solution was added to each well, and the plate was further incubated at 37 °C for 30 min. Subsequently, the fluorescence intensity of each well was measured at excitation and emission wavelengths of 360 and 460 nm, respectively [19].
The inhibition rate (%) is calculated using the formula:

$$\text{Inhibition Rate (\%)} = \frac{(\text{Fcontrol} - \text{Fsample})}{(\text{Fcontrol} - \text{Fblank})} x\ 100\%$$

Fblank: The fluorescence intensity of blank well.
Fsample: The fluorescence intensity of sample well.
Fcontrol: The fluorescence intensity of control well.

3 Results and Discussion

3.1 Determination of yields, total phenolic content (TPC), total flavonoid content (TFC)

The extraction results of 250 g of dried *S. cristaefolium* simplicial with ethanol solvent using the maceration method are shown in Table 1. The extraction yield of *S. cristaefolium* varied significantly depending on the solvent used. Ethanol extraction resulted in a sticky extract weight of 8.19 ± 0.92 grams, corresponding to a yield of 3.27 ± 0.37%. In contrast, the ethyl acetate fraction produced a sticky extract weight of only 1.89 ± 0.38 grams, with a much lower yield of 0.75 ± 0.15%.

Based on the literature, *Sargassum*.sp contains various chemical compounds, including terpenoids, saponins, phlorotannin, cardiac glycosides, phenols, and flavonoids [24]. This difference in yield can be attributed to the polarity of the solvent used. Ethanol, a polar solvent, is capable of extracting a broad range of compounds, including polar and semi-polar constituents, such as polyphenols, flavonoids, and other hydrophilic bioactive compounds commonly found in Sargassum species. These compounds were highly soluble in ethanol, resulting in higher extraction yields. In contrast, ethyl acetate is a semi-polar solvent with limited solubility for highly polar compounds. It is more selective for extracting less polar or semi-polar constituents, such as terpenoids or certain lipophilic compounds. The lower yield observed with the ethyl acetate fraction is consistent with the selective nature of this solvent, which targets a narrower range of bioactive compounds [21].

Table 1. Yield of Ethanol Extract and Ethyl Acetate Fraction from *S.cristaefolium* Sample Extract

Sample Extract	Dry weight of simplicial (g)	Weight of sticky extract ± SEM (g)	Yield ± SEM (%)
Ethanol	250	8.19 ± 0.92	3.27 ± 0.37
Ethyl Acetate Fraction	250	1.89 ± 0.38	0.75 ± 0.15

The results showed that the total phenolic content (TPC) and total flavonoid content (TFC) varied between the ethanol extract and ethyl acetate fraction of *S. cristaefolium* (shown in Table 2). The ethanol extract exhibited lower TPC (1.04 ± 0.02% GAE) and TFC (1111.70 ± 48.91 µg QE/g) than the ethyl acetate fraction, which had higher TPC (7.33 ± 0.04% GAE) and TFC (3529.01 ± 54.32 µg QE/g).

Table 2. Total Phenolic Contents (Gallic Acid Equivalent) and Total Flavonoid Contents (Quercetin Equivalent) of Ethanol Extract and Ethyl Acetate Fraction

Sample Extract	Average TPCs ± SEM (% GAE)	Average TFCs ± SEM (µgQE/g)
Ethanol	1.04 ± 0.02	1111.70 ± 48.91
Ethyl Acetate Fraction	7.33 ± 0.04	3529.01 ± 54.32

The results reflect differences in the content of chemical compounds between the ethanol extract and ethyl acetate fraction. This disparity can be attributed to the polarity of the solvents used and their efficacy in the extraction of phenolic and flavonoid compounds. Ethanol is a polar solvent that is proficient in extracting a broad spectrum of bioactive compounds, including both polar and semi-polar constituents. However, ethyl acetate, a semi-polar solvent, is more selective and efficient for extracting phenolic and flavonoid compounds, particularly those with moderate polarity [22, 23]. Studies have indicated that ethyl acetate is effective for extracting phenolic compounds because of its intermediate polarity, which aligns well with the polarity of many phenolic compounds.

Previous research on *Sargassum polycystum* explains that extraction with ethyl acetate solvent results in higher total phenolic and total flavonoid contents than extraction using ethanol solvent [28]. A comparative study between the ethanol extract and ethyl acetate fraction of green tea leaves also indicated that the ethyl acetate fraction contained the most significant amount of flavonoid compounds [25]. Polyphenols and flavonoids were found in all extracts and fractions of *S.cristaefolium*, indicating that this plant is rich in antioxidant compounds. These phenolic and flavonoid compounds play an essential role in protecting cells from damage caused by free radicals, because they have an ideal chemical structure that allows them to effectively counteract the harmful reactivity of free radicals [26].

3.2 DPPH radical scavenging activity

Antioxidants are believed to act by donating hydrogen, which is why they are effective at scavenging DPPH compounds. An indicator of antioxidant activity is the concentration of antioxidants required to reduce the initial DPPH concentration by 50% (IC_{50}). A lower IC_{50} value corresponds to higher antioxidant power. The ethyl acetate fraction exhibited a lower IC_{50} value of 0.469 ± 0.0003 mg/mL, indicating stronger antioxidant activity than the ethanol extract, which had an IC_{50} value of 1.389 ± 0.0021 mg/mL (Tables 3 and 4).

This enhanced antioxidant activity in the ethyl acetate fraction was correlated with its higher TPC and TFC. Phenolic and flavonoid compounds are known for their antioxidant properties, primarily because of their ability to donate hydrogen atoms or electrons to neutralize free radicals. The higher concentrations of these compounds in the ethyl acetate fraction likely contributed to their superior radical-scavenging activity [27]. Additionally, the

specific phenolic profile present in the ethyl acetate fraction may play a role in its potent antioxidant activity. Ethyl acetate tends to extract phenolic compounds with moderate polarity, which are associated with strong antioxidant activity [28].

Table 3. Antioxidant (DPPH) of the Ethanol Extract of *S.cristaefolium*

Concentration (mg/mL)	Average Free Radical Scavenging ± SEM (%)	IC_{50} ± SEM (mg/mL)
0.288	12.283 ± 0.038	1.390 ± 0.0021
0.576	25.813 ± 0.038	
1.153	44.407 ± 0.100	
1.729	62.396 ± 0.136	
2.306	76.757 ± 0.236	

Table 4. Antioxidant (DPPH) of the Ethyl Acetate Fraction of *S.cristaefolium*

Concentration (mg/mL)	Average Free Radical Scavenging ± SEM (%)	IC_{50} ± SEM (mg/mL)
0.113	27.322 ± 0.000	0.469 ± 0.0003
0.226	35.993 ± 0.037	
0.452	49.654 ± 0.037	
0.678	65.027 ± 0.000	
0.904	73.734 ± 0.036	

In the ethyl acetate fraction of *S.cristaefolium*, more significant activity in the sequestration of free radicals was observed. This activity increased with increasing concentrations. Flavonoid compounds contribute to the antioxidant activity. The higher the number of flavonoid compounds, the higher is the antioxidant ability of the ethanol and ethyl acetate fractions. The ability of the ethyl acetate fraction to contain more flavonoids may indicate that this fraction has significant potential to function as an effective antioxidant agent in protecting cells from damage caused by free radicals.

3.3 GC/MS chemical profiling

GC-MS analysis allows for the identification of major non-volatile compounds, particularly with high accuracy when compounds are derivatized (Figures 1, 2, Table 5 and 6) [29]. In the ethanol extract and ethyl acetate fraction, prominent peaks were observed, indicating the presence of major compounds, such as hexadecanoic acid methyl ester (RT 17.46) and trans-13-octadecenoic acid methyl ester (RT 19.14). The chemical structures of the main bioactive compounds are shown in Figure 3. These compounds exhibited higher peak intensities in the ethyl acetate fraction, highlighting their predominance in this fraction.

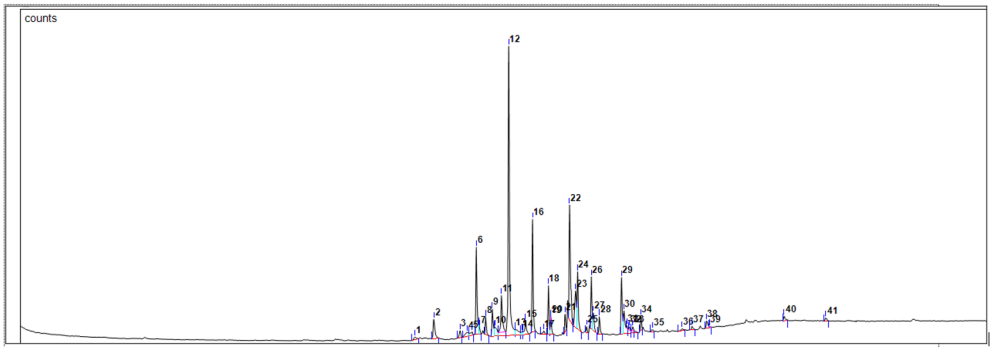


Fig. 1. GC-MS Chromatogram of the Ethanol Extract of *S.cristaefolium*

Table 5. Compounds Identified in the Ethanol Extract of *S.cristaefolium* by GC-MS

No.	Ret.Time (min)	Compound Name	Mol. Weight	Area %
1	14,88	[1,1'-Bicyclopropyl]-2-octanoic acid, 2'-hexyl-, methyl ester	322	0,43
2	15,40	Methyl tetradecanoate	242	2,20
3	16,13	Tetradecanoic acid, ethyl ester	256	0,65
4	16,33	6-Hydroxy-4,4,7a-trimethyl-5,6,7,7a-tetrahydrobenzofuran-2(4H)-one	196	0,92
5	16,45	Cyclopropanebutanoic acid, 2-[[2-[[2-[(2-pentylcyclopropyl)methyl]cyclopropyl]methyl]cyclopropyl]methyl]-, methyl ester	374	0,56
6	16,57	Neophytadiene	278	6,47
7	16,66	[1,1'-Bicyclopropyl]-2-octanoic acid, 2'-hexyl-, methyl ester	322	0,52
8	16,83	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	296	1,24
9	17,02	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	296	2,25
10	17,09	2-Methyl-E,E-3,13-octadecadien-1-ol	280	1,45
11	17,27	(Z)-Methyl hexadec-11-enoate	268	3,55
12	17,46	Hexadecanoic acid, methyl ester	270	26,19
13	17,65	Cyclopropanebutanoic acid, 2-[[2-[[2-[(2-pentylcyclopropyl)methyl]cyclopropyl]methyl]cyclopropyl]methyl]-, methyl ester	374	1,24
14	17,83	Cyclopropanebutanoic acid, 2-[[2-[[2-[(2-pentylcyclopropyl)methyl]cyclopropyl]methyl]cyclopropyl]methyl]-, methyl ester	374	0,35
15	17,93	Ethyl 9-hexadecenoate	282	1,57
16	18,13	Hexadecanoic acid, ethyl ester	284	7,44
17	18,43	Cyclopropanedodecanoic acid, 2-octyl-, methyl ester	366	0,44
18	18,56	Arachidonic acid	304	2,97
19	18,63	cis-5,8,11,14,17-Eicosapentaenoic acid	302	0,01
20	18,63	cis-5,8,11,14,17-Eicosapentaenoic acid	302	1,37
21	19,02	Methyl stearidonate	290	1,20
22	19,14	trans-13-Octadecenoic acid, methyl ester	296	10,08
23	19,31	Phytol	296	3,87
24	19,37	Heptadecanoic acid, 16-methyl-, methyl ester	298	4,20
25	19,62	9,12,15-Octadecatrienoic acid, 2,3-dihydroxypropyl ester, (Z,Z,Z)-	352	0,37
26	19,74	(E)-9-Octadecenoic acid ethyl ester	310	3,38
27	19,80	(E)-9-Octadecenoic acid ethyl ester	310	1,48
28	19,97	Heptadecanoic acid, 15-methyl-, ethyl ester	312	1,19
29	20,58	5,8,11,14-Eicosatetraenoic acid, methyl ester, (all-Z)-	318	4,20
30	20,65	cis-5,8,11,14,17-Eicosapentaenoic acid	302	4,00
31	20,73	6,9,12,15-Docosatetraenoic acid, methyl ester	346	0,15
32	20,80	6,9,12,15-Docosatetraenoic acid, methyl ester	346	0,21
33	20,89	cis-13-Eicosenoic acid	310	0,26
34	21,11	5,8,11,14-Eicosatetraenoic acid, ethyl ester, (all-Z)-	332	0,95
35	21,43	Ethyl iso-allocholate	436	0,31
36	22,25	Spirost-8-en-11-one, 3-hydroxy-, (3ß,5a,14ß,20ß,22ß,25R)-	428	0,32
37	22,52	Ethyl iso-allocholate	436	0,36
38	22,92	Hexa-t-butylselenatrisiletane	506	0,78
39	23,01	Ethyl iso-allocholate	436	0,04
40	25,06	Ethyl iso-allocholate	436	0,49
41	26,20	Octasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15-hexadecamethyl-	578	0,37

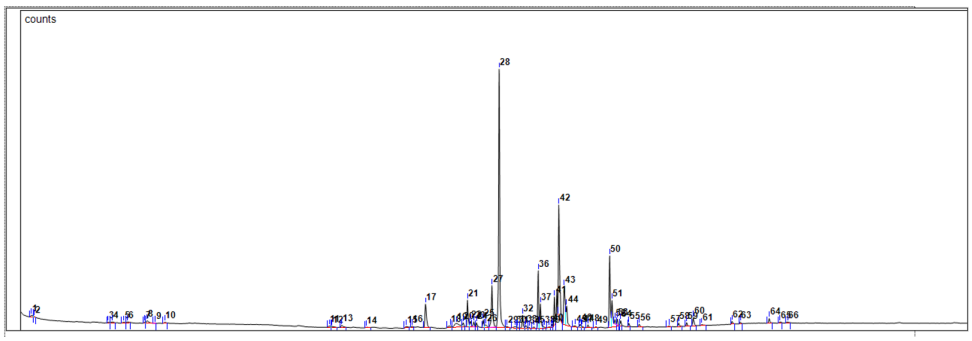
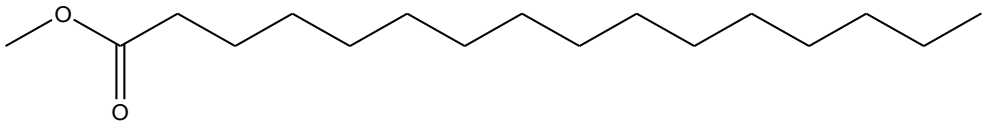


Fig. 2. GC-MS chromatogram of the Ethyl Acetate Fraction of *S.cristaefolium*

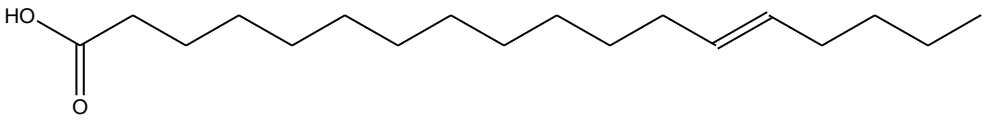
Table 6. Compounds Identified in the Ethyl Acetate Fraction of *S.cristaefolium* by GC-MS.

No	Ret.Time (min)	Compound Name	Mol. Weight	Area %
1	4,31	Isoxazolidine-3,5-dicarboxylic acid, dimethyl ester	189	0,40
2	4,39	Isoxazolidine-3,5-dicarboxylic acid, dimethyl ester	189	0,14
3	6,46	9-Octadecenoic acid (Z)-, hexyl ester	366	0,13
4	6,58	9-Octadecenoic acid, (2-phenyl-1,3-dioxolan-4-yl)methyl ester, cis-	444	0,25
5	6,91	9-Octadecenoic acid (Z)-, 2-(acetyloxy)-1-[(acetyloxy)methyl]ethyl ester	440	0,14
6	7,01	9-Octadecenoic acid (Z)-, 2-(acetyloxy)-1-[(acetyloxy)methyl]ethyl ester	440	0,17
7	7,50	9-Octadecenoic acid (Z)-, 2-(acetyloxy)-1-[(acetyloxy)methyl]ethyl ester	440	0,15
8	7,56	trans-Verbenyl caprate	306	0,62
9	7,79	9-Octadecenoic acid (Z)-, hexyl ester	366	0,03
10	8,06	9-Octadecenoic acid, (2-phenyl-1,3-dioxolan-4-yl)methyl ester, cis-	444	0,19
11	12,68	9-Octadecenoic acid (Z)-, 2-(acetyloxy)-1-[(acetyloxy)methyl]ethyl ester	440	0,29
12	12,76	Hexadecane, 1,1-bis(dodecyloxy)-	594	0,27
13	13,04	2,4-Di-tert-butylphenol	206	0,43
14	13,73	Estra-1,3,5(10)-trien-17ß-ol	256	0,16
15	14,86	Methyl 3-hydroxydodecanoate	230	0,26
16	15,02	1-Hexadecanol, 2-methyl-	256	0,14
17	15,39	Methyl tetradecanoate	242	3,37
18	16,10	1-Hexadecanol, 2-methyl-	256	0,51
19	16,26	6-Hydroxy-4,4,7a-trimethyl-5,6,7,7a-tetrahydrobenzofuran-2(4H)-one	196	1,76
20	16,45	Tetradecanoic acid, 12-methyl-, methyl ester	256	0,40
21	16,57	Neophytadiene	278	2,63
22	16,66	2-Pentadecanone, 6,10,14-trimethyl-	268	0,85
23	16,75	1,3-di-iso-propylnaphthalene	212	0,44
24	16,83	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	296	0,33
25	17,02	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	296	0,77
26	17,09	cis-13-Eicosenoic acid	310	0,67
27	17,26	9-Hexadecenoic acid, methyl ester, (Z)-	268	4,96
28	17,46	Hexadecanoic acid, methyl ester	270	27,98

No	Ret.Time (min)	Compound Name	Mol. Weight	Area %
29	17,69	Cyclopropanebutanoic acid, 2-[[2-[[2-[(2-pentylcyclopropyl)methyl]cyclopropyl]methyl]cyclopropyl]methyl]-, methyl ester	374	0,33
30	17,92	Cyclopropanebutanoic acid, 2-[[2-[[2-[(2-pentylcyclopropyl)methyl]cyclopropyl]methyl]cyclopropyl]methyl]-, methyl ester	374	0,37
31	17,98	12,15-Octadecadiynoic acid, methyl ester	290	0,16
32	18,12	Hexadecanoic acid, ethyl ester	284	1,32
33	18,22	cis-10-Heptadecenoic acid, methyl ester	282	0,37
34	18,31	Cyclopropanedodecanoic acid, 2-octyl-, methyl ester	366	0,13
35	18,44	Hexadecanoic acid, 14-methyl-, methyl ester	284	0,25
36	18,56	5,8,11,14-Eicosatetraenoic acid, methyl ester, (all-Z)-	318	4,93
37	18,63	Doconexent	328	2,40
38	18,73	cis-5,8,11,14,17-Eicosapentaenoic acid	302	0,16
39	18,88	Cyclopropanebutanoic acid, 2-[[2-[[2-[(2-pentylcyclopropyl)methyl]cyclopropyl]methyl]cyclopropyl]methyl]-, methyl ester	374	0,16
40	18,95	6,9,12-Octadecatrienoic acid, methyl ester	292	0,25
41	19,02	Methyl stearidonate	290	2,36
42	19,14	trans-13-Octadecenoic acid, methyl ester	296	12,90
43	19,30	Phytol	296	5,66
44	19,36	Heptadecanoic acid, 16-methyl-, methyl ester	298	2,21
45	19,61	6,9,12,15-Docosatetraenoic acid, methyl ester	346	0,32
46	19,74	trans-9-Octadecenoic acid, pentyl ester	352	0,31
47	19,80	9-Octadecenoic acid (Z)-, 2-hydroxy-1-(hydroxymethyl)ethyl ester	356	0,17
48	19,96	Ethyl 14-methyl-hexadecanoate	298	0,36
49	20,19	Ethyl iso-allocholate	436	0,15
50	20,57	5,8,11,14-Eicosatetraenoic acid, methyl ester, (all-Z)-	318	6,79
51	20,64	5,8,11,14,17-Eicosapentaenoic acid, methyl ester, (all-Z)-	316	3,84
52	20,72	6,9,12,15-Docosatetraenoic acid, methyl ester	346	0,42
53	20,79	Methyl 8,11,14,17-eicosatetraenoate	318	0,52
54	20,88	Methyl 9-eicosenoate	324	0,53
55	21,11	Cyclopropanebutanoic acid, 2-[[2-[[2-[(2-pentylcyclopropyl)methyl]cyclopropyl]methyl]cyclopropyl]methyl]-, methyl ester	374	0,32
56	21,40	Ethyl iso-allocholate	436	0,34
57	22,25	Spirost-8-en-11-one, 3-hydroxy-, (3ß,5a,14ß,20ß,22ß,25R)-	428	0,17
58	22,52	Methyl 11-docosenoate	352	0,38
59	22,74	Docosanoic acid, methyl ester	354	0,37
60	22,92	Diisooctyl phthalate	390	0,94
61	23,17	Methyl glycocholate, 3TMS derivative	695	0,21
62	24,02	Spirost-8-en-11-one, 3-hydroxy-, (3ß,5a,14ß,20ß,22ß,25R)-	428	0,31
63	24,25	Methyl glycocholate, 3TMS derivative	695	0,20
64	25,06	Ethyl iso-allocholate	436	0,54
65	25,36	Octasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15-hexadecamethyl-	578	0,17
66	25,58	Methyl glycocholate, 3TMS derivative	695	0,21



hexadecanoic acid, methyl ester [30]



trans-13-octadecenoic acid, methyl ester [31]

Fig. 3. Major bioactive compounds

Hexadecanoic acid, known as palmitic acid, possesses antioxidant, anti-inflammatory, hypocholesterolemic, and 5-α reductase inhibitory properties, preserves beta-cell function, and inhibits α-amylase and intestinal α-glucosidase activity in vitro [32, 33, 34]. Trans-13-octadecenoic acid has anti-inflammatory effects [35]. Given the significant role of reactive oxygen species (ROS) in the pathogenesis of type-2 diabetes mellitus (T2DM), understanding the interplay among diabetes mellitus, aging, inflammation, ROS, and oxidative stress is important [36]. Many flavonoids and phenolic compounds, renowned for their anti-inflammatory and antioxidant actions, mitigate ROS effects, thereby potentially alleviating diabetes-related complications over the long term [32].

3.4 DPP-IV assay

In the in-vitro DPP-IV inhibition assay, sitagliptin was employed as the positive control alongside two samples consisting of the ethanol extract and ethyl acetate fraction of *S.cristaefolium*, detailed results are presented in Table 7. Sitagliptin functions as an inhibitor of dipeptidyl peptidase-IV (DPP-IV, also known as CD26), which is a clinically approved treatment for diabetes mellitus. Sitagliptin exhibited antioxidant properties and specifically inhibited DPP-IV [37]. Inhibiting DPP-IV enzyme is an effective strategy to maintain incretin hormones in the bloodstream, thereby enhancing insulin-dependent glucose disposal from the blood [38].

Table 7. DPP-IV inhibitory activity

Sample	Concentration (ppm)	Inhibition (%)
Sitagliptin	40	85.55 ± 1.64
Ethanol Extract	500	92.07 ±1.47
Ethyl Acetate Fraction	62.5	95,87 ± 2,54

These findings indicated that the ethyl acetate fraction exhibited superior DPP-IV inhibitory activity compared to the ethanol extract (Table 7). This aligns with previous observations, highlighting the high antioxidant capacity of the ethyl acetate fraction. Additionally, GC-MS analysis revealed increased concentrations of hexadecanoic acid methyl ester and trans-13-octadecenoic acid methyl ester in the ethyl acetate fraction. These compounds may contribute to the observed DPP-IV inhibitory activity, possibly enhanced by the antioxidant, anti-inflammatory, and antidiabetic properties of the sample.

4 Conclusions

This study shows that the *Sargassum cristaefolium* extract contains beneficial compounds, including antioxidants, total phenols, and flavonoids, which may help manage diabetes. The

research highlights the potential of marine algae as natural inhibitors of the DPP-IV enzyme, which plays a role in blood sugar regulation. The ethyl acetate fraction demonstrated stronger antioxidant activity than the ethanolic extract. Additionally, chemical analysis (GC-MS) identified key compounds, such as hexadecanoic acid, methyl ester, and trans-13-octadecenoic acid, methyl ester, in the ethyl acetate fraction. These compounds may contribute to its ability to inhibit DPP-IV activity, which, combined with its antioxidant effects, suggests potential antidiabetic benefits.

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References

[1] H. Sun, P. Saeedi, S. Karuranga, M. Pinkepank, K. Ogurtsova, B.B. Duncan, et al. Diabetes Res Clin Pract. **183**, 109119 (2022).

[2] W. Setyani, R. Murwanti, T.N.S. Sulaiman, T. Hertiani. J Adv Pharm Technol Res.**14**, 4 (2023).

[3] A. Petersmann, D. Müller-Wieland, U.A. Müller, R. Landgraf, M. Nauck, G. Freckmann, et al.**127**,S 01,S1–7, (2019).

[4] A. M. Abd-Elkader, S. Labib, T.F. Taha, F. Althobaiti, A. Aldhahrani, H. M. Salem, et al. Saudi J Biol Sci.**29**,3,1428–33 (2022).

[5] S. Bhattacharya, P.C. Sil. React Oxyg species. **5**,13,15–34 (2018).

[6] H. Ramos, P. Bogdanov, J. Huerta, A. Deàs-Just, C. Hernández, R. Simó. Antioxidants. **11** (2022).

[7] J.O. Unuofin, S.L. Lebelo. Oxid Med Cell Longev.**1**,1356893 (2020).

[8] M.A. Nauck, J.J. Meier. Diabetes, Obes Metab.**20**:5–21 (2018).

[9] A.K. Singh, D. Yadav, N. Sharma, & J.O. Jin. Pharmaceuticals*,* **14**(6)*,* 586 (2021).

[10] A.E. Nugroho, G. Kusumaramdani, A. Widyaninggar, D.P. Anggoro, S. Pramono. Int Food Res J. **21**,2,785 (2014).

[11] S. Pramono, A.E. Nugroho. Int Food Res J. **22**,4 (2015).

[12] R.R. Remya, A.V. Samrot, S.S. Kumar, V. Mohanavel, A. Karthick, V.K. Chinnaiyan, et al. Adsorpt Sci Technol. 9104835(2022).

[13] S. Lim, A-H. Choi, M. Kwon, E-J. Joung, T. Shin, S-G. Lee, et al. Food Chem.**278**:178–84 (2019).

[14] J. Liu, S. Luthuli, Y. Yang, Y. Cheng, Y. Zhang, M. Wu, et al. T. Food Science and Nutrition. **8 (**2020).

[15] K. Hira, H. Farhat, N. Sohail, M. Ansari, J. Ara, S. Ehteshamul-Haque. Clin Phytoscience.**7**,1,40 (2021).

[16] W. Ko, H. Lee, N. Kim, H.G. Jo, E-R. Woo, K. Lee, et al. Antioxidants. **10** (2021).

[17] I. Isnindar, S. Luliana. Maj Obat Tradis. **25,**3,140–5(2020).

[18] E. Lukitaningsih, R. Rumiyati. Borneo J Pharm.**4**,1,29–35(2021).

[19] H.S. Parmar, P. Jain, D.S. Chauhan, M.K. Bhinchar, V. Munjal, M. Yusuf, K. Choube, A. Tawani, V. Tiwari, E. Manivannan, A. Kumar. Diabetes Research and Clinical Practice. 1;**97**(1):105-11 (2012).

[20] M.K.A. Sobuj, Islam MA, Haque MA, Islam MM, Alam MJ, Rafiquzzaman SM. J

Food Meas Charact.**15**,1653–65(2021).

[21] E. Edison, A. Dirahmi, N.M. Ariani, M. Ilza. Jurnal Pengolahan Hasil Perikanan Indonesia. **30**;23(1):58-66(2020).

[22] A. Altemimi, N. Lakhssassi, A. Baharlouei, D.G. Watson, & D.A. Lightfoot. Plants, **6**(4), 42 (2017).

[23] T. Lefebvre, E. Destandau, E. Lesellier. Journal of Chromatography A. Jan **4**;1635:461770 (2021).

[24] A. Arsianti, A. Bahtiar, V.K. Wangsaputra, N.N. Azizah, W. Fachri, L.D. Nadapdap, et al. Pharmacogn J.**12**,1 (2020).

[25] K. Indarti, E.F. Apriani, A.E. Wibowo, P. Simanjuntak. Pharmacogn J.**11**,4 (2019).

[26] D. Bešlo, N. Golubić, V. Rastija, D. Agić, M. Karnaš, D. Šubarić, et al. Antioxidants.**12**,6:1141 (2023).

[27] M.D. Catarino, R. Silva-Reis, A. Chouh, S. Silva, S. S. Braga, A. M. S. Silva, & S. M. Cardoso. Marine drugs, **21**(3), 172 (2023).

[28] S. Mariem, F. Hanen, J. Inès, S. Mejdi, K. Riadh. South African Journal of Botany. 1;**94**:114-21 (2014).

[29] A. A. Shalini, M.S. Ali, V. Anuradha, N. Yogananth, P. Bhuvana. Biocatal Agric Biotechnol.**19**,101148(2019).

[30] National Institute of Standards and Technology (NIST). (n.d.). Chemistry WebBook. Retrieved January 26, 2025, from https://webbook.nist.gov/cgi/cbook.cgi?ID=C112390&Mask=8

[31] National Institute of Standards and Technology (NIST). (n.d.). Chemistry WebBook. Retrieved January 26, 2025, from https://webbook.nist.gov/cgi/inchi?ID=U333613&Mask=200

[32] O.R. Olasehinde, O.R. Afolabi, O.V. Owolabi, A.B. Akawa, O.B. Omiyale. Sci African.**16**,e01178 (2022).

[33] R. Agada, D. Thagriki, D. E. Lydia, A. Khusro, J. Alkahtani, M.M. Al-Shaqha, et al. J King Saud Univ - Sci.**33**,2,101342 (2021).

[34] D.C. Tan, N.K. Kassim, I.S. Ismail, M. Hamid, M.S.A. Bustamam. Biomed Res Int.**1**, 7603125(2019).

[35] M.S. Khan, M. Uzair, M. Hanif, M. Jabeen, B.A. Chaudhary. Pak J Pharm Sci.**35**,6 (2022).

[36] M. Halim, A. Halim. Diabetes Metab Syndr Clin Res Rev.**13**,2,1165–72(2019).

[37] X. Wang, J. Ke, Y. Zhu, B. Cao, R. Yin, Y. Wang, et al. Cell Death Discov. **7**,1:236 (2021).

[38] R. Mahmood, W.K. Kayani, T. Ahmed, F. Malik, S. Hussain, M. Ashfaq, et al. BMC Complement Med Ther.**20**,1–17 (2020).