

Comparative Analysis of Phenolic Compounds, Carotenoid, and Antioxidant Activity of Leaf and Stem of *Acanthus ebracteatus* Vahl (Sea Holly)

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ABSTRACT

Introduction: In Thailand, *Acanthus ebracteatus* Vahl (Sea Holly), known locally as ‘Nguak Pla Mo,’ has been used for anti-inflammatory, analgesic, and diuretic properties. This study aimed to explore Sea Holly’s antioxidant and phytochemical attributes, open avenues for pharmaceutical applications, and emphasize the importance of conserving Thailand’s ethnobotanical wisdom and biodiversity. The primary objectives of this study were to characterize the amount of carotenoid, total phenolic compounds, and antioxidant activity in the leaf and stem of Sea Holly, as well as identify the main chemical constituents in plant extracts.

Methods: The plant extracts were analyzed for their carotenoid and total phenolic content. Their antioxidant activity was evaluated using ABTS, FRAP, and DPPH assays, and the chemical constituents were characterized using GC-MS and IR spectroscopy.

Results: Sea Holly leaf extract contains higher amounts of both carotenoid (0.2261 mg/g sample) and total phenolic compounds (344.67 mg GAE/g DW) than its stem, which has 0.0103 mg/g sample for carotenoid and 92.75 mg GAE/g DW for total phenolic compounds. Consequently, the extraction from leaves exhibits higher antioxidant activity (%DPPH: 18.73; %ABTS: 81.4 and FRAP: 3.46 mmolFe²⁺/g sample) than stem extract (%DPPH: 14.48; %ABTS: 69.0 and FRAP: 2.40 mmolFe²⁺/g sample). The GC-MS and IR analysis reveals that the main phytochemicals contained in both stem and leaf extracts are hexadecenoic acid, triolein, campesterol, stigmasterol, and γ -sitosterol.

Conclusion: Sea Holly leaf contains higher total phenolic compounds, carotenoid content, and antioxidant activity than Sea Holly stem, a potential application for treatment in oxidative-stress-related diseases and a promising opportunity to develop alternative healthcare and medicinal products.

Keywords: Antioxidant activity; *Acanthus ebracteatus* Vahl; Carotenoid; Total Phenolics Compound; Sea Holly

Introduction

Acanthus ebracteatus Vahl (Sea Holly) belongs to the Acanthaceae family and has been used in Thai traditional medicine for healing skin diseases, treatment of asthma, diabetes, hepatitis, and various types of cancer, as well as health promotion and longevity. Some reports have shown that the extract of Sea Holly displays an immunostimulatory effect on macrophage cells in vitro. A study by Prasansuklab and Tencomnao [1] reported that the Sea Holly leaf extract can diminish the intracellular reactive oxygen species and HT22 cell death induced by glutamate. Olatunji et al. [2] reported that extracting Sea Holly leaf displayed more potent

antibacterial activity against *Escherichia coli* and *Listeria monocytogenes* than stem extraction. Moreover, it also exhibits anticancer and antiproliferative activity against oral squamous carcinoma cells. The extract of Sea holly contains many bioactive constituents such as alkaloids, flavonoids, phenolic compounds, lignans, steroids, and terpenoids [3-4]; therefore, it can be used as a natural food supplement since it consists of many antioxidant constituents. However, only a few researches reported the difference in chemical constituents and antioxidant activity between the stem and leaf of Sea Holly.

Evidence shows that many diseases are caused by oxidative stress, which results from an imbalance between the formation and neutralization of reactive free radicals. These reactive free radicals are generated either by endogenous sources, by-products of normal metabolic processes for ATP production, or exogenous sources: air pollution, smoking, UV radiation, etc. [5]. The free radicals cause oxidative damage of macromolecules in the human body, such as protein, lipid, and nucleic acid, resulting in various degenerative diseases such as diabetes, Alzheimer's, Parkinson's, cardiovascular diabetes, as well as programmed cell death, i.e., aging [6]. In recent years, the interest in finding some antioxidant phytochemicals in plants, also known as natural antioxidants, has increased since they can inhibit the propagation of the oxidative stress process [7]. Many investigations reported the amount of antioxidant species in plant extracts. However, only a few reports provided details of the phytochemical and antioxidant activity of the leaf and stem of sea holly. A report on the antioxidant activity of Sea Holly grown in southern Thailand (Surat Thani province) shows that the antioxidant activity of Sea Holly Leaf extracted by the sedimentation process is 1113.2 $\mu\text{mol TE/g}$ [2]. Another report from Rakkhitawatthana et al. [8] shows that the extract of Sea Holly harvesting from Rayong Province exhibits antioxidant activity at 277.986 $\pm$ 38.308 mg of Trolox equivalent/g sample. Moreover, the antioxidant of Sea Holly collected from the northern part of Thailand, such as Nan and Phetchabun Province, was reported at IC₅₀ of 59.05 $\pm$ 0.23 $\mu\text{g/mL}$ [9] and 25.57 $\pm$ 6.41 $\mu\text{g/mL}$ [10], respectively. The chemical constituents in plants vary depending on their habitat.

This work collected a Sea Holly plant sample from Surin Province, the northeast part of Thailand. The phytochemical constituents in the leaf and stem of sea holly, including carotenoid, total phenolics compounds, and their antioxidant efficiency, were examined. In addition, the chemical compositions of plant extract were also analyzed.

Methods

Chemicals and Reagents

Methanol, ethanol, and diethyl ether were purchased from Qchemical Co., Ltd (New Zealand). Sodium chloride, sodium sulphate anhydrous, Folin-Ciocalteu reagent, sodium carbonate, and potassium persulphate were purchased from Loba Chemie pvt. Ltd. (Mumbai, India). Standard gallic acid, 2,2'-azinobis (3-ethylbenzothiazoline-6-sulfonic acid (ABTS solution), Trolox, Iron (III) chloride hexahydrate, 2, 4, 6-Tripyridyl-s-triazin (TPTZ), Iron (II) Sulfate-Heptahydrate and 1,1-diphenyl-2-picrylhydrazyl radical (DPPH) were purchased from Sigma Aldrich (St. Louis, MO, USA). All chemicals and solvents used were analytical grades and used as purchased.

Plant Materials

The leaves and stems of sea holly were collected from a regional botanical garden in Prasart District, Surin Province, in January 2022. The botanical certification of the samples was conducted by experts from the Faculty of Agriculture and Technology at Rajamangala University of Technology Isan, Surin Campus. Plant materials were washed with tap water and dried at room temperature. The dried samples (50.00 g) were chopped into small pieces and extracted in 80% methanol for 24 h. The extractions were evaporated using a rotary evaporator, and the crude extracts were kept at 4°C for further use.

Determination of Carotenoid content

Carotenoid content in plant extracts was determined according to the process adapted from Sommer et al. [11]. A 0.2 g dried sample was extracted with 10 ml of 95% ethanol and 1 ml of 60% KOH and then sonicated for 5 minutes. The extraction was incubated at 50 °C for 5 min and cooled down to room temperature before centrifuge for 10 min. The supernatant was transferred to the Erlenmeyer flask and wrapped with Aluminum foil to prevent light exposure, and then 15 ml of diethyl ether and 15 ml of 9%NaCl were added. The solution in the flask was stirred and left until it split into two layers. The upper yellow layer containing carotenoid was transferred to another flask, and then sodium sulphate anhydrous was added to adsorb water. The absorption of the solutions was determined at 450 nm using a UV-Vis Spectrometer (Thermo Scientific, Genesys 10S), and the following equation calculated the amount of carotenoid.

$$\text{total carotenoid content} = \frac{D_{450} \cdot V}{m \cdot l \cdot A_{\beta\text{-car-tab}}}$$

where D_{450} is the absorbance of the sample at 450 nm

V is the volume of the extract.

m is the mass of the sample.

l is a path length

$A_{\beta\text{-car-tab}}$ is the table extraction coefficient of the predominant carotenoid in the sample = 2590

Determination of Total Phenolic content

The overall phenolic content was quantified with the Folin-Ciocalteu test [11]. The plant extract was made by adding 50 ml of 80% methanol with 0.5g of dried sample, followed by sonication for 20 min and subsequent centrifugation. The supernatant was stored in dark glass bottles at -20 °C until utilized. An amount of 2 ml of plant extract was carefully placed into a 25 ml volumetric flask containing 2 ml of Folin-Ciocalteu reagent. Subsequently, a solution consisting of 10 ml of 7%Na₂CO₃ was introduced into the flask, enhancing the total volume to 25 ml. The solution was incubated at

ambient temperature for 90 min, after which the absorbance at 750 nm was determined. A calibration curve was prepared using a standard gallic acid ($\geq 98.0\%$, CAS no. 149-91-7). The findings were quantified in terms of mg gallic acid equivalents/gram of dried sample. All the samples underwent triplicate analysis.

ABTS Radical Scavenging Assay

ABTS Radical Scavenging activity of plant samples was carried out similarly to the methodology by Arnao et al. [12]. The ABTS⁺ cation radical was synthesized by reacting the ABTS solution with potassium persulfate. The ABTS free radical was stored in a light-restricted environment at room temperature for 14 h. Subsequently, the solution underwent dilution with ethanol to achieve an absorbance reading of 0.700 at 734 nm. A 100 μ L of plant extract was introduced into a 100 μ L diluted ABTS solution. The solution was then stored in a dark environment for 6 min. After that, the absorbance at 734 nm was measured, with ethanol serving as a blank. %ABTS Inhibition was calculated using the following equation:

$$\%ABTS \text{ Inhibition} = \frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}} \times 100$$

where A_{control} is the absorbance of ABTS solution without the sample.

A_{sample} is the absorbance of the DPPH solution with the sample.

The antioxidant is also expressed in terms of Trolox equivalent using Trolox (97.0%, CAS no. 53188-07-1) as a standard. The calibration curve was plotted between the effective absorbance and Trolox concentration (1.1725-18.75 μ g/mL). All measuring procedures were triplicated.

Ferric Reducing Antioxidant Power (FRAP)

The Ferric Reducing Antioxidant Power (FRAP) assay was employed to evaluate the antioxidant activity by measuring the reduction of ferric ion (Fe^{3+}) to ferrous (Fe^{2+}) complex in an acidic setting. The FRAP assay was conducted by the methodology outlined by Benzie et al. [13]. The FRAP reagent was produced by mixing 10 ml of acetate buffer (300 mM, pH = 3.6), 10 ml of 0.02M $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$, and 1 ml of 10 mM TPTZ solution (dissolving 31.2 mg TPTZ in 10 ml of 40 mM HCl). A 96-well plate was used for the assay. Freshly prepared FRAP reagent (200 μ L) was mixed with 10 μ L of plant extract and 30 μ L of DI water. An intense blue color complex was formed when the ferric tripyridyl triazine (Fe^{3+} TPTZ) complex in FRAP reagent was reduced to ferrous (Fe^{2+}) form, and the absorbance at 600 nm was recorded against a reagent blank. All determinations were performed in triplicates, and the calibration curve was prepared by plotting the absorbance at 600 nm versus the different concentrations of $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ (99.0%, Cas no. 7782-63-0).

DPPH Free Radical-Scavenging Assay

The assessment of the antioxidant activity of plant extracts involved the examination of their scavenging effects on the DPPH (1,1-diphenyl-2-picrylhydrazyl) radical (97%, Cas no. 1707-75-1), using the method outlined by M. Allothman et al. [14]. Next, 10 ml of plant extract aliquot was combined with 90 ml of DI water and 4 ml of a 25 mM solution of DPPH in methanol. The mixture was mixed in a vortex thoroughly and incubated in the dark for 30 min. The absorbance of the solution was quantified at 515 nm against a blank of methanol without DPPH. The %DPPH Scavenging was calculated using the following equation:

$$\%DPPH \text{ Scavenging} = \frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}} \times 100$$

Where A_{control} is the absorbance of the DPPH solution without the sample.

A_{sample} is the absorbance of the DPPH solution with the sample.

Fourier transform infrared spectroscopy (FTIR) Analysis

Fourier transform infrared spectroscopy (FTIR) was employed to identify functional groups in plant extracts. FT-IR spectra were obtained through a Bruker Tensor 27 attenuated total reflectance Fourier transform infrared (ATR-FTIR) spectrometer in the 4000-600 cm^{-1} range.

Gas Chromatography - Mass Spectroscopy Analysis

The GC-MS analysis was conducted at the central laboratory of Ubon Ratchatani University. The analysis was performed with the Perkin-Elmer Clarus 680 system equipped with a fused silica column, which contained Elite-5MS (30.0 m in length x 320 μ m in diameter x 0.25 μ m in thickness) with He as a carrier gas at a flow rate of 1.5 ml/min. The spectral detection was collected from 50 to 500 Da with an injection volume of 1 μ L (split ratio 10:1) and an injection temperature at 250 $^{\circ}\text{C}$. The initial temperature of the column oven was set to 60 $^{\circ}\text{C}$ for 1 min and was then gradually increased to 180 $^{\circ}\text{C}$ at a rate of 5 $^{\circ}\text{C}/\text{min}$. The temperature was maintained for 1 min and subsequently raised at a rate of 10 $^{\circ}\text{C}/\text{min}$ until it reached 300 $^{\circ}\text{C}$, and finally held for 2 min. The chemical constituents contained in the analyzed samples were identified based on a comparison of retention time, peak area, peak height, and mass spectra patterns with the database of standard compounds in the National Institute of Standards and Technology (NIST) library.

Results and Discussion

Phytochemical constituents: Carotenoid and total phenolic content

Since Sea Holly has been reported as an herb used for traditional medicinal treatment and has also been shown to possess antioxidant activity, this research was carried out to investigate the presence of β -carotene and phenolic compounds, which are known to exhibit antioxidant activity. Carotenoids have been widely recognized as a safe chemical for food supplements due to their notable coloring capacity and their function as precursors for vitamin A. Table 1 presents carotenoid contents of the plant extract. The result shows that Sea Holly exhibits a higher carotenoid concentration in its leaf than in its stems. Carotenoids play an essential role in the photosynthetic process of organisms as they serve two primary functions. Firstly, they act as photoprotective agents, which prevents the occurrence of harmful photodynamic reactions. Secondly, they function as accessory light-harvesting pigments, expanding the spectral range of light used for photosynthesis [15]. Hence, the carotenoid concentration in leaves is significantly greater compared to stems. This is because photosynthetic activity takes place extensively in leaves. The results were consistent with various previous studies in a similar discipline. For

example, a study by Lisiewska et al. [16], who investigated the levels of β -carotene in dill, revealed that the concentration of β -carotene in the leaf portion (5620 $\mu\text{g}/100\text{ g}$) was significantly greater compared to the stem portion (250 $\mu\text{g}/100\text{ g}$). The total carotenoid concentration is influenced by various physiological, genetic, and biochemical factors inherent to the plant, including species, plant part, maturity level at harvest, and post-harvest management. Comparing carotenoid content with other plants, the carotenoid concentration in Sea Holly leaves, at 0.2261 mg/g, surpasses that of pomegranate leaves (*Punica granatum*), which have a carotenoid content of approximately 0.180 mg/g [17]. This indicates a significant presence of carotenoids in Sea Holly compared to pomegranate leaves. Despite this, Sea Holly's carotenoid levels fall short compared to pumpkin, which boasts a remarkable 0.404 mg/g of carotenoids [18], highlighting a vast difference in their phytochemical profiles. In another comparison, Sea Holly leaves' carotenoid content exceeds that found in kale leaves (*Brassica oleracea* var. *acephala*), which is recorded at 0.152 mg/g [19]. Yet, the carotenoid levels in Sea Holly are not as high as those in carrot leaves (*Daucus carota*), where the concentration ranges from 8 to 15 mg/g fresh weight, illustrating a significant gap in carotenoid content between these species [20].

Table 1 The amount of Carotenoid, TPC, and antioxidant activity in Sea Holly leaf and stem extracts. Values are expressed as mean $\pm$ SD for each parameter

Plant Samples	Phytochemical constituents			Antioxidant Activity		
	Carotenoid (mg/g)	TPC (mg GAE/g DW)	DPPH (%)	% ABTS Inhibition	Trolox equivalent/g sample (mgTE/g)	FRAP (mmol Fe ²⁺ /g sample)
Sea Holly (leaf)	0.2261 $\pm$ 0.0020	344.67 $\pm$ 2.06	18.73 $\pm$ 0.42	81.4 $\pm$ 0.9	245.02 $\pm$ 0.13	3.46 $\pm$ 0.04
Sea Holly (stem)	0.0103 $\pm$ 0.0009	92.75 $\pm$ 0.42	14.48 $\pm$ 0.44	69.0 $\pm$ 1.7	2440.84 $\pm$ 0.65	2.40 $\pm$ 0.05

The amount of total phenolic compounds in plant extract was also determined to identify the source of potential physiological activity of medicinal plants. Phenolic chemicals are significant ingredients possessing redox characteristics that play an important role in antioxidant actions. The presence of the hydroxyl group in phenolic compounds plays a crucial role in enabling the process of free radical scavenging. Table 1 presents the level of total phenolic in plant extracts examined using the Folin-Ciocalteu reagent and expressed in gallic acid equivalent. Results indicate that the leaf of Sea Holly contains much higher total phenolic content than the stem.

Normally, the extraction procedure and solvent are responsible for dissolving total phenolic in a plant. The phenolic compounds are polar structures due to the

presence of a hydroxyl group. Thus, polar solvents such as methanol are suitable for extraction. Prapasanobol et al. [21] determined the amount of total phenolic acid in *C. monantha* Jacobs extracted using different solvents, including hexane, dichloromethane, acetone, and methanol. They found that acetone is the most suitable solvent for extracting total phenolic compounds, followed by methanol, dichloromethane, and hexane. Rakkhitawattana et al. [22] reported that the total phenolic content in the ethanolic extract of Sea Holly leaf is 679.75 mgGEA/g. In contrast, the report by Olatunji et al. [2] revealed that the total phenolic compounds in ethanolic extract of Sea holly leaf and stem were found to be 30.49 $\pm$ 0.1 and 138.20 $\pm$ 0.10 mg GAE/g dry extract, respectively. The value of phenolic content in plants varied compared to those in the

literature. This might be caused by different amounts of sugars, carotenoids, and ascorbic acid, or the duration of harvesting, geographical variation, or extraction method, which affect the number of phenolics [23].

Regarding phenolic content, Sea Holly leaves exhibit a higher concentration (344.67 mg GAE/g) than both valerian (*Valeriana officinalis*) with 38 mg GAE/g [24] and bitter melon leaves (*Momordica charantia*), which contain 115 mg GAE/g of phenolics [25]. This demonstrates Sea Holly's superior potential as a source of antioxidants. However, it is noteworthy that clove buds (*Syzygium aromaticum*) contain significantly higher levels of phenolics, at 1,034 mg GAE/g [26], placing them at the upper end of the spectrum for phenolic content. Moreover, Sea Holly's phenolic compound concentration stands higher than German valerian leaves but is considerably lower than the phenolic content found in mango peel (*Mangifera indica*), which has an exceptionally high concentration of 842 mg GAE/g [27]. This juxtaposition emphasizes the variability in phenolic content among different plant sources and highlights Sea Holly's position within this range.

Antioxidant Activity

Various herbal plants exhibit antioxidant activity, defined as the ability to donate a single electron to free radicals to stop the chain reaction of free radicals, thus stopping the chain reaction of free radicals that causes cell damage. In this study, the in vitro antioxidant activity assays were carried out to estimate the capacity of plant extracts to scavenge free radicals, including 1,1-Diphenyl-2-picrylhydrazyl (DPPH), 2,2'-azino-bis (3-ethylbenzthiazoline-6-sulphonic acid (ABTS) and the ability to reduce Fe^{3+} to Fe^{2+} . The antioxidant activity is usually measured in %DPPH, %ABTS, and FRAP values. The higher the value of all three, the better antioxidant activity. Table 1 shows the antioxidant capacity of Sea Holly leaf and stem extracts. The leaf extract exhibited antioxidant activity at $18.73 \pm 0.42\%$ for the DPPH assay, $81.4 \pm 0.90\%$ for the ABTS assay, and 3.46 ± 0.04 mmol Fe^{2+} /g sample for the FRAP assay. In contrast, the stem extract showed

antioxidant activity at $14.48 \pm 0.44\%$, $69.0 \pm 1.7\%$, and 2.40 ± 0.05 mmol Fe^{2+} /g samples for the DPPH, ABTS, and FRAP assays. The results indicate that the antioxidant activity of the leaf extract is higher than that of the stem extract for all three assays, which is in good agreement with the findings reported by Olatunji et al. [2]. They found that the antioxidant activity of the root and stem of the *Acanthus ebracteatus* Vahl examined by DPPH Assay is 91.90 ± 0.40 μ mol TE/g dry extract. In contrast, the antioxidant activity of *Acanthus ebracteatus* Vahl leaf is 448.10 ± 0.40 μ mol TE/g dry extract. Several Thai herbs have been studied for their antioxidant activity.

In comparison with the antioxidant activity of *Acanthus ebracteatus* Vahl, some plants show higher antioxidant activity, such as Holy Basil (64.2-82.5% DPPH Scavenging) [28] and Turmeric (79.12% DPPH Scavenging) [29]. However, some plants exhibit more minor antioxidant activity than *Acanthus ebracteatus* Vahl, such as *Pistia stratiotes* L (7.69% DPPH Scavenging) [30] and *Bougainvillea glabra* leaves (6.56% DPPH Scavenging) [31]. While Sea Holly exhibits antioxidant properties, it appears to have a lower antioxidant capacity and bioactive compound content than other well-known antioxidant-rich plants. It is widely used as herbal medicine and elixir, so it may contain other important substances with medicinal properties that do not have antioxidant activity. Moreover, factors such as bioavailability, safety, and cost-effectiveness should also be considered when evaluating the potential of Sea Holly as a source of antioxidants for various applications [32].

Chromatographic analysis of plant extracts

Phytochemicals contained in plant extracts were analyzed by GC-MS. By means of comparing the spectrum of unknown compounds with the spectrum of known compounds in the NIST library. Phytochemical compounds, as well as their retention time, molecular formula, molecular weight, and peak area (%), were presented in tables 2, 3, and figure 1.

Table 2 GC-MS analysis of Sea Holly stem extract

No	Retention Time	Tentative Compounds in Sea Holly stem extract	Molecular formula	Molecular weight (g/mol)	Peak Area %
1	5.357	Glycerin	$C_3H_8O_3$	92.09	0.191
2	26.887	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	270.5	0.228
3	29.497	9,12-Octadecadienoic acid, methyl ester	$C_{19}H_{34}O_2$	294.5	0.268
4	29.594	10-Octadecenoic acid, methyl ester	$C_{19}H_{36}O_2$	296.5	0.242
5	31.363	Butanoic acid	$C_{24}H_{34}O_6$	418.5	0.215
6	31.914	Hexadecanoic acid,	$C_{36}H_{58}O_6$	586.8	0.336
7	32.131	Cyclopropanedodecanoic acid, 2-octyl-, methyl ester	$C_{24}H_{46}O_2$	366.6	0.306

No	Retention Time	Tentative Compounds in Sea Holly stem extract	Molecular formula	Molecular weight (g/mol)	Peak Area%
8	32.477	Lupeol	C ₃₀ H ₅₀ O	426.7	0.618
9	32.609	Lupeol	C ₃₀ H ₅₀ O	426.7	0.789
10	32.804	Trilinolein	C ₅₇ H ₉₈ O ₆	879.4	0.305
11	32.987	Hexadecanoic acid	C ₃₆ H ₅₆ O	584.8	0.576
12	33.192	Hexa-t-butylselenatrisiletane	C ₂₄ H ₅₄ SeSi	505.9	0.444
13	33.325	4'-Apo- α , ψ i.-carotenoic acid	C ₃₅ H ₄₆ O ₂	498.7	0.337
14	33.492	Olean-12-ene-3,15,16,21,22,28-hexo	C ₃₀ H ₅₀ O ₆	506.7	0.271
15	33.813	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester	C ₁₉ H ₃₈ O ₄	330.5	1.197
16	33.925	9,12,15-Octadecatrienoic acid, 2-phenyl-1,3- dioxan-5-yl ester	C ₂₈ H ₄₀ O ₄	440.6	0.256
17	35.001	Trilinolein	C ₅₇ H ₉₈ O ₆	879.4	4.564
18	35.254	9-Octadecenoic acid (Z)-, 2-hydroxy-1-(hydroxymethyl)ethyl ester	C ₂₁ H ₄₀ O ₄	356.5	1.719
19	35.454	1,1',2,2'-tetrahydro-1,1'- dimethoxy	C ₄₂ H ₆₄ O ₂	601	1.438
20	35.877	9-Octadecenamide	C ₁₈ H ₃₅ NO	281.5	2.092
21	36.290	9,19-Cyclolanost-24-en-3-ol, acetate	C ₃₂ H ₅₂ O ₂	468.8	4.015
22	38.259	Cholestan-3-one, cyclic 1,2-ethanediyl aetal	C ₂₉ H ₅₀ O ₂	430.7	23.579
23	38.969	Campesterol	C ₂₈ H ₄₈ O	400.7	15.882
24	39.177	Stigmasterol	C ₂₉ H ₄₈ O	412.7	5.553
25	39.690	γ -Sitosterol	C ₂₉ H ₅₀ O	414.7	23.621

Table 3 GC-MS analysis of Sea Holly leaf extract

No	Retention Time	Tentative Compounds in Sea Holly stem extract	Molecular formula	Molecular weight (g/mol)	Peak Area%
1	4.234	2-Furancarboxaldehyde	C ₆ H ₆ O ₂	110.11	0.208
2	5.515	4-Propylcyclohexylamine	C ₉ H ₁₉ N	141.25	0.218
3	5.977	1-Amino-2,6-dimethylpiperidine	C ₇ H ₁₆ N ₂	128.22	0.473
4	6.893	Furaneol	C ₆ H ₈ O ₃	128.13	0.527
5	7.173	Undecane	C ₁₃ H ₂₈	184.36	0.205
6	10.889	5-Hydroxymethylfurfural	C ₆ H ₆ O ₃	126.11	0.430
7	27.733	n-Hexadecanoic acid	C ₁₆ H ₃₂ O ₂	256.42	2.165
8	29.564	9,12,15-Octadecatrienoic acid, methyl ester	C ₁₉ H ₃₂ O ₂	292.5	0.225
9	30.127	Linoelaidic acid	C ₁₈ H ₃₂ O ₂	280.4	1.185
10	30.197	9,12,15-Octadecatrienoic acid, (Z,Z,Z)-	C ₁₈ H ₃₀ O ₂	278.4	3.681
11	30.508	Octadecanoic acid	C ₁₈ H ₃₆ O ₂	284.5	0.395
12	35.064	1-Heptatriacotanol	C ₃₇ H ₇₆ O	537	0.340
13	35.274	Trilinolein	C ₅₇ H ₉₈ O ₆	879.4	0.905
14	35.417	Methyl glycocholate, 3TMS derivative	C ₃₆ H ₆₉ NO ₆ Si ₃	696.2	0.364
15	35.552	Olean-12-ene-3,15,16,21,22,28-hexol,	C ₃₀ H ₅₀ O ₆	506.7	0.412
16	35.714	Pregn-5-ene-3,11-dione	C ₂₅ H ₃₄ O ₇	446.5	0.420
17	35.882	9-Octadecenamide	C ₁₈ H ₃₅ NO	281.5	0.857
18	35.985	Hexadecanoic acid,	C ₃₆ H ₅₈ O ₆	586.8	0.357
19	36.080	Carotene, 1,1',2,2'-tetrahydro-1,1'-dimethoxy	C ₄₂ H ₆₄ O ₂	601	0.406
20	36.320	Trilinolein	C ₅₇ H ₉₈ O ₆	879.4	1.332
21	37.999	Ergosta-5,22-dien-3-ol, acetate, (3 α ,22E)-	C ₃₀ H ₄₈ O ₂	440.7	17.391

No	Retention Time	Tentative Compounds in Sea Holly stem extract	Molecular formula	Molecular weight (g/mol)	Peak Area%
22	38.254	dl- α -Tocopherol	C ₂₉ H ₅₀ O ₂	430.7	5.767
23	38.984	Campesterol	C ₂₈ H ₄₈ O	400.7	14.859
24	39.180	Stigmasterol	C ₂₉ H ₄₈ O	412.7	6.491
25	39.690	γ -Sitosterol	C ₂₉ H ₅₀ O	414.7	23.668

Among the phytochemicals identified, hexadecenoic acid, trilinolein, campesterol, stigmasterol, and γ -sitosterol were found in both the leaf and stem of Sea holly. Based on the peak area, campesterol, stigmasterol, and γ -sitosterol are the main components in both leaf and stem extracts. All three components are cholesterol from plants, so-called phytosterol. They are reported to be able to prevent cholesterol absorption in the human intestinal tract [33]. Plant sterols can help reduce cholesterol because they have a structure similar to animal cholesterol. It will compete with the absorption of cholesterol in the small intestine. As a result, the amount of cholesterol entering the bloodstream can be reduced. The report from Yoshida and Niki [34] suggested that beta-sitosterol, stigmasterol, and campesterol also exerted antioxidant effects on the oxidation of methyl linoleate in solution. The antioxidant activity of campesterol is almost equal to beta-sitosterol but higher than stigmasterol. Normally, *Acanthus ebracteatus* Vahl is a mangrove plant grown under tidal inundation, but the specimen analyzed in this work was grown under normal conditions. It is apparent that the content of steroid compounds in *Acanthus ebracteatus* Vahl extract differs depending on its habitat conditions. The *Acanthus ebracteatus* Vahl grown in mangrove condition (East Sumatra) has been shown to increase their lipid content, such as hydrocarbon, wax ester, steryl esters, triglycerides, and steroids [35]. MITRA, S. et al. [36] reported the occurrence of sterols in *Acanthus ebracteatus* Vahl grown in a mangrove area. The relative composition of Campesterol, Stigmasterol, and γ -Sitosterol were about 15%, 15%, and 60%, respectively. The amount of Campesterol, Stigmasterol, and γ -Sitosterol from this work was found to be 15.88%, 5.55%, and 23.62%, respectively. Apparently, plants regularly submerged in water produce more short-chain hydrocarbons, *n*-alcohols, and unsaturated fatty acids. This is likely because these compounds help the plants stay flexible in the cold water, typically colder than the air temperature.

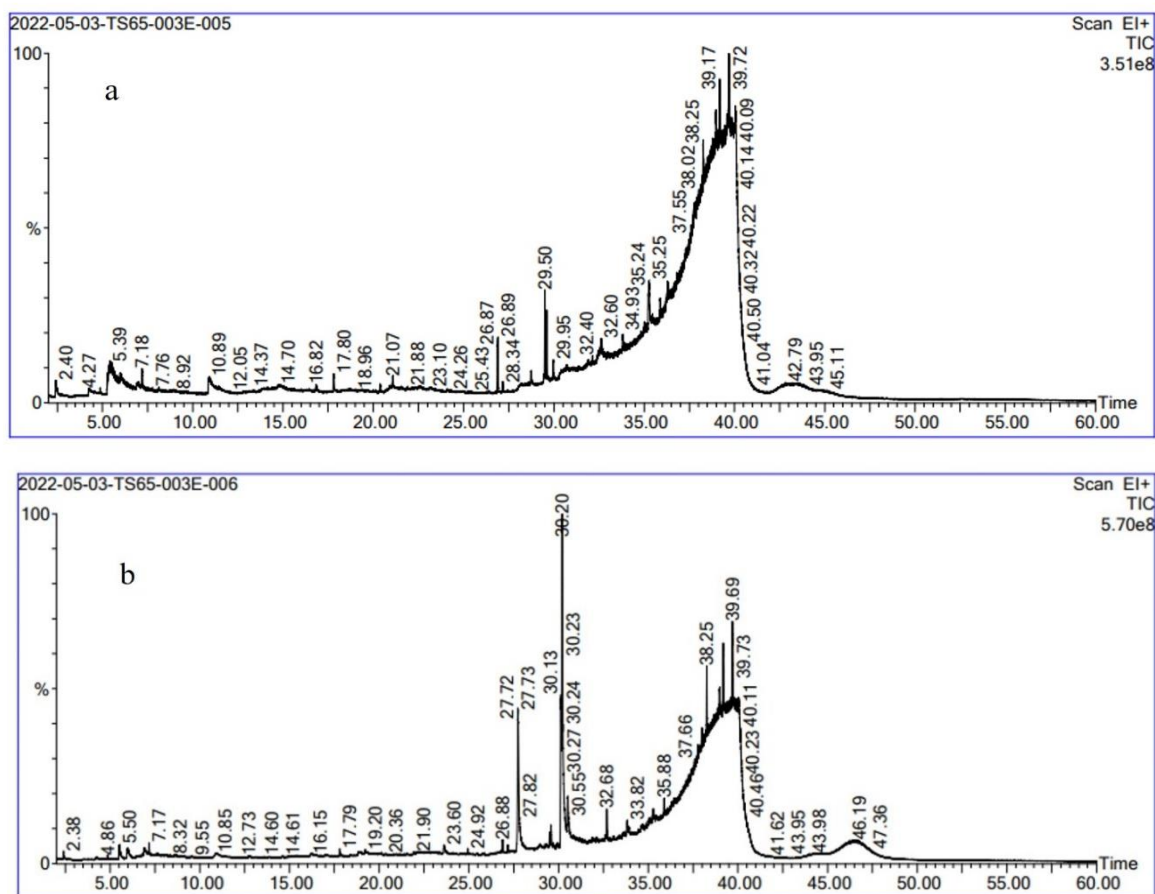


Figure 1 GC-MS chromatogram of Sea Holly extract; stem (a) and leaves (b)

FTIR Analysis of Plant Extracts

The functional groups of active components present in methanolic extracts were identified by FT-IR spectroscopy, as shown in figure 2. Possible functional groups of plant extract were assigned based on the literature [37-39]. IR Spectrum of the extraction from leaf and stem are almost identical. Both spectra present a band between 600-1000 cm^{-1} , corresponding to the C=C bending of alkene, the band at 1041-1048 cm^{-1} due to C-N stretching, and the bands at around 1200 – 1300 cm^{-1} , corresponding to C-O stretching. The bands belonging to C-H stretching at 1049 cm^{-1} were observed in both spectra. The band was also found due to C-H bending at 1450-1502 cm^{-1} and at 2933 cm^{-1} for C-H in CH_2/CH_3 . Moreover, both spectra present bands corresponding to C=C of the aromatic compound at 1591-1629 cm^{-1} . The broad band at 3200-3400 cm^{-1} belonging to the O-H stretching of water was also found.

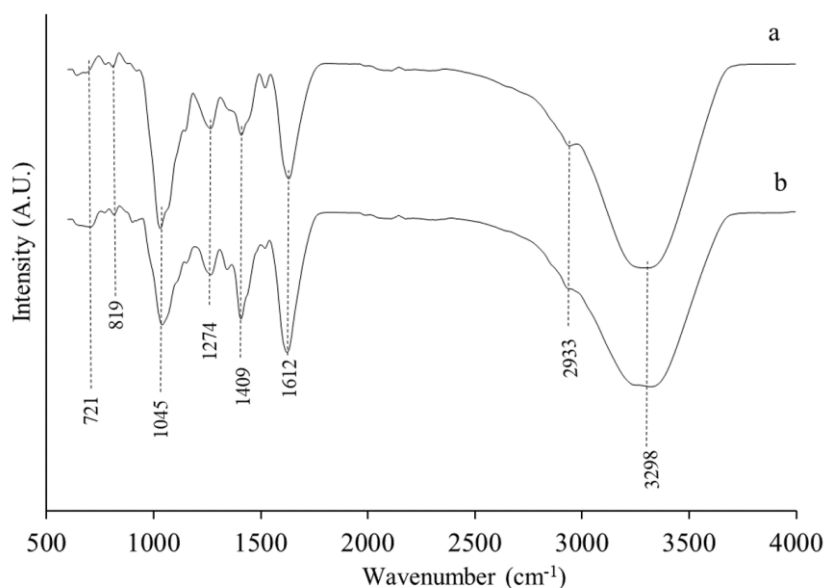


Figure 2 FT-IR spectra representing potential bands in the methanolic extract of Sea Holly stem (a) and leaf (b)

Conclusion

The amount of carotenoid and total phenolic compounds in the leaves and stem of *Acanthus ebracteatus* Vahl (Sea Holly) were evaluated and correlated with the antioxidant activity. The finding shows that the leaves of Sea Holly contain higher carotenoid and phenolic compounds than its stem. The antioxidant activity of Sea Holly leaf extract is also higher than that of the stem. GC-MS and IR spectroscopy characterized plant extract and indicated that the main constituents in plant extract are campesterol, stigmasterol, and γ -sitosterol. The data provided in this study may be used in further health-care products or pharmaceutical industries.

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