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Article

Antioxidant and antidiabetic effects *in vitro* of extract from the above-ground parts of *Acanthus ilicifolius*

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ABSTRACT

The *in vitro* antioxidant and antidiabetic activity of the extract from the above-ground parts of *Acanthus ilicifolius* was evaluated to assess the potential of this plant as a probable natural medicinal source for disease treatment. The aqueous extract from the above-ground parts of this plant demonstrated the existence of alkaloids, polyphenols, flavonoids, steroids, triterenes, tannins, saponins, and glycosides, providing promising results in all the tests performed. The antioxidant activity of *A. ilicifolius*'s above-ground parts extract was graded by five methods, including total antioxidant capacity (TAC), reducing power (RP), ferric reducing antioxidant power (FRAP), 1,1-diphenyl-2-picrylhydrazyl (DPPH), 2,2'-azino-bis-3-ethylbenzothiazoline-6-sulphonic acid ($ABTS^{\bullet +}$), and the results showed that the extract has strong antioxidant effects. The inhibitory activity of two enzymes, α-amylase and α-glucosidase enzymes was assessed and compared with the standard acarbose. Extract from *A. ilicifolius*'s above-ground parts could inhibit the enzyme α-amylase with an EC_{50} value of 136.35 ± 2.11 µg/mL and α-glucosidase with an EC_{50} value of 49.81 ± 0.37 µg/mL. The results confirmed that *A. ilicifolius* can be an important natural medicinal source with antioxidant, α-amylase, and α-glucosidase inhibitory properties.

Keywords: *Acanthus ilicifolius*; antioxidant; antidiabetic; α-amylase; α-glucosidase; medicinal.

INTRODUCTION

Uncontrolled free radicals can oxidize lipids, proteins, and nucleic acids in cells [1]. Studies and empirical data have demonstrated that diabetes is exacerbated by unchecked free radicals activity. According to Khalid et al. [2], oxidative stress plays an indispensable role in the development of diabetes mellitus, mostly through oxidation, non-enzymatic protein glycation, and protein oxidative degradation. Plant-derived secondary metabolites are thought to provide an alternative to manufactured medications in the treatment of diabetes because of their antioxidant qualities, which help mitigate the negative effects of hyperglycemia and improve glucose metabolism and absorption. As a result, medical researchers have been and continue to be interested in gaining knowledge about the antioxidant and antidiabetic properties of secondary metabolites derived from plants.

Around the world, mangroves were a prospective source of secondary metabolites used in ethnopharmaceuticals [3]. Secondary metabolites produced by mangrove species have been established to have certain medicinal values [4]. In Ca Mau Province, Vietnam, there was a species of *Acanthus ilicifolius* that grew and developed on saline land, exposing great medicinal potential. This plant was a small tree species in the Acanthaceae family, recognized by Vietnamese people for its effects in treating liver pain, enlarged liver and spleen, menorrhagia, antiseptics, rheumatism, and aches and pains [5]. Scientific investigations throughout the world have revealed that *A. ilicifolius* has numerous significant pharmacological impacts on human health, including anti-inflammatory [6], liver protection [7], anti-cancer [8], anti-ulcer, anti-osteoporosis [9], anti-epileptic [10] and anti-atherosclerotic circuit [11]. Different *A. ilicifolius* components, including roots, leaves, stems, and fruit peels, were extracted using ethanol, methanol, chloroform, and *n*-hexane. It was discovered that these extracts included a variety of secondary metabolites, such as alkaloids [12], triterpenoids, saponins [13], sterols [14], lignans [15], glycosides [16], fatty acids, and coumaric acid derivatives [9]. Secondary metabolites in *A. ilicifolius* provide conclusive evidence of important pharmacological effects on human health. This plant is exploited and used in medicine by many nations around the world, including India and China [17]. In Vietnam, there has been little investigation into the chemical composition and medicinal potential of Ca Mau's *A. ilicifolius*. Under *in vitro* circumstances, we conducted early investigations on the chemical composition, antioxidant, and antidiabetic properties of the above-ground parts of *A. ilicifolius*. The research contributes to giving a scientific foundation for the therapeutic characteristics of this plant in Ca Mau, as well as assisting the Province with a direction to utilize the medicinal value of *A. ilicifolius*.

MATERIAL AND METHODS

Research object: Halofai Saltwater Agriculture Co., Ltd. provided the above-ground parts of *A. ilicifolius*, which was around one year old, in June 2019. *A. ilicifolius* (Figure 1.) is cultivated in Tan Thanh hamlet, Phu Tan Commune, Phu Tan District, Ca Mau Province, Viet Nam in the raw material area of Halofai Saltwater Agriculture Co., Ltd. This plant was identified based on morphological characteristics following the Vietnamese herb classification system [5] with the support of Dr. Nguyen Thi Kim Hue (Deputy Dean of Biology, College of Natural Sciences, Can Tho University).

Figure 1. *Acanthus ilicifolius*

Equipment: freeze dryer (Biobase BK-FD10PT, Japan), spectrophotometer (Thermo Scientific Multiskan GO, Finland), rotary vacuum evaporator (Heidolph, Germany), cold centrifuge (Mikro 12- 24, Hettich, Germany), analytical balance (AB104-S, Mettler Toledo, Switzerland), drying oven (BE 200, Memmert, Germany), incubation tank (Memmert, Germany), vortex machine (ZX3, Velp, Italy) , micropipette (Thermol Labsystems) and several other devices.

Chemicals: Potassium persulphate (Merck), sodium carbonate (Merck), gallic acid (Merck), quercetin (Merck), Folin-Ciocalteu's phenol reagent (Merck), potassium ferricyanide (Merck), trichloroacetic acid (Merck), ferric chloride (Sigma-Aldrich), sodium nitrite (Xilong), aluminum chloride hexahydrate (Xilong), 2, 2-diphenyl-1-picrylhydrazyl (Sigma-Aldrich), 2,2'-azino-bis (3-ethylbanzthiazoline-6-sulphonic acid (Roche Diagnostics), 2, 4, 6-tripyridyl-s-triazine (Sigma-Aldrich), ammonium heptamolybdate tetrahydrate (Xilong), dimethyl sulfoxide (Merck), α-amylase enzyme (Sigma-Aldrich), α-glucosidase enzyme (Sigma-Aldrich), acarbose (Sigma-Aldrich), thibarbituric acid (Merck) and some other chemicals.

Processing and preparation of the above-ground parts of *A. ilicifolius*

After collection, *A. ilicifolius*'s above-ground parts are picked and impurities (vines, soil, sand, dirt) are removed. The sample will then be allowed to dry naturally before being processed into powder, which is steeped in distilled water at a 1:10 (w/v) ratio in a thermostatic bath at 100°C for 2 hours with a stirrer. After soaking for 2 hours in distilled water, filter to get the extract. To eliminate water, the extract was evaporated using a rotary evaporator. The residual extract was then frozen at −18°C for 24 hours, after that, the extract was freeze-dried using a Biobase BK-FD10PT device for 48 hours to remove water to obtain the aqueous fraction, which will be stored in glass jars at 4°C for subsequent experiments.

Determination of moisture in powder and the extract from the above-ground parts of *A. ilicifolius*

The moisture content of the powder and *A. ilicifolius*'s above-ground parts extract was determined by using heat to evaporate the water vapor in the sample. Weighed the sample both before and after it had dried, and then estimated the proportion of water in it. The ceramic-glazed mortar was dried at 105°C for 15 minutes until the weight remained constant, cooled in a desiccator, and weighed. Before drying, weigh 1 g of herb powder in a ceramic-glazed mortar and compute the mass. Then, this glazed mortar containing the dried herb powder samples was dried at 105°C for 2 hours to a constant weight, cooled in a desiccator, and weighed. Herb powder samples' moisture content is reckoned using the formula below:

$$\text{Humidity (\%)} = \frac{A - B}{A - C} \times 100 \qquad (1)$$

Note: C (g) was the mass of the ceramic-glazed mortar after drying, A (g) was the mass of the ceramic-glazed mortar after adding powder or the extract from the above-ground parts of *A. ilicifolius*, B (g) was the mass of the ceramic-glazed mortar and herb powder or *A. ilicifolius*'s above-ground parts extract after drying.

Preliminary phytochemical screening

A. ilicifolius's above-ground parts extract will be submitted to preliminary phytochemical screening, as described by Farnsworth [18], to determine the presence of several chemical groups such as alkaloids, polyphenols, flavonoids, sterols, triterpenes, saponins, and tannins.

Quantification of chemical components of *A. ilicifolius*'s above-ground parts

Determination of alkaloids content

The alkaloid content was determined using the complex formation method with bromocresol green (BCG), which yielded a yellow result. *A. ilicifolius*'s above-ground parts extract (1 mL) was combined with 1 mL of 2N HCl solution. The above solution was filtered through filter paper to get rid of the residue after the reaction had been going on for 5 minutes. Place the aforementioned solution in the separator and add 5 mL of BCG and 5 mL of phosphate buffer solution (pH = 4.7). Ultimately, the blend was vigorously swirled within an extraction flask holding 10 millilitres of chloroform solution. After 2 minutes of reaction at room temperature, the 470 nm wavelength spectral absorbance was measured. The alkaloid concentration in *A. ilicifolius*'s above-ground parts extract was calculated using the atropine standard curve equation [19].

Determination of polyphenols content

The polyphenol content was determined following Singleton et al.'s method [20], with some modifications. The reaction mixture contained 250 L of *A. ilicifolius*'s above-ground parts extract, 250 µL of deionized water, and 250 µL of Folin-Ciocalteu reagent, well mixed. Add 250 µL of 10% Na_2CO_3 and incubate in a thermostatic bath at 40°C for 30 minutes. At 765 nm, the reaction mixture's spectral absorbance was measured. Gallic acid was used as a positive control to generate a standard curve equation. The polyphenols content of *A. ilicifolius*'s above-ground parts extract was measured using the gallic acid standard curve equation.

Determination of flavonoids content

Bag et al.'s $AlCl_3$ colorimetric method was used to evaluate flavonoid concentration [21], with adjustment. The reaction mixture consists of 1 mL of *A. ilicifolius*'s above-ground parts extract combined with 1 mL of deionized water and shaken well. The reaction mixture was treated with 200 µL of 5% $NaNO_2$ for 5 minutes, followed by 200 µL of 10% $AlCl_3$ and vigorous shaking. After 6 minutes of incubation, 2 mL of 1M NaOH was added to the reaction mixture. At 510 nm, the absorbance was measured after adding 5 mL of water. Quercetin worked as a positive control. The total flavonoid content of *A. ilicifolius*'s above-ground parts extract was measured using the quercetin standard curve equation.

Determination of tanins content

Tannins content was evaluated using the description provided by Bhat et al. [22]. The reaction mixture consists of 1 mL of *A. ilicifolius*'s above-ground parts extract and 5 mL of reagent solution. For 20 minutes, the reaction mixture was maintained at room temperature. Next, the spectral absorbance at 500 nm was determined. The tannins concentration of *A. ilicifolius*'s above-ground parts extract was calculated using the catechin standard curve equation.

Investigation of *in vitro* antioxidant activity of *A. ilicifolius*'s above-ground parts extract

2,2-Diphenyl-1-picrylhydrazyl (DPPH) method

The 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical neutralization activity of *A. ilicifolius*'s above-ground parts extract was measured using the DPPH free radical neutralization method reported by Sharma & Bhat [23], with some modifications. There were 960 µL of extract and 40 µL of DPPH (1000 µg/mL) in the reaction mixture. This mixture was incubated in the dark at 30°C for 30 minutes. Then, measure DPPH's spectral absorbance at 517 nm.

2,2'-Azino-bis (3-ethylbenzthiazoline-6-sulfonic acid) ($ABTS^{\bullet+}$) method

The free radical neutralizing activity of 2,2'-azino-bis (3-ethylbenzthiazoline-6-sulfonic acid) ($ABTS^{\bullet+}$) was measured using Nenadis et al.'s $ABTS^{\bullet+}$ decolorization method [24]. $ABTS^{\bullet+}$ was created by combining 7 mM ABTS and 2.45 mM potassium persulfate. The combination was incubated for 16 hours at room temperature in the dark before use. After diluting the combination, the spectral absorbance at 734 nm was determined to be 0.70 ± 0.05. To conduct the survey, react 10 µL of *A. ilicifolius*'s above-ground parts extract with 990 µL of $ABTS^{\bullet+}$ at room temperature for 6 minutes. The spectral absorbance of the reaction mixture was then measured at 734 nm.

Ferric reducing-antioxidant power (FRAP) method

The reduction potential of *A. ilicifolius*'s above-ground parts extract was calculated using Benzie & Strain's description [25], with changes. The principle of this method is based on the reduction of the ferric-tripyridyltriazine complex. *A. ilicifolius*'s above-ground parts extract (10 µL) was mixed with FRAP solution (990 µL) for 30 minutes under light-obscured circumstances. Determine the experimental solution's spectral absorbance at 593 nm.

Total antioxidant capacity (TAC) method

The total antioxidant activity of *A. ilicifolius*'s above-ground parts extract was assessed using the method published by Prieto et al. [26]. *A. ilicifolius*'s above-ground parts extract (300 µL) was mixed with 900 µL test solution (0.6 M sulfuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate). For 90 minutes, the reaction solution was incubated at 95°C. The solution's spectral absorbance was measured at 695 nm.

Nitric oxide ($NO^{\bullet}$) method

The ability of *A. ilicifolius*'s above-ground parts extract to inhibit the formation of nitric oxide ($NO^{\bullet}$) was investigated according to Alisi & Onyeze [27], with changes. The reaction mixture included 200 µL of *A. ilicifolius*'s above-ground parts extract and 400 µL of sodium nitroprusside (5 mM). The reaction mixture was incubated at 25°C for 60 minutes before centrifugation at 11.000 rpm for 15 minutes. The centrifuge was supplemented with 600 µL of Griess reagent. The sample was then incubated for a further 5 minutes before being tested for spectral absorbance at 546 nm.

Vitamin C essence served as a positive control in the procedures described above for testing antioxidant activity. The *in vitro* antioxidant activity of the extract from the above-ground parts of *A. ilicifolius* was compared to a vitamin C standard using the concentration (µg/mL) that decreased, neutralized, or blocked 50% free radicals (EC_{50}-effective concentration of 50%). The EC_{50} values for *A. ilicifolius*'s above-ground parts extract and vitamin C were calculated as stated by Piaru et al. [28].

Investigation of *in vitro* antidiabetic activity of *A. ilicifolius*'s above-ground parts extract

Inhibiting activity of α-amylase enzyme

The capacity of *A. ilicifolius*'s above-ground parts extract to inhibit α-amylase enzyme was done as stated by Kwon et al. [29], with adjustments. The reaction mixture included 50 µL of phosphate buffer (pH = 7), 50 µL of *A. ilicifolius*'s above-ground parts extract, and 50 µL of α-amylase enzyme (3 U/L), incubated at 37 °C for 5 minutes. Next, add 50 µL of starch (2 mg/mL) to the mixture and incubate for 15 minutes at 37 °C. The process was stopped by adding 200 µL of strong HCl solution. Finally, 300 µL of iodine reagent solution was added to determine the amount of residual starch based on the distinctive blue response. At a wavelength of 660 nm, the above mixture was measured for the spectral absorbance of the starch-iodine complex.

Inhibiting activity of α-glucosidase enzyme

The *A. ilicifolius*'s above-ground parts extract's ability to block the α-glucosidase enzyme was tested using modifications from Shai et al. [30]. 40 µL of *A. ilicifolius*'s above-ground parts extract, 20 µL of α-glucosidase enzyme (1 U/L), and 100 µL of phosphate buffer (100 mM, pH = 6.8) were all included in the reaction mixture. For 15 minutes, the reaction mixture was incubated at 37 °C. After adding 40 µL of *p*-nitro-phenyl-α-D-glucopyranoside (5 mM), the mixture was incubated for an additional 20 minutes at 37°C. To halt the reaction, 100 µL of 0.1 M Na_2CO_3 was added. At a wavelength of 405 nm, the spectral absorbance of the *p*-nitrophenol molecule created during the reaction was calculated.

In the above methods to investigate *in vitro* antidiabetic activity, acarbose essence was used as a positive control. The *in vitro* antidiabetic potential of the extract from the above-ground parts of *A. ilicifolius* was compared with that of acarbose using the concentration (µg/mL) at which the standard or *A. ilicifolius*'s above-ground parts extract reduced or neutralized 50% of the free radicals (EC_{50}-effective concentration of 50%). The EC_{50} value of *A. ilicifolius*'s above-ground parts extract and acarbose was determined as reported by Alqahtani et al. [31].

Processing and analyzing data

All experimental data are shown as mean ± standard error. The gathered data was analyzed using one-factor ANOVA and Tukey's test in Minitab 16 software, with the p value less than 0.05 indicating a significant difference.

RESULTS AND DISCUSSION

Processing results of adjusting the extract from the above-ground parts of A. ilicifolius

The above-ground parts of *A. ilicifolius* weighed 9000 g. Through processing, 1000 g of the powder of the above-ground parts of *A. ilicifolius* are obtained with a particle size of ≤60 mesh and a moisture content of 6.30 ± 0.17%. This powder with a particle size meeting Vietnam Pharmacopoeia V standards is extracted with 10,000 mL of distilled water at 100 °C for 2 hours, filtered to remove medicinal powder residue, and obtained as 6000 mL of extract. Then, concentrate the extract to 600 mL, freeze the remaining extract at −18°C for 24 hours, and freeze-dry (DC-801, Yamato, Japan) to obtain 95 g of the aqueous fraction. The research team has determined that the efficiency of preparing the aqueous fraction according to the above process was 9.50% and the moisture content of it was 4.30 ± 0.44%. The aqueous fraction of *A. ilicifolius*'s above-ground parts was dark green in color, had a thick consistency, and had the characteristic scent of a medicinal herb.

The weight of the above-ground parts of *A. ilicifolius* (g)			**The moisture content of the above-ground parts of *A. ilicifolius* (%)**		**The efficiency of preparing the extract (%)**
Fresh	Powder	Extract	Powder	Extract	
9000	1000	95	6.30 ± 0.17	4.30 ± 0.44	9.50

Table 1. Results of processing and preparing *A. ilicifolius*'s above-ground parts extract

Results of preliminary phytochemical screening

Preliminary qualitative results on the chemical composition of *A. ilicifolius's* above-ground parts extract revealed the presence of many classes of chemicals, including alkaloids, polyphenols, flavonoids, steroids, triterpenes, tannins, saponins, and glycosides (Table 2). Karim et al. [11] discovered comparable categories of substances in our study, including alkaloids, flavonoids, phenols, glycosides, steroids, terpenoids, and saponins. Those were key families of secondary metabolites that have been utilized to treat a variety of serious human ailments.

Phytochemicals	**Test**	**Inference**
Alkaloids	Mayer's Test	+
	Dragendroff's Test	+
	Wagner's test	−
Flavonoids	Ferric chloride (5%) Test	+
	Sulfuric acid concentrate Test	+
	1% NaOH/Ethanol Test	+
Steroids	Salkowski's Test	+
Triterpenoids	Liebermann Burchard's Test	+
	Rosenheim's Test	−
Tanins	Stiasny's Test	−
	Saturated Lead Acetate Test	+
	Gelatin Test	+
Saponins	NaOH 0,1N (pH = 13)	+
	HCl 0,1N (pH = 1)	+
Glycosides	Fehling's Test	+
	Keller-Killiam's Test	−
Note: (+): indicates presence, (-): indicates absence		

Table 2. Results of preliminary phytochemical screening of *A. ilicifolius's* above-ground parts extract

Results of quantification of chemical components

Groups of chemicals known as alkaloids, polyphenols, flavonoids, and tannins have a variety of significant pharmacological impacts on human health. These chemical groups have a role in controlling the body's metabolic processes and exhibit antioxidant activity. Therefore, the research team determined the alkaloids, polyphenols, flavonoids, and tannins content in *A. ilicifolius*'s above-ground parts extract. The quantitative results of alkaloids, polyphenols, flavonoids, and tannins in that extract are shown in Figure 2. The alkaloid content of the extract obtained from the above-ground portions of *A. ilicifolius* was found to be 205.24 ± 6.82 mg AE/g extract. Alkaloids have a great deal of physiological impact on the bodies of both humans and animals, with the nervous system being particularly affected. Alkaloids-rich medicinal herbs are useful in the treatment of hepatitis, kidney stones, and cancer, and improving kidney function [33]. Even though it was a miraculous treatment, alkaloids could occasionally prove harmful if taken in excess or at the wrong dosage. Therefore, research on the dosage of alkaloid-rich medicinal herbs needs attention. Polyphenols, flavonoids, and tannins are antioxidants that might have many positive effects on humans's health. The body is shielded from toxins and everyday stresses by the circulation of polyphenols, flavonoids, and tannins, which also aid in the regulation of cellular activity and the battle against free radicals that cause oxidative stress [34]. The research team determined the polyphenols, flavonoids, and tannins content in *A. ilicifolius*'s above-ground parts extract to be 63.51 ± 1.16 mg GAE/g extract, 22.47 ± 0.23 mg QE/g extract, and 71.50 ± 2.50 mg CE/g extract. It was evident from that extract's first qualitative and quantitative analysis of its chemical makeup that it had a wide variety of groupings of components that may be used to produce foods that are beneficial to health.

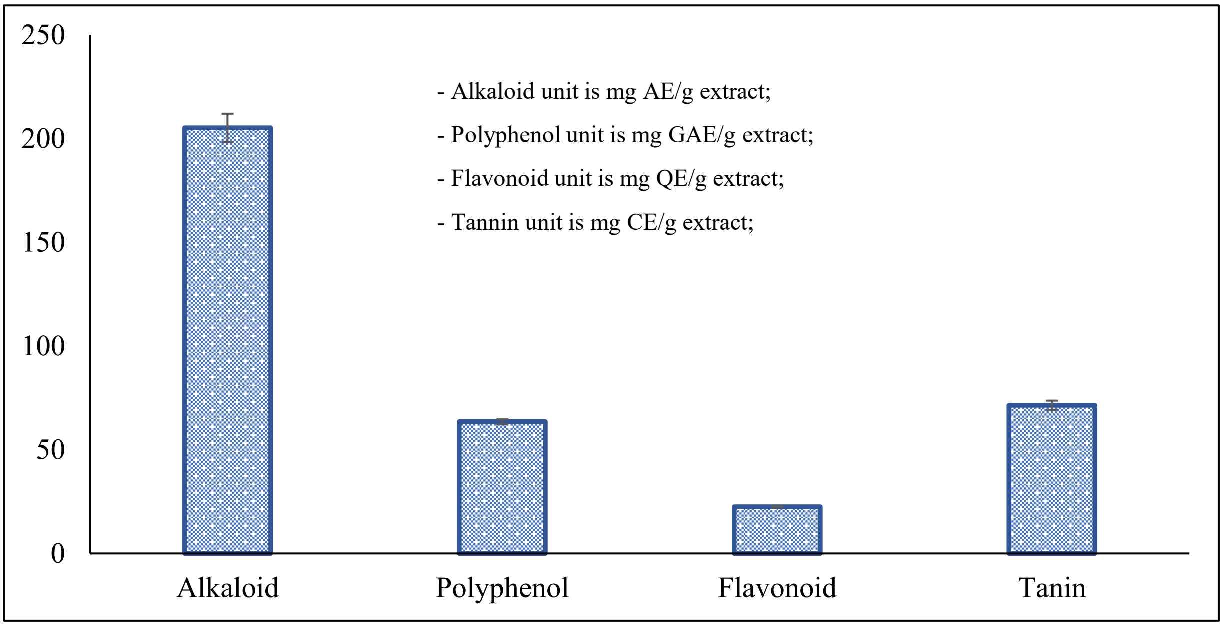

Figure 2. Results of quantification of chemical components

Prasansuklab et al. gathered leaf samples from *Acanthus ebracteatus*, a plant of the same genus as *A. ilicifolius*, in Rayong Province, Thailand, and measured the amount of polyphenols and flavonoids in them. The results demonstrated that the leaves of *Acanthus ebracteatus* had polyphenol and flavonoid content of 84.86 ± 3.69 mg GAE/g extract and 20.22 ± 3.69 mg QE/g extract, respectively [35]. Our research showed that the above-ground parts of *A. ilicifolius* had a lower polyphenol content than *Acanthus ebracteatus* leaves, but had a higher flavonoid content than *Acanthus ebracteatus* leaves. Chinnathambi et al. [36] found that methanol extract from *A. ilicifolius* stems taken from the Madras Presidency, India, had polyphenols content (42.3 ± 0.08 mg GAE/g) and alkaloids (9.23 ± 0.09 mg AE/g) that were 4.85 and 7.10 times lower than our study. Most research in the globe focuses on the alkaloids, polyphenols, and flavonoid content of *A. ilicifolius*. For the first time, our research has helped to provide additional scientific information regarding the tannin content of *A. ilicifolius*'s above-ground parts cultivated in Ca Mau, Vietnam.

Results of investigation of in vitro antioxidant activity

Many biological processes in the bodies of humans and other animals produce free radicals, which, if left unchecked, could turn into agents that encourage the onset of numerous illnesses [37]. Free radicals in the body mainly belong to the groups of reactive oxygen species (ROS), reactive nitrogen species (RNS), and toxic chemotherapeutic metal ion complexes. The total antioxidant activity of *A. ilicifolius*'s above-ground parts extract has been assessed using synthetic free radicals from the ROS group, such as DPPH, $NO^{\bullet}$, and RNS groups, such as $ABTS^{\bullet+}$, as well as hazardous covalent metal ion complexes using techniques like FRAP (Fe^{2+}), TAC (Mo^{6+}). The results of determining the antioxidant activity of *A. ilicifolius*'s above-ground parts extract on many different free radicals and metal complexes are shown in Table 3.

Methods	Samples	Linear regression-equation	EC_{50} (µg/mL)
DPPH	AiAGPE	$y = 0.336x - 0.1512$ ($R^2 = 0.9994$) (2)	$149.26^a \pm 0.13$
	Vitamin C	$y = 6.4651x + 1.2128$ ($R^2 = 0.9984$) (3)	$7.55^b \pm 0.03$
$ABTS^{\bullet+}$	AiAGPE	$y = 0.5392x + 1.4789$ ($R^2 = 0.9921$) (4)	$89.98^a \pm 0.94$
	Vitamin C	$y = 10.306x + 1.0889$ ($R^2 = 0.9979$) (5)	$4.75^b \pm 0.03$
$NO^{\bullet}$	AiAGPE	$y = 0.743x - 0.8459$ ($R^2 = 0.9985$) (6)	$68.44^b \pm 0.70$
	Vitamin C	$y = 0.5521x + 0.8817$ ($R^2 = 0.9989$) (7)	$88.97^a \pm 0.57$
FRAP	AiAGPE	$y = 0.0058x - 0.0034$ ($R^2 = 0.9965$) (8)	$86.30^a \pm 0.67$
	Vitamin C	$y = 0.0678x + 0.0029$ ($R^2 = 0.9995$) (9)	$7.34^b \pm 0.01$
TAC	AiAGPE	$y = 0.0051x + 0.0231$ ($R^2 = 0.994$) (10)	$93.51^a \pm 0.17$
	Vitamin C	$y = 0.011x + 0.0025$ ($R^2 = 0.9979$) (11)	$45.04^b \pm 0.22$
Note: Values followed by the same letter (a, b) in the same method are not statistically different ($p>0.05$); AiAGPE is *A. ilicifolius*'s above-ground parts extract.			

Table 3. The concentration of neutralizing or eliminating 50% of free radicals of *A. ilicifolius*'s above-ground parts extract, extract, and vitamin C

The study compared the antioxidant activity of *A. ilicifolius*'s above-ground parts extract with vitamin C essence using the concentration (µg/mL) at which vitamin C or that extract neutralizes, reduces, or inhibits 50% free radicals (EC_{50}-half maximal effective concentration). The extract from the above-ground portions of *A. ilicifolius* was able to neutralize DPPH and $ABTS^{\bullet+}$ free radicals, as seen in Table 3, with EC_{50} values of 149.26 ± 0.13 and 89.98 ± 0.94 µg/mL, respectively. That extract had a weaker ability to neutralize free radicals DPPH and $ABTS^{\bullet+}$ than vitamin C ($EC_{50,\ DPPH} = 7.55 \pm 0.03$ µg/mL; $EC_{50,\ ABTS\bullet+} = 4.75 \pm 0.03$ µg/mL) at 19.77 and 18.94 times, respectively. The extract from the above-ground parts of *A. ilicifolius* not only could neutralize free radicals such as DPPH and $ABTS^{\bullet+}$, but it also showed promise in preventing the production of $NO^{\bullet}$, a free radical that causes inflammation. *A. ilicifolius*'s above-ground parts extract ($EC_{50} = 68.44 \pm 0.70$ µg/mL) could inhibit the formation of $NO^{\bullet}$ free radicals more effectively than vitamin C ($EC_{50} = 88.97 \pm 0.57$ µg/mL) by 1.30 times.

The ability of the extract from the above-ground parts of *A. ilicifolius* and vitamin C to form complexes with metal ions, particularly iron and copper, may be used to assess their reducing potential. Among them, iron-containing complexes were often widely used in evaluating antioxidant activity in the direction of reducing Fe^{3+} ions to Fe^{2+}. The reduction of the colorless Fe^{3+}-TPTZ complex to the blue Fe^{2+}-TPTZ complex provided the basis for the iron reduction potential. An antioxidant that gives H^+ electrons in an acidic environment forms the Fe^{2+}-TPTZ complex. The amount of Fe^{2+}-TPTZ produced was positively associated with the color intensity of the solution following the reaction of Benzie & Strain [25]. In our research, the reducing potential of *A. ilicifolius*'s above-ground parts extract (EC_{50} = 86.30 ± 0.67 µg/mL) was determined to be weaker than vitamin C (EC_{50} = 7.34 ± 0.01 µg/mL) by 11.76 times.

Based on the creation of a blue phosphate/Mo (V) complex and the reduction of Mo (VI) to Mo (V) by antioxidant chemicals, total antioxidant activity (TAC) was calculated. Table 3 shows the capacity of vitamin C and *A. ilicifolius*'s above-ground parts extract to decrease Mo (VI) to Mo (V). The antioxidant activity of that extract was demonstrated by the data shown in Table 3. overall, at 93.51 ± 0.17 µg/mL, however it was still 2.08 times less potent than vitamin C essence (EC_{50} = 45.04 ± 0.22 µg/mL), a statistically significant difference ($p < 0.05$). Therefore, harmful metal ion complexes can be reduced by the extract from the above-ground parts of *A. ilicifolius* to a less hazardous or non-toxic state.

Blois found that the sample with EC_{50} values less than 50 µg/mL had very strong antioxidants, 50 − 100 µg/mL had strong antioxidants, 101 − 150 µg/mL had moderate antioxidants, and EC_{50} values more than 150 µg/mL had weak antioxidants [38]. According to research findings, *A. ilicifolius*'s above-ground parts extract exhibited extremely significant antioxidant activity against free radicals in the following methods: FRAP (EC_{50} = 86.30 ± 0.67 µg/mL), TAC (EC_{50} = 93.51 ± 0.17 µg/mL), $NO^{\bullet}$ (EC_{50} = 68.44 ± 0.70 µg/mL), and $ABTS^{\bullet+}$ (EC_{50} = 89.98 ± 0.94 µg/mL). In the meantime, *A. ilicifolius*'s above-ground parts extract (EC_{50} = 149.26 ± 0.13 µg/mL) is thought to have moderate antioxidants in the DPPH method. The majority of free radicals utilized in the study are susceptible to the potent antioxidant properties of the extract from this plant. The study of Sravya et al. showed that an aqueous extract from *A. ilicifolius* leaves collected in Gilakaladindi (6 km east of Machilipatnam, Andhra Pradesh, India) effectively neutralized DPPH free radicals with an EC_{50} value of 164.8 µg/mL [39]. Thus, our *A. ilicifolius*'s above-ground parts extract in our research exhibits 1.10 times the DPPH free radical neutralizing activity of Sravya et al.'s (2023) aqueous extract of *A. ilicifolius* leaves. Antioxidants can be used to control free radicals by (1) inhibiting their formation; (2) decomposing oxidants; (3) converting toxic free radicals into less toxic substances; (4) inhibiting the production of secondary toxic metabolites and inflammatory mediators; (5) inhibiting the generation of secondary oxidation; (6) repairing oxidatively damaged molecules; and (7) activating and stimulating the endogenous antioxidant defense system. Our research demonstrated that the extract from the above-ground parts of *A. ilicifolius* contains secondary metabolites that can neutralize or reduce free radicals and metal complexes to a less toxic state.

Results of investigation of in vitro or in vitro antidiabetic activity

The study's α-amylase and α-glucosidase enzymes shared structural and molecular similarities with human enzymes. The enzymes α-amylase and α-glucosidase are involved in the metabolism of carbohydrates and can be used to set up *in vitro* tests to look into their potential antidiabetic effects. The inhibitory effects of *A. ilicifolius*'s above-ground parts extract on α-amylase and α-glucosidase enzymes were compared in our investigation using acarbose essence as a positive control. Enzyme inhibition capacity may be assessed using the concentration at which that extract blocks 50% of enzyme activity (EC_{50} values). *A. ilicifolius*'s above-ground parts extract capacity to inhibit an enzyme was increased with a reduced EC_{50} value and vice versa. The findings displayed in Table 4 demonstrated that acarbose had a stronger inhibitory effect on the enzymes α-amylase and α-glucosidase than does acarbose. The enzymes α-amylase (EC_{50} = 136.35 ± 2.11 µg/mL) and α-glucosidase (EC_{50} = 49.81 ± 0.37 µg/mL) may be inhibited by *A. ilicifolius*'s above-ground parts extract more effectively than acarbose ($EC_{50,\ \alpha\text{-amylase}}$ = 27.60 ± 0.22 µg/mL; $EC_{50,\ \alpha\text{-glucosidase}}$ = 7.92 ± 0.03 µg/mL) by 4.94 and 6.29 times, respectively.

Ogundajo et al. studied *Acanthus montanus*, a plant from the same genus as *A. ilicifolius*, gathered in the Badagry Area of Lagos, Nigeria, for α-amylase and α-glucosidase inhibitory activity in leaf samples. The results showed that methanol and ethyl acetate extracts from the leaves of *Acanthus montanus* had IC_{50} values of 2870 and 1650 µg/mL, respectively, for α-amylase enzyme inhibitory activity; IC_{50} values were 2390 and 7100 µg/mL for α-glucosidase enzyme inhibitory activity of 84.86 ± 3.69 mg GAE/g extract and 20.22 ± 3.69 mg QE/g extract, respectively [40]. The results of our study indicated that *A. ilicifolius*'s above-ground parts had α-amylase enzyme inhibitory activity 21.05 and 12.10 times stronger than methanol and ethyl acetate extracts from *Acanthus montanus* leaves, respectively; α-glucosidase enzyme inhibitory activity was 86.59 and 257.25 times stronger than methanol and ethyl acetate extracts from *Acanthus montanus* leaves, respectively.

Compared to *Azadirachta indica* leaf extract (EC_{50} = 235.80 ± 0.56 µg/mL) also extracted with water as a solvent (Phuong et al. [41]), *A. ilicifolius*'s above-ground parts extract had inhibitory activity α-glucosidase enzyme was 4.73 times stronger. Some other plant species such as *Annona muricata* leaves (EC_{50} = 55.73 µg/mL) and *Psidium guajava* leaves (EC_{50} = 97.47 µg/mL) extracted with the solvent ethanol (Duy [42]) were also available α-glucosidase enzyme inhibitory activity was weaker than *A. ilicifolius*'s above-ground parts extract in our study. However, the ability to inhibit the α-amylase enzyme of *A. ilicifolius*'s above-ground parts extract is weaker than that of *Annona muricata* leaves (EC_{50} = 64.85 µg/mL) and *Psidium guajava* leaves (EC_{50} = 42.92 µg/mL). Numerous investigations indicated that in the metabolism of carbohydrates, the α-glucosidase enzyme was more significant than the α-amylase enzyme. Thus, compared to some plant species, *A. ilicifolius*'s above-ground parts extract had superior α-glucosidase enzyme inhibitory activity and maybe a more efficient diabetes management agent than common plant species. However, additional investigation into the antidiabetic action of *A. ilicifolius*'s above-ground parts extract in animal models was required to have more scientific proof.

Methods	Samples	Linear regression-equation	EC_{50} (µg/mL)
α-Amylase	AiAGPE	$y = 0.3448x + 2.9843$ ($R^2 = 0.9943$) (12)	$136.35^a \pm 2.11$
	Acarbose	$y = 1.83x - 0.5131$ ($R^2 = 0.9997$) (13)	$27.60^b \pm 0.22$
α-Glucosidase	AiAGPE	$y = 0.9325x + 3.5573$ ($R^2 = 0.9962$) (14)	$49.81^a \pm 0.37$
	Acarbose	$y = 6.007x + 2.4259$ ($R^2 = 0.9964$) (15)	$7.92^b \pm 0.03$

Note: Values followed by the same letter (a, b) in the same method are not statistically different (p>0.05); AiAGPE is *A. ilicifolius*'s above-ground parts extract.

Table 4. The concentration that inhibits 50% of enzyme activity of *A. ilicifolius*'s above-ground parts extract extract and acarbose

Our research revealed that groupings of alkaloid chemicals, polyphenols, flavonoids, steroids, triterpenes, tannins, saponins, and glycosides were present in *A. ilicifolius*'s above-ground parts extract. *A. ilicifolius*'s above-ground parts had potent *in vitro* antidiabetic effects because of their alkaloids, polyphenols, flavonoids, and tannins in particular. Numerous studies have shown that plant extracts could reduce the activity of α-amylase and α-glucosidase depending on the secondary metabolite level. The above-mentioned compounds' hydroxyl groups, both in number and location, block the enzymes α-amylase and α-glucosidase. According to Ken et al. (2015), the hydroxyl groups in these groups' molecular structures could hydrogen bond with the -OH group in the enzyme's functional amino acid active side chain, inhibiting the α-amylase enzyme reaction and the carbohydrates' ability to hydrolyze [43].

The plant species employed in the study, *A. ilicifolius*, was planned by Halofai Saltwater Agriculture Co., Ltd. in Ca Mau, Vietnam, in mangrove material regions. Numerous investigations have been carried out globally to examine the chemical makeup and biological properties of *A. ilicifolius*. Nonetheless, research focuses mostly on the chemical makeup and biological activities of *A. ilicifolius* in its natural habitat. There hasn't been any research done on the chemical makeup and biological activity of this plant that is grown by people in the arid environment of Ca Mau, Vietnam. In comparison to earlier research, our results demonstrated that the extract from the above-ground parts of *A. ilicifolius* includes several secondary metabolites in appreciable amounts. Moreover, *in vitro* tests of *A. ilicifolius*'s above-ground parts extract sectioned reveal strong antioxidant and antidiabetic properties. This might have something to do with *A. ilicifolius*'s growing habitat. Vietnam's Ca Mau province is a mangrove area with a hard environment and soil that borders the sea. To resist these unfavorable circumstances, *A. ilicifolius* cultivated in Ca Mau, Vietnam, must enhance metabolism to develop numerous secondary chemicals.

This is the first study to publish results on the chemical composition and biological activities of the extract from the above-ground parts of *A. ilicifolius* plant grown in Ca Mau, Vietnam. Our study is important not only because it publishes results on the concentration of plant secondary metabolites in *A. ilicifolius* but also because it investigates the diverse medicinal potential of cultivated *A. ilicifolius* in Ca Mau, Vietnam, for the international community.

CONCLUSIONS

The results obtained in our study show that the above-ground part of *A. ilicifolius* grown in the raw material area of Halofai Saltwater Agriculture Co., Ltd. in Ca Mau Province, Vietnam, is a source of important secondary metabolites from the plant to prevent oxidation and inhibit the activity of enzymes α-amylase and α-glucosidase. As can be seen from the data above, *A. ilicifolius*'s above-ground parts had the potential to be a source of natural medicinal herbs that were already being used to treat diabetes. Further research is needed to investigate the effectiveness of the *A. ilicifolius* extract in animal models of diabetes and to determine the safety of the extract for human consumption.

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