

Antioxidant, anti-diabetic activities and chemical composition of n-hexane extract of artichoke flowers (*Cynara scolymus* L.)

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(Received: 16 January 2026; Revised: 05 March 2026; Accepted: 06 April 2026)

Abstract. This study investigated the chemical composition and antioxidant and antidiabetic activities of the n-hexane extract of artichoke flowers (*Cynara scolymus* L.). The GC-MS analysis of the extract identified 17 compounds belonging to different groups, including hydrocarbons, esters, alcohols and polyols, heterocyclic compounds, silicon-containing compounds, and carboxylic acids. Among these groups, carboxylic acids and esters accounted for the majority, including n-hexadecanoic acid methyl ester, oleic acid, elaidic acid, palmitic acid, linoleic acid methyl ester, 9-octadecenoic acid, and linoleic acid methyl ester. *In vitro* bioassays show that the artichoke extract exhibited significant antioxidant activity with an IC₅₀ of 43.8 ± 1.75 µg/mL by using the DPPH scavenging method and 45.2 ± 2.01 µg/mL with the ABTS method. In addition, the artichoke extract exhibited significant anti-diabetic activity with an IC₅₀ of 696.7 ± 13.63 µg/mL through the ability to inhibit α-amylase and 643.2 ± 9.45 µg/mL through the ability to inhibit α-glucosidase. These findings indicate the antioxidant and antidiabetic potential of artichoke flower extracts under *in vitro* conditions. Further studies are required to isolate and identify the key active constituents, elucidate their mechanisms of action, and evaluate their safety and efficacy *in vivo* to determine their biological relevance and potential for future applications.

Keywords: *Cynara scolymus*, n-hexane, antioxidants, antidiabetes, GC-MS

1 Introduction

Cynara scolymus L., commonly known as artichoke, is a plant species belonging to the family Asteraceae. It is native to the Mediterranean region and is capable of adapting to a wide range of soil types and climatic conditions [1]. Artichoke has been extensively utilised in both culinary applications and traditional medicine. The immature flower heads are commonly consumed as nutrient-rich food, whereas the leaves are primarily employed for medicinal purposes. This species is characterised by low caloric content, high dietary fibre levels, and a substantial supply of essential vitamins and minerals, including vitamin

C, folate, magnesium, phosphorus, and potassium [2]. In medical contexts, artichoke exhibits diverse biological activities, such as digestive support, diuretic effects, hepatoprotective properties, lipid-lowering activity, anti-inflammatory effects, and antioxidant capacity [2].

Chemical studies have demonstrated that artichokes contain numerous bioactive compounds, including polyphenols, flavonoids, sesquiterpenes, triterpenes, sterols, tannins, and fatty acids. However, the content and chemical composition of these compounds vary considerably among different plant parts and cultivars [2]. In Vietnam, several studies have been

conducted to isolate and identify chemical constituents from various parts of the artichoke plant. Representative compounds include β -sitosterol, luteolin glycosides, caffeoylquinic acid derivatives, flavonoids, and sesquiterpene lactones [3].

Evaluations of biological activity have shown that aqueous and ethanol extracts from different parts of the artichoke plant exhibit strong antioxidant activity, as evidenced by their ability to scavenge DPPH free radicals and reduce levels of malondialdehyde (MDA), a final product of lipid peroxidation commonly used as a biomarker of oxidative stress in the body [3]. In addition, the compounds isolated from artichoke cultivated in Vietnam have also demonstrated effective DPPH radical-scavenging activity and a significant reduction in MDA levels [3]. This paper reports the results of an investigation into the chemical constituents as well as the antioxidant and antidiabetic activities of the n-hexane extract obtained from the flower heads of artichoke (*Cynara scolymus* L.).

2 Materials and methods

2.1 Materials

Fresh artichoke flower heads (green cultivar) were harvested from Da Lat, Lam Dong Province, in 3/2025. The scientific name of the plant specimen was identified by Dr. Luu Dam Ngoc Anh at the Vietnam National Museum of Nature, VAST. The voucher specimen, coded CS-2025-01, is deposited at the Mien Trung Institute of Scientific Research.

Chemicals and reagents: n-hexane and ethanol were purchased from Merck (Darmstadt, Germany). 2,2-Diphenyl-1-picrylhydrazyl (DPPH), 2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonate) (ABTS), ascorbic acid, soluble starch azure, α -amylase, α -glucosidase, p-nitrophenyl- α -D-glucopyranoside, and acarbose were obtained from Sigma-Aldrich (St. Louis, MO, USA). All

chemicals and reagents were of analytical grade and used without further purification.

2.2 Methods

Preparation of artichoke extract

The samples were washed with distilled water to remove surface impurities, air-dried at ambient temperature to constant weight, and ground into fine powder. For extraction, 100 g of powdered material were extracted with 2.0 L of n-hexane (1:20, w/v) at 25 ± 2 °C for 16–18 h. The extraction was repeated three times under identical conditions. After filtration (Whatman No. 1), the combined extracts were then concentrated under reduced pressure at 40 °C with a rotary evaporator to obtain the crude extract. The crude extract was weighed to determine the extraction yield and stored at 4 °C in amber bottles until use.

Antioxidant assays

DPPH radical-scavenging assay

The DPPH (1,1-diphenyl-2-picrylhydrazyl) radical-scavenging activity of the extracts was evaluated according to the method described by Dat et al. [4]. The reaction mixture consisted of 10 μ L of the extract and 190 μ L of DPPH solution (0.1 mg/mL) in 96-well microplates. The mixture was incubated at ambient temperature for 30 min; after that, the absorbance was measured at 517 nm with an ELx800 microplate reader (BioTek Instruments, USA). Ascorbic acid was used as the positive control. The DPPH radical-scavenging activity was calculated by using the following equation:

$$\text{DPPH scavenging activity (\%)} = (1 - A/A_c) \times 100$$

where A represents the absorbance of the sample, and A_c is the absorbance of the control. The concentration of extract required to scavenge 50% of DPPH radicals (IC_{50}) was calculated by using GraphPad Prism software version 8.0.

ABTS radical-scavenging assay

The ABTS [2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonate)] radical-scavenging activity of the extracts was determined according to the method described by Dat et al. [4]. A stock ABTS radical solution was prepared by mixing ABTS (7 mM) with potassium persulfate ($K_2S_2O_8$, 2.45 mM) and incubating the mixture in the dark at ambient temperature for 16 h prior to use to generate ABTS radicals. The resulting ABTS radical solution was then diluted with ethanol to obtain an absorbance of 0.700 ± 0.02 at 734 nm. The reaction mixture consisted of 10 μ L of the extract and 190 μ L of the ABTS radical solution in 96-well microplates. The reaction plate was incubated at ambient temperature for 10 min; after that, the absorbance was measured at 734 nm by using an ELx800 microplate reader (BioTek Instruments, USA). Ascorbic acid was used as the positive control. The ABTS radical-scavenging activity was calculated as follows:

$$\text{ABTS scavenging activity (\%)} = (1 - A/A_c) \times 100$$

where A is the absorbance of the sample, and A_c represents the absorbance of the control. The concentration of extract required to scavenge 50% of ABTS radicals (IC_{50}) was calculated by using GraphPad Prism software version 8.0.

Antidiabetic assays

α -Amylase inhibitory assay

The α -amylase inhibitory activity of the extract was evaluated via a 96-well microplate assay as previously described [4]. The reaction mixture, consisting of 50 μ L of the extract, 50 μ L of starch azure, and 25 μ L of α -amylase solution (2 U/mL in Tris-HCl buffer), was incubated at 37 °C for 15 min. The reaction was terminated by adding 75 μ L of 50% acetic acid to each well. After termination, the absorbance was measured at 595 nm by using an ELx800 microplate reader (BioTek Instruments, USA). Acarbose was used as the positive control.

The α -amylase inhibitory activity was calculated using the following equation:

$$\alpha\text{-Amylase inhibition (\%)} = (1 - A/A_c) \times 100$$

where A represents the absorbance of the sample, and A_c is the absorbance of the control. The concentration of extract required to inhibit 50% of α -amylase activity (IC_{50}) was calculated by using GraphPad Prism software version 8.0.

α -Glucosidase inhibitory assay

The α -glucosidase inhibitory activity of the extract was evaluated via a 96-well microplate assay as previously described [4]. The reaction mixture, consisting of 50 μ L of the extract and 25 μ L of α -glucosidase (0.5 U/mL in 0.1 M potassium phosphate buffer, pH 6.8), was pre-incubated at 37 °C for 10 min. The reaction was then initiated by adding 50 μ L of 5 mM *p*-nitrophenyl- α -D-glucopyranoside and incubated at 37 °C for 10 min. The reaction was terminated by adding 75 μ L of 0.2 M Na_2CO_3 . The absorbance was measured at 405 nm by using an ELx800 microplate reader (BioTek Instruments, USA). Acarbose was used as the positive control. The α -glucosidase inhibitory activity was calculated as follows:

$$\alpha\text{-Glucosidase inhibition (\%)} = (1 - A/A_c) \times 100$$

where A is the absorbance of the sample, and A_c is the absorbance of the control. The concentration of extract required to inhibit 50% of α -glucosidase activity (IC_{50}) was calculated by using GraphPad Prism software version 8.0.

Gas chromatography-mass spectrometry analysis

Volatile and semi-volatile compounds in the artichoke extract were analysed by using gas chromatography-mass spectrometry (GC-MS) on an Agilent 7890B GC system coupled with an Agilent 5977A mass selective detector (Agilent Technologies, Santa Clara, CA, USA) equipped with an HP-5MS capillary column (30 m $\times$ 0.250 mm $\times$ 0.25 μ m) [4]. The sample was diluted in

hexane (1:10), and 1 μL was injected into the GC system at a flow rate of 1 mL/min. Helium (purity 99.999%) was used as the carrier gas at a flow rate of 1 mL/min in splitless mode. The oven temperature was initially set at 60 $^{\circ}\text{C}$ and held for 2 min, then ramped to 260 $^{\circ}\text{C}$ at 5 $^{\circ}\text{C}/\text{min}$ and held for 1 min. The injector temperature was set at 260 $^{\circ}\text{C}$, and the ion source temperature was 280 $^{\circ}\text{C}$. The solvent delay was 3.00 min. The MS detector was operated in electron impact (EI) mode at 70 eV, scanning from m/z 50–550 in full-scan mode. The data were processed by using Agilent ChemStation C.01.10 software (Agilent Technologies, Santa Clara, CA, USA). Compounds were identified by comparing their mass spectra with those in the built-in MS libraries (W8N08 and NIST08). The relative percentage of individual components was calculated from the GC peak areas.

3 Results and discussion

3.1 Antioxidant activity of the n-hexane extract from artichoke flowers

The results indicated that the artichoke extract exhibited strong antioxidant activity, as demonstrated by its ability to scavenge DPPH and ABTS free radicals (Table 1). The extract showed DPPH radical-scavenging activity with an IC_{50} of 43.8 ± 1.75 $\mu\text{g}/\text{mL}$ and ABTS radical-scavenging activity with an IC_{50} of 45.2 ± 2.01 $\mu\text{g}/\text{mL}$, compared with the positive control, ascorbic acid, which exhibited IC_{50} values of 14.4 ± 0.42 $\mu\text{g}/\text{mL}$ for DPPH and 12.3 ± 0.39 $\mu\text{g}/\text{mL}$ for ABTS. These results suggested that the n-hexane extract possesses bioactive compounds capable of neutralising free radicals, supporting its potential use as a natural antioxidant source.

The findings of the present study are consistent with previous reports highlighting the strong antioxidant potential of artichoke extracts. In particular, a review of animal studies demonstrated that artichoke extracts could

modulate antioxidant capacity and reduce lipid peroxidation. These effects are attributed to the ability of the extracts to increase the levels of superoxide dismutase, catalase, glutathione, and glutathione peroxidase in the liver, while simultaneously decreasing malondialdehyde concentrations in both liver and plasma of experimental animals [5]. These results further confirm the potential application of artichoke as a natural source of bioactive compounds with antioxidant properties, supporting its use in functional foods, nutraceuticals, and health-promoting interventions.

Table 1. DPPH and ABTS radical-scavenging activity of artichoke extract

Sample	IC_{50} ($\mu\text{g}/\text{mL}$)	
	DPPH	ABTS
Artichoke extract	43.8 ± 1.75	45.2 ± 2.01
Ascorbic acid	14.4 ± 0.42	12.3 ± 0.39

3.2 Antidiabetic activity of n-hexane extract from artichoke flowers

The antidiabetic potential of the artichoke extract was evaluated *in vitro* through its inhibitory effects on key enzymes involved in diabetes, namely, α -amylase and α -glucosidase (Table 2). The results showed that the extract exhibited moderate antidiabetic activity, with IC_{50} values of 696.7 ± 13.63 $\mu\text{g}/\text{mL}$ for α -amylase inhibition and 643.2 ± 9.45 $\mu\text{g}/\text{mL}$ for α -glucosidase inhibition, compared with the positive control, acarbose, which displayed IC_{50} values of 105.3 ± 3.23 $\mu\text{g}/\text{mL}$ (α -amylase) and 129.6 ± 3.57 $\mu\text{g}/\text{mL}$ (α -glucosidase).

Previous *in vitro* and *in vivo* studies using diabetic mouse models also demonstrated the antidiabetic potential of artichoke extracts. The extracts from artichoke leaves showed a significant *in vitro* inhibition of α -amylase activity with an IC_{50} of 72.22–95.78 $\mu\text{g}/\text{mL}$ compared with $\text{IC}_{50} = 14.83$ $\mu\text{g}/\text{mL}$ acarbose [6]. Administration of the extract

at doses of 200–400 mg/kg for 28 days significantly reduced ($p < 0.001$) serum α -amylase levels compared with untreated diabetic control mice [6]. Furthermore, treatments with the artichoke extracts markedly improved plasma lipid profiles in diabetic mice, with reductions of 18.11% in total cholesterol (T-Ch), 60.47% in triglycerides (TG), and 37.77% in low-density lipoprotein cholesterol (LDL-C) [6]. These findings indicate that artichoke extract may exert beneficial effects in controlling postprandial blood glucose and in preventing complications associated with type 2 diabetes.

Table 2. α -Amylase and α -glucosidase inhibitory activity of artichoke extract

Sample	IC ₅₀ (μg/mL)	
	α -Amylase	α -Glucosidase
Artichoke extract	696.7 ± 13.63	643.2 ± 9.45
Acarbose	105.3 ± 3.23	129.6 ± 3.57

3.3 Chemical composition of n-hexane extract from artichoke flowers

GC-MS analysis of the n-hexane extract from artichoke flowers identified 17 compounds belonging to various chemical classes, with carboxylic acids and esters as the predominant constituents (Table 3). Major components included n-hexadecanoic acid methyl ester and C18 fatty acids, such as oleic, elaidic, and linoleic derivatives.

Many of the compounds detected in this extract were previously reported to exhibit diverse biological activities, such as antioxidant, antibacterial, antifungal, anti-inflammatory, anti-allergic, antitumor, hepatoprotective, and antiviral effects (Table 3), highlighting the potential of artichoke in the prevention and management of various diseases. The presence of bioactive compounds with multiple functional properties

suggests the multifunctional potential of artichoke extracts.

Notably, several antioxidant constituents (compounds **2**, **4**, **9**, **12**, and **15**) identified in the n-hexane extract of artichoke flowers likely contribute to the *in vitro* antioxidant activity observed in the present study. In addition, numerous fatty acids and related lipophilic compounds identified in the n-hexane extract of artichoke flowers were associated with the inhibition of carbohydrate-digesting enzymes relevant to diabetes management. For example, palmitic acid (**11**) was identified as a bioactive component with mixed-type inhibitory effects on α -amylase, supporting its potential role in modulating carbohydrate digestion [19]. In particular, fatty acids, such as palmitic acid (**11**) and oleic acid (**15**), demonstrated a significant *in vitro* inhibition of α -glucosidase, with a stronger activity than the pharmaceutical standard acarbose and primarily acting as competitive inhibitors, while exhibiting comparatively weaker α -amylase inhibition [20]. Together, these findings suggest that the fatty acid constituents of the artichoke extract may contribute to its observed *in vitro* antidiabetic enzyme inhibitory activity. The available literature on methyl ester derivatives, including n-hexadecanoic acid methyl ester (**9**), linoleic acid methyl ester (**13**), and linolelaidic acid methyl ester (**17**), is limited; however, structural analogues imply that esterification can alter enzyme affinity and solubility, as well as modify inhibitory effects. Therefore, comprehensive biochemical evaluations (*in vitro*, *in vivo*, and *in silico*) are warranted to clarify the potential biological roles of the remaining constituents in the extract for which direct evidence of antidiabetic enzyme inhibitory or antioxidant activities is currently unavailable.

Table 3. Chemical composition of n-hexane extract from artichoke flowers

No	Compound	Percentage (%)	Reported bioactivity
1	2,2-Dimethoxybutane	0.466	
2	2,4-Dihydroxy-2,5-dimethyl-3-furanon	0.906	Antioxidant, antibacterial, antifungal [7, 8]
3	Undecane	0.383	Anti-inflammatory, anti-allergic [9]
4	5-Hydroxymethyl-2-furaldehyde	0.662	Antioxidant [10]
5	Dodecamethylcyclohexasiloxane	2.165	
6	Tetradecamethylcycloheptasiloxane	0.849	Antifungal [12]
7	Ethyl 4-ethoxybenzoate	0.711	
8	3,4-Dimethoxyphenylacetone	0.426	
9	n-Hexadecanoic acid methyl ester	18.425	Antibiotic, antioxidant, anti-inflammatory, hepatoprotective [13, 14]
10	9-Octadecenoic acid	6.461	Anti-inflammatory, antitumor [15]
11	Palmitic acid	13.192	Antitumor, anti-inflammatory, antibacterial, antiviral, anti-diabetic [16–19]
12	3,5-Di-tert-butyl-4-hydroxyphenylpropionic acid	1.805	Antioxidant [21]
13	Linolelaidic acid methyl ester	7.138	
14	Methyl dihydromalvalate	0.389	
15	Oleic Acid	15.966	Antibiotic, anti-inflammatory, antioxidant, anti-diabetic [22–24]
16	Elaidic acid	12.409	
17	Linoleic acid methyl ester	5.937	Antifungal, anti-diabetic, melanin biosynthesis inhibition [24, 26]
Total		83.498	

4 Conclusions

This study investigated the chemical composition and biological activities of the n-hexane extract from artichoke flowers (*Cynara scolymus* L.). GC-MS analysis of the extract identified 17 compounds belonging to various chemical classes, including hydrocarbons, esters, alcohols, heterocyclic compounds, and carboxylic acids, with fatty acids and esters being the predominant constituents. *In vitro* assays demonstrated that the extract exhibited strong antioxidant activity, as evidenced by DPPH and ABTS radical-scavenging capacities with IC₅₀ values of 43.8 ± 1.75 µg/mL and 45.2 ± 2.01 µg/mL,

respectively. In addition, the extract showed potential antidiabetic activity through the inhibition of α-amylase (IC₅₀ = 696.7 ± 13.63 µg/mL) and α-glucosidase (IC₅₀ = 643.2 ± 9.45 µg/mL). These findings indicate the antioxidant and antidiabetic potential of artichoke flower extracts under *in vitro* conditions. Nevertheless, further investigations are required to isolate and identify the key active constituents, clarify their mechanisms of action, and assess their safety and efficacy *in vivo*. Such studies are essential to substantiate the potential application of artichoke flower extracts in nutraceutical, functional food, or pharmaceutical development.

Acknowledgement

We are grateful to Dr. Luu Dam Ngoc Anh, Vietnam National Museum of Nature, for identifying the scientific name of *Cynara scolymus*.

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