



## Method Development for Simultaneous Quantification of Polyphenol Compounds in Artichoke (*Cynara scolymus* L.) Leaf Dry Extract by UPLC-PDA

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### ABSTRACT

Artichoke (*Cynara scolymus* L.) is renowned for its cholesterol-lowering, diuretic, liver-protective, and antioxidant attributes, primarily attributed to its abundance of caffeoylquinic acid derivatives and flavonoids. However, a deficiency remains in the quality control of bioactive ingredients within artichoke. This study's core objective was to establish and validate a ultra-high-performance liquid chromatography-photodiode array detector (UPLC-PDA) method capable of quantifying 12 specific polyphenol compounds as 1-caffeoylquinic acid, 3-caffeoylquinic acid, chlorogenic acid or 5-caffeoylquinic acid, 4-caffeoylquinic acid, cynarine or 1,3-dicaffeoylquinic acid, 3,4-dicaffeoylquinic acid, 3,5-dicaffeoylquinic acid, 1,5-dicaffeoylquinic acid, 4,5-dicaffeoylquinic acid, caffeic acid, scolymoside and cynaroside present in both artichoke extracts and supplements. The method demonstrated commendable system suitability, specificity, and a linear range spanning 1-500 µg/mL (R<sup>2</sup> = 0.99). Furthermore, we effectively applied this developed method to assess the polyphenol content in 19 distinct artichoke supplements. This concurrent quantification strategy offers a reliable avenue for evaluating the polyphenolic constituents across various artichoke preparations.

**Keywords:** Artichoke, *Cynara scolymus*, Compositae family, Polyphenol, UPLC-PDA

### Introduction

Artichoke (*Cynara scolymus* L.) is an herbaceous plant native to the Mediterranean region, belonging to the Compositae family.<sup>1</sup> Its leaves have been traditionally used for treating liver complaints,<sup>2-5</sup> hyperlipidemia,<sup>6-10</sup> and irritable bowel syndrome (IBS) symptoms.<sup>11-13</sup> These therapeutic effects are attributed to caffeoylquinic acids (CQAs) such as chlorogenic acid (5-CQA), cynarine (1,3-CQA), and luteolin derivatives.<sup>14-17</sup> The cultivation of artichoke plants worldwide contributes significantly to the agricultural and pharmaceutical industries.<sup>18,19</sup>

In Vietnam, artichoke plants were introduced in the early 20th century and are currently grown in Da Lat and Sapa.<sup>20</sup> The development of herbal medicines from artichoke aligns with the national policy and strategy on traditional medicines set by the Vietnamese Ministry of Health, which prioritizes investment in 40 medicinal plants, including artichoke. Various artichoke extract preparations, such as capsules, tablets, and tea bags, are available in both foreign and domestic pharmaceutical markets.<sup>21-23</sup>

Artichoke (*Cynara scolymus* L.), also known as *Cynara cardunculus* L., belongs to the herbaceous plant family Asteraceae and is native to the Mediterranean region.<sup>1,2</sup>

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Revered for its medicinal and functional properties, artichoke is employed to address liver disorders, bile-related issues, and lower blood lipid levels.<sup>3</sup> Its leaves have been traditionally used for treating liver complaints,<sup>2-5</sup> hyperlipidemia,<sup>6-10</sup> and irritable bowel syndrome (IBS) symptoms.<sup>11-13</sup> These therapeutic effects are attributed to caffeoylquinic acids (CQAs) such as chlorogenic acid (5-CQA), cynarine (1,3-CQA), and luteolin derivatives.<sup>14-17</sup> The cultivation of artichoke plants worldwide contributes significantly to the agricultural and pharmaceutical industries.<sup>18,19</sup> Consequently, the cultivation of artichoke has gained prominence globally, particularly in countries such as Italy, Egypt, and Spain.<sup>1,2</sup>

In Vietnam, regions like Da Lat, Sa Pa, and Tam Dao are notable for their substantial artichoke cultivation. This herb has garnered significant scientific interest due to its multifarious benefits.<sup>20</sup> On the global front, there exists a plethora of research studies centered around artichoke flowers and leaves. Noteworthy themes encompass investigations into chemical composition, pharmacological impacts, quantitative methodologies, and select clinical trials. Within the domestic landscape, studies on the chemical composition of Dalat artichoke remain relatively limited. Published compounds include sterols, cynarine, and flavonoids.<sup>20</sup> In Vietnam, the exploration and exploitation of medicinal properties in artichoke aligns harmoniously with national drug policies and the Ministry of Health's strategic emphasis on herbal medicine development. Artichoke is among the 40 priority medicinal plants designated for focused investment and growth.<sup>21</sup> Presently, both the Vietnamese and global markets offer a diverse array of artichoke-based products, ranging from tea bags and sugar-coated tablets to capsules and infusions. However, certain clinical investigations have produced disparate outcomes when employing distinct branded artichoke preparations with identical experimental models. This variance can be attributed to the lack of standardization in artichoke leaf extracts (ALEs). Differences arise due to varying production methodologies across preparations, which subsequently affects ingredient composition.<sup>21-23</sup> Unfortunately, these protocols are not widely disseminated in national or international journals. Preliminary analysis of main polyphenol content, specifically

cynarin and chlorogenic acid, in market-available preparations revealed considerable variations. Some products displayed minimal or even undetectable levels of these active ingredients. Notably, the majority of domestic preparations exhibited lower active ingredient content when compared to Chophytol, an imported preparation from France that has found a place in the Vietnamese market.

However, inconsistent results in clinical studies on the bioactivity of artichoke preparations have been observed, partly due to the lack of standardization and quality control of artichoke leaf extracts.<sup>24</sup> Therefore, the objective of this study was to develop a ultra-high-performance liquid chromatography-photodiode array detector (UPLC-PDA) method for simultaneous quantification of 12 polyphenols including 1-caffeoylquinic acid, 3-caffeoylquinic acid, chlorogenic acid or 5-caffeoylquinic acid, 4-caffeoylquinic acid, cynarine or 1,3-dicaffeoylquinic acid, 3,4-dicaffeoylquinic acid, 3,5-dicaffeoylquinic acid, 1,5-dicaffeoylquinic acid, 4,5-dicaffeoylquinic acid, caffeic acid, scolymoside and cynaroside in artichoke dry extract. This method aims to provide an overview of the quality of commercial artichoke preparations by analyzing their polyphenol components.

## Materials and methods

### Materials

The spray-dried artichoke extract (5.3% moisture content) used in this study was provided by the Saigon Pharmaceutical Sciences and Technology Center (SAPHARCEN) at the University of Medicine and Pharmacy in Ho Chi Minh City, Vietnam. The extract was obtained from fresh artichoke leaves using an aqueous extraction method. Nineteen commercial supplements presented artichoke leaf extract in various formulations (tablets, capsules, liquid, and concentrated

extracts) were purchased from local markets in France, USA, Germany, and Vietnam. These product's information is provided in Table 1.

### Chemicals

Three reference standards, namely chlorogenic acid (96.0%, lot.2779), cynarine (96.0%, lot.857), and cynaroside (96.0%, lot.7842), were obtained from Phytolab company in Germany. Additionally, nine working standards [1-CQA (99.4%), 3-CQA (95.2%), 4-CQA (94.7%), caffeic acid (99.2%), scolymoside (97.4%), 1,5-diCQA (96.3%), 3,4-diCQA (96.1%), 3,5-diCQA (95.1%), 4,5-diCQA (93.5%)] were supplied by SAPHARCEN J.S.C, Ho Chi Minh City, Vietnam. The solvents used, including methanol (Scharlau), acetonitrile (Merck), trifluoroacetic acid (Scharlau), and formic acid (Baker), were of HPLC grade.

### Optimization of sample preparations

To optimize the recovery and extraction of the 12 polyphenols, the dried artichoke extract was dissolved in various concentrations of methanol and ethanol solutions (20%, 40%, 60%, 80%, and 100%). It was observed that different methanol-water or ethanol-water mixtures resulted in different extraction outcomes, with 40% methanol proving to be the most effective solvent. The use of an ultrasonic temperature of 40°C facilitated the dissolution of polyphenolic components in the artichoke dry extract without compromising substance yield. However, at higher temperatures, such as 50°C, certain phenolic compounds, including chlorogenic acid and cynarine, were found to be unstable during the extraction process. Additionally, an ultrasonic time of 10 minutes was determined to be the optimal choice for sample preparation.

**Table 1:** Product information of 19 artichoke preparations

No.	Preparations	Dosage forms	Contents (mg) <sup>a</sup>	Made in	Mfg. Date <sup>b</sup>
1	F.P1	Capsule	500	USA	2019
2	F.P2	Capsule	600	USA	2018
3	F.P3	Sugar-coated tablet	200	France	2019
4	F.P4	Sugar-coated tablet	200	France <sup>c</sup>	2019
5	F.P5	Capsule	350	Germany	2020
6	F.P6	Capsule	700	Germany	2021
7	F.P7	Capsule	550	Germany	2020
8	F.P8	Capsule	400	Germany	2019
9	V.P9	Sugar-coated tablet	100	Vietnam	2019
10	V.P10	Liquid extract	280	Vietnam	2020
11	V.P11	Liquid extract	200	Vietnam	2020
12	V.P12	Liquid extract	900	Vietnam	2020
13	V.P13	Liquid extract	200	Vietnam	2019
14	V.P14	Liquid extract	200	Vietnam	2020
15	V.P15	Condensed extract	100 <sup>d</sup>	Vietnam	2019
16	V.P16	Condensed extract	100 <sup>d</sup>	Vietnam	2020
17	R.P17	Film-coated tablet	200	Vietnam	2016
18	R.P18	Film-coated tablet	200	Vietnam	2016
19	R.P19	Film-coated tablet	200	Vietnam	2016

<sup>a</sup>Artichoke leaf extract (mg) per unit; <sup>b</sup>Mfg. Date: Manufacture date; <sup>c</sup>Imported product was purchased in Vietnam; <sup>d</sup>Extract (g) per package; F.P(1-9): Foreign products; V.P(9-16): Vietnamese products; R.P(17-19): Researched product.

Consequently, the results indicated successful extraction of the phenolic compounds from 20 mg of artichoke dry extract using 2 mL of 40% methanol under the established extraction conditions. The established sample preparation method involved accurately weighing (Ohaus PX224E, Ohaus, USA) 20 mg of artichoke dry extract into a 1.5 mL eppendorf tube. The extract was then mixed with 1 mL of 40% methanol, vortexed (MX-F – 8031101000, DLAB, China) for 1 minute, sonicated (Q700, Qsonica, USA) at 40°C for 10 minutes, and centrifuged (LC500-24, Joan Lab, China) at 12,000 × g for 2 minutes. The resulting supernatant was transferred, and the residue underwent the same extraction steps. The extracts were combined and transferred to a 5 mL volumetric flask, where the volume was adjusted using 40% methanol. Finally, the solution was filtered through a 0.22 µm filter (MF-Millipore™ Membrane Filter, Germany) before being subjected to UPLC analysis.

#### Optimization of UPLC conditions

Sample and standard solutions were analyzed using a Waters UPLC system equipped with a 2996 PDA detector (MA, USA) and a Thermo Fisher C18 column (100 × 2.1 mm; 2.6 µm) (MA, USA). The chromatographic parameters were optimized as follows: The mobile phase consisted of 0.1% trifluoroacetic acid (pump A) and acetonitrile (pump B). The elution method employed a gradient program with specific time intervals and percentages of pump B (see supporting information). The flow rate was set at 0.8 mL/min, and the column temperature was maintained at 35°C. The UV detection wavelengths were 325 nm for CQA compounds (1-CQA, 3-CQA, chlorogenic acid or 5-CQA, 4-CQA, cynarine or 1,3-diCQA, 3,4-diCQA, 3,5-diCQA, 1,5-diCQA, 4,5-diCQA, and caffeic acid) and 349 nm for flavonoids (scolymoside and cynaroside). The UPLC-PDA chromatograms of the sample solutions and standard mixture are presented in supporting information (Table S1).

#### Method validation

To optimize the sample preparation, various variables including extraction methods, extraction solvents (water, ethanol, and methanol), temperatures, extraction time, and number of extractions were tested to achieve an optimal extraction of the 12 polyphenols. The UPLC method was validated according to the International Conference on Harmonization (ICH) guidelines for the validation of analytical procedures.<sup>25</sup> The validation included assessing linearity, limit of detection (LOD), limit of quantification (LOQ), system suitability, intra-day and inter-day precision, and accuracy. Since some polyphenols were present in limited quantities, chlorogenic acid and cynarine were used as substitutes to quantify the respective classes of compounds in the artichoke leaf dry extract.<sup>24,25</sup> The system suitability tests of both standard and sample solutions were evaluated by injecting each solution in six replicates. The results demonstrated that the system was suitable for quantitative analysis as the peaks of the 12 polyphenols met all the assessment requirements, including retention time (Rt), peak area (S), and theoretical plate number (N), with a relative standard deviation (RSD%) of ≤ 2, a value of  $0.8 \leq A_s \leq 1.5$ , and a resolution (Rs) > 1.5. Furthermore, the comparison of peaks between the sample, blanks, and standards confirmed that the 12 polyphenol peaks could be effectively separated and detected in the artichoke dry extract. For linearity assessment, stock solutions of each standard compound were prepared in 40% methanol at a concentration of 1000 µg/mL. Calibration curves were constructed by combining appropriate volumes of the standard stock solutions to create mixtures of eight standards. These mixtures were then diluted to obtain seven different concentrations ranging from 1 to 500 µg/mL. Each mixture standard solution was analyzed in duplicate, and linearity equations were calculated using linear regression in Microsoft Excel. The calibration results indicated good linearity ( $R_2 \geq 0.999$ ) for all compounds within the working concentration range. The LODs and LOQs were determined by diluting standard solutions until they reached concentrations that exhibited a signal-to-noise ratio (S/N) of 2-3 in the chromatograms. The LOQ was calculated as  $LOQ = LOD \times 3.3$ . The precision was assessed by calculating the relative standard deviation (RSD%) based on six replicates of sample analysis. The intra-day and inter-day precision were determined by analyzing the

prepared samples on the same day and on two different days, respectively. The accuracy of the method was evaluated through recovery experiments. Artichoke dried extract samples were spiked with three different amounts (80%, 100%, and 120%) of the standard compounds prior to extraction.

#### Application of method to commercial products

For the quantitative analysis of the 12 polyphenols in artichoke supplements, approximately 25 tablets or capsules were used, and a portion of the powder equivalent to 20 mg of the labeled amount of artichoke dry leaf extract was accurately weighed. In the case of liquid extracts, 100 mL of extract were freeze-dried to obtain the dry extract before sample preparation. The prepared samples were analyzed using the established UPLC conditions, and the sample solutions were filtered through a 0.22 µm PTFE membrane filter.

#### Statistical analysis

The statistical analysis utilized in this study involved Microsoft Office Excel (2021) and the Statistical Package for Social Sciences (SPSS; version 21.0) software. Descriptive statistics were generated, including measures of mean and standard deviation (SD). An analysis of variance (ANOVA) was conducted, with significance evaluated at a level of  $p \leq 0.05$  to establish statistical significance.

## Results and discussion

#### Optimization of UPLC conditions

The artichoke leaf dry extract was quantitatively analyzed for twelve phenolic compounds, including nine CQAs, one caffeic acid, and two flavonoids. The UV spectra of the four mono-CQAs, five di-CQAs, and caffeic acid exhibited similar shapes with three absorption maximum wavelengths due to their common caffeic acid derivative structure. These compounds showed the highest absorptivity at the wavelength range of 321-328 nm compared to 216-217 nm and 241-242 nm (Figure 1, Table 2).

On the other hand, the UV spectrum of cynaroside showed a similar shape to that of scolymoside, as they are both luteolin derivatives. The absorption at 349 nm provided better absorbance and less interference compared to 254 nm and 206 nm, making it the selected wavelength for quantifying the two flavonoids. Two mobile phase systems, acetonitrile-water and methanol-water were compared to determine the optimal separation conditions, and the gradient prepared from acetonitrile-water was found to be the best solvent system. To eliminate peak tailing, different types of acid modifiers such as acetic acid (AA), formic acid (FA), and trifluoroacetic acid (TFA) were added to the mobile phase. TFA was chosen as the modifier. The final chromatographic conditions involved a gradient elution system using acetonitrile and 0.1% (v/v) aqueous TFA. Satisfactory separation of the twelve phenolic compounds was achieved within 40 minutes with a flow rate of 0.8 mL/min. The chromatographic parameters, including peak purity, asymmetry, resolution, and theoretical plate, met the requirements for method validation (Figure 2, Table S2).

#### Method validation

**The system suitability testing (SST):** When injecting the dry sample of *C. scolymus* (artichoke) six times consecutively, the relative standard deviation (RSD %) of the chromatographic parameters, including peak area (S), ranged from 0.12-0.9%, retention time (Rt) ranged from 0.12-1.17%; the resolution of the quantified peaks ranged from 1.6-37.1, and the asymmetry factor had values between 1.00-1.39. Therefore, the process meets the requirements for system suitability (Figure S1, Table S1-2).

**Specificity assessment:** The results of specificity evaluation showed that the blank sample did not have peaks of the targeted substances; the UV spectra and retention time (Rt) of the 12 quantified polyphenols in the sample, standard sample, and additional spiked sample were equivalent; all quantified substances exhibited pure peaks. Hence, the quantification process is specific (Figure S2-4).

**Linearity, the LODs, and the LOQs:** The linear relation between peak areas and concentration of 8 working standards and their LODs and LOQs values under the present chromatographic conditions could be

observed in Table 3, Figure S6. The correlation coefficients ( $r$ ) of each calibration curve were greater than 0.99, as required by ICH guidelines (Figure S5, Table S3).

Table 3.

**The precision and accuracy:** The intra-day precision ranged from 0.57% to 1.17%, while the inter-day precision ranged from 0.90% to 2.51% (Table 4, Table S6, Figure S7-9). For the accuracy, the spiked samples were then extracted and analyzed in triplicate under the optimized conditions. The recovery of the eight compounds ranged from 90.0% to 106.3%, with RSD values ranging from 0.39% to 2.53% (Table 5, Table S7-8). These results indicate that the developed quantitative method is both precise and accurate.

**Quantitative determination of 12 polyphenols in some Artichoke preparations**

The developed UPLC method was used to analyze a total of 19 preparations, including 8 foreign commercial artichoke preparations

(F.P1-8) purchased from the USA, France, and Germany, 8 domestic preparations made in Vietnam (V.P9-16), and 3 research preparations (R.P17-19) supplied by SAPHARCEN (Table 1). The results of the analysis are presented in Figures 3, 4, and Table S9.

Regarding the results of applying the process to analyze 12 polyphenols in different formulations, although the formulations vary in complexity, there is no significant difference in the retention time of the quantified peaks. The chromatograms of foreign formulations are quite similar to domestic formulations. However, the chromatograms of foreign formulations also exhibit some distinct characteristics compared to Vietnamese formulations, mainly due to the presence of additional components, albeit at low signal intensities. These compositional differences may arise from variations in the genetic makeup or cultivation regions of *C. scolymus*, as *C. scolymus* is a polyploid species ( $2n=2x=34$ ) with genetic diversity, and there are numerous *C. scolymus* varieties worldwide 26,27 28.

**Table 2:** The values of maximum absorption ( $\lambda_{\max}$ ) of 12 polyphenols in artichoke leaf extract

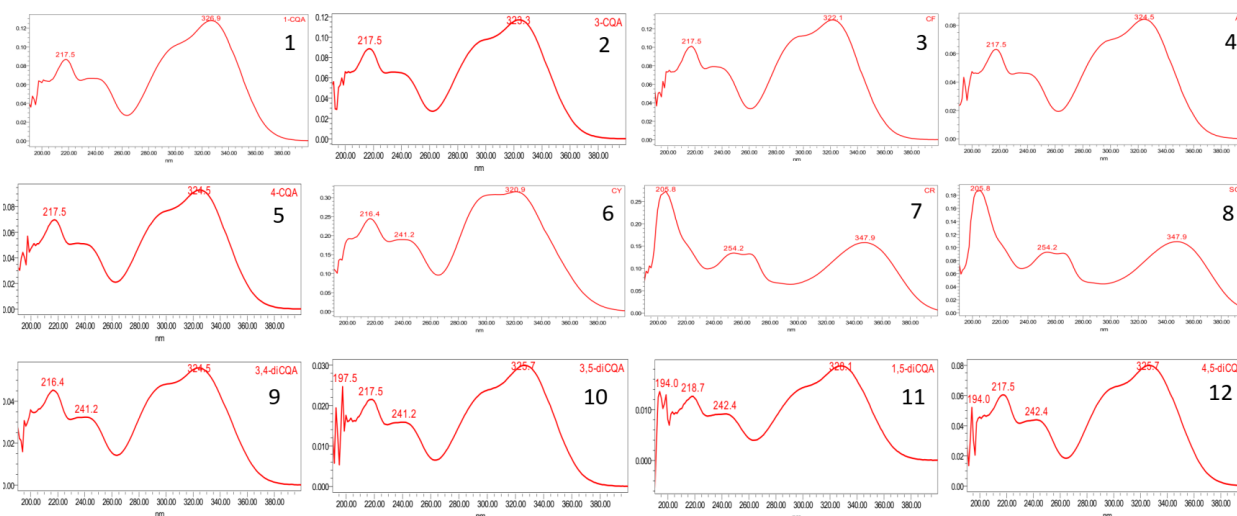
$\lambda_{\max}$	1	2	3	4	5	6	7	8	9	10	11	12
$\lambda_{\max 1}$	217.5	217.5	217.5	217.5	217.5	216.4	205.8	205.8	216.4	217.5	218.7	217.5
$\lambda_{\max 2}$	-	-	-	-	-	241.2	254.2	254.2	241.2	241.2	242.4	242.4
$\lambda_{\max 3}$	326.9	323.3	322.1	324.5	324.5	320.9	347.9	347.9	324.5	325.7	326.1	325.7

1: 1-caffeoylquinic acid; 2: 3-caffeoylquinic acid; 3: Caffeic acid; 4: Chlorogenic acid; 5: 4-caffeoylquinic acid; 6: Cynarine; 7: Cynaroside; 8: Scolymoside; 9: 3,4-dicaffeoylquinic acid; 10: 3,5-dicaffeoylquinic acid; 11: 1,5-dicaffeoylquinic acid; 12: 4,5-dicaffeoylquinic acid

**Table 3:** Linearity, LODs, and LOQs of 8 standards

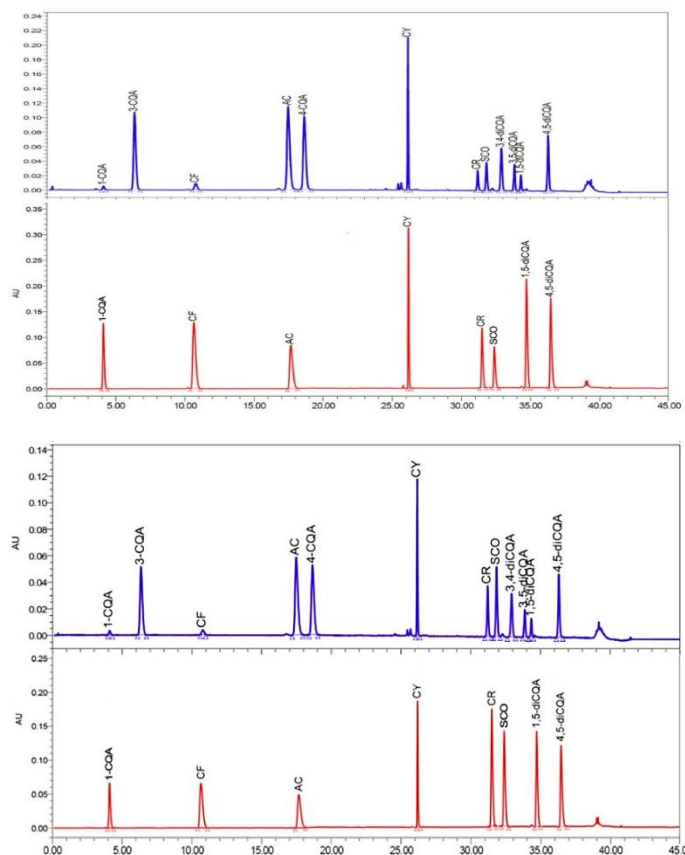
Compounds	Equations <sup>a</sup>	R <sup>2</sup>	Concentration range (µg/mL)	LOD (µg/mL)	LOQ (µg/mL)
1-CQA	$y = 13433 x$	0.9999	1 - 500	0.125	0.412
CF	$y = 25533 x$	0.9998	1 - 500	0.125	0.412
AC	$y = 17659 x$	0.9999	1 - 500	0.125	0.412
CY	$y = 17657 x$	0.9998	1 - 500	0.050	0.165
CR	$y = 16497 x$	0.9999	1 - 500	0.0625	0.206
SCO	$y = 12151 x$	0.9998	1 - 500	0.0625	0.206
1,5-diCQA	$y = 22109 x$	0.9999	1 - 500	0.0625	0.206
4,5-diCQA	$y = 20950 x$	0.9999	1 - 500	0.0625	0.206

<sup>a</sup> Regression equation is compatible ( $F > F_{0.05}$ ), coefficient b is not statistically significant



**Figure 1.** UV spectra of 12 phenolic compounds in artichoke leaf extract

1: 1-caffeoylquinic acid; 2: 3-caffeoylquinic acid; 3: Caffeic acid; 4: Chlorogenic acid; 5: 4-caffeoylquinic acid; 6: Cynarine; 7: Cynaroside; 8: Scolymoside; 9: 3,4-dicaffeoylquinic acid; 10: 3,5-dicaffeoylquinic acid; 11: 1,5-dicaffeoylquinic acid; 12: 4,5-dicaffeoylquinic acid



**Figure 2:** UPLC-PDA chromatogram of artichoke dried extract (a, c) and mixture of standards (b, d) at wavelength of 325 and 349 nm. CQA (caffeoylquinic acid); CF (caffeic acid); AC (chlorogenic acid); CY (cynarine); CR (cynaroside); SCO (scolymoside).

The quantification process is suitable for determining the 12 polyphenols in most domestic formulations. However, for foreign formulations, the process is appropriate for quantifying 9 phenolic compounds, excluding cynaroside, scolymoside, and 4,5-diCQA, as most foreign formulations may contain additional components that appear near these peaks, resulting in impure peaks and inadequate resolution for quantification (Figure S10-11).

In general, mono-CQAs were the major components in terms of total phenolic content in all samples, ranging from 0.04% to 7.14%. They were followed by di-CQAs (0.02% to 3.58%) and flavonoids (0.02% to 1.87%). These findings are consistent with previous studies.<sup>21-24</sup> Chlorogenic acid was the predominant mono-CQA compound, accounting for the highest percentage among the mono-CQAs (0.01% to 2.73%). Cynarine was the predominant di-CQA compound (0.01% to 2.61%), and cynaroside was the predominant flavonoid (0.009% to 1.32%). Figure 3 compares the amounts of mono-CQAs in the preparations. Chlorogenic acid (5-CQA) was the most abundant, followed by 4-CQA or 3-CQA, and 1-CQA. Notably, 1-CQA was not detected in preparations F.P8 and V.P12 and was found only in minor amounts compared to the other mono-CQAs. According to the European Pharmacopoeia 10.0, the minimum content of chlorogenic acid in artichoke leaf dry extract should be 0.6%<sup>29</sup>. The results showed that half of the foreign preparations (F.P1, 5, 6, 7) met this criterion, while only three-eighths of the domestic preparations met the minimum requirement. Four preparations (F.P1, F.P5, V.P14, V.P15) and three research preparations (R.P17-19) had remarkably high yields of chlorogenic acid.

Figure 4 illustrates the di-CQA content in the preparations. Cynarine (1,3-caffeoylquinic acid) was generally the major compound among the total di-CQAs. Cynarine is known to be an artifact resulting from the isomerization of 1,5-di-CQA during aqueous extraction<sup>21, 30,31</sup>, and it was found in preparations with significant concentrations (34.2% to 72.8%) of total di-CQAs<sup>22</sup>. The present analysis of 19 preparations from artichoke leaf dry extract showed similar results. Cynarine was popularly mentioned on the labels of foreign artichoke preparations, with amounts ranging from 0.01% to 0.92%. Domestic preparations contained cynarine amounts between 0.01% and 0.29%, generally lower than those of foreign preparations. However, all three research products (R.P17-19) exhibited abundant cynarine content ranging from 1.66% to 2.61%. Other derivatives of di-CQAs, including 1,5-diCQA, 4,5-diCQA, 3,4-diCQA, and 3,5-diCQA, were also determined within the di-CQA group. These findings indicate the varying content of polyphenols, including mono-CQAs, di-CQAs (with a focus on cynarine), and flavonoids, in different artichoke preparations. The results provide insights into the composition and potential pharmacological activities of these preparations. In addition to hydroxycinnamic acids, the flavonoid contents of artichoke preparations, specifically cynaroside (luteolin 7-O-glucoside) and scolymoside (luteolin 7-O-rutinoside) were present in amounts ranging from 0.009% to 1.32%, while scolymoside ranged from 0.017% to 1.10% (Figure 4).

**Table 4:** Inter-day and intra-day precision of the method

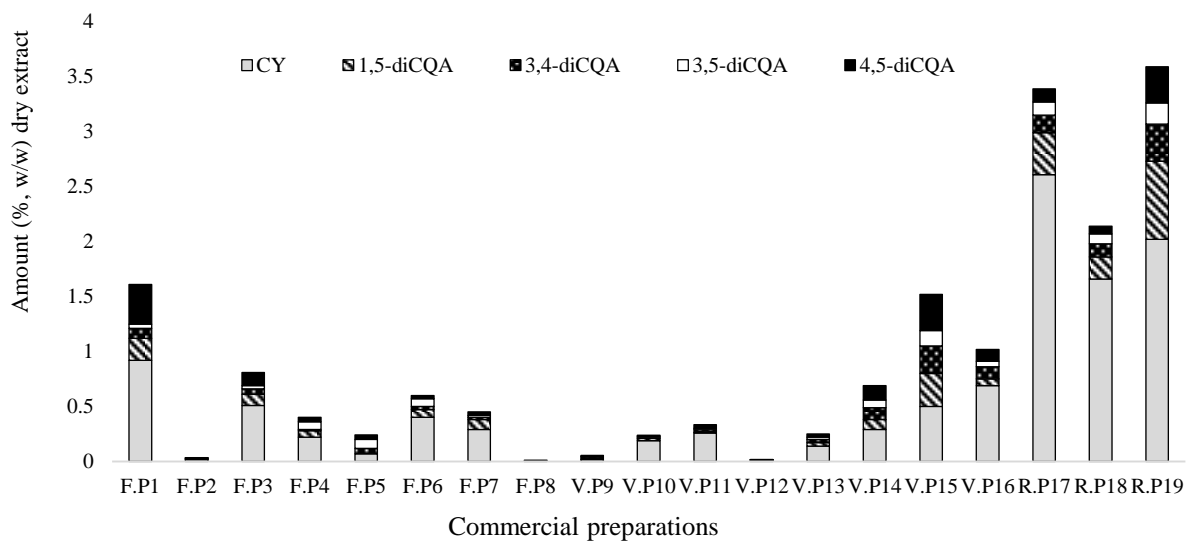
No.	Compounds	$\bar{X} \pm SD$ (n = 6) (mg/g)	Amount (%)	Precision (%RSD)	
				Intra-day (n=6)	Inter-day (n=12)
1	1-CQA	8.902 ± 0.02	0.089	0.70	0.91
2	3-CQA	18.261 ± 0.41	1.826 <sup>a</sup>	0.78	1.20
3	CF	12.803 ± 0.03	0.128	0.84	2.01
4	AC	23.972 ± 0.58	2.397	0.96	1.24
5	4-CQA	19.384 ± 0.03	1.938 <sup>a</sup>	0.62	1.05
6	CY	10.57 ± 0.20	1.057	0.76	1.27
7	CR	4.253 ± 0.13	0.425	1.16	2.38
8	SCO	8.374 ± 0.22	0.837	1.09	1.59
9	3,4-diCQA	6.930 ± 1.03	0.693 <sup>b</sup>	0.62	1.60
10	3,5-diCQA	3.331 ± 0.05	0.333 <sup>b</sup>	0.71	1.22
11	1,5-diCQA	1.423 ± 0.03	0.142	0.90	1.24
12	4,5-diCQA	6.890 ± 0.15	0.689	0.83	1.36

\* Amounts (%) of phenolic compounds were shown as means (n = 3). The amounts were calculated following the regression equation of chlorogenic acid <sup>a</sup> and cynarine <sup>b</sup>

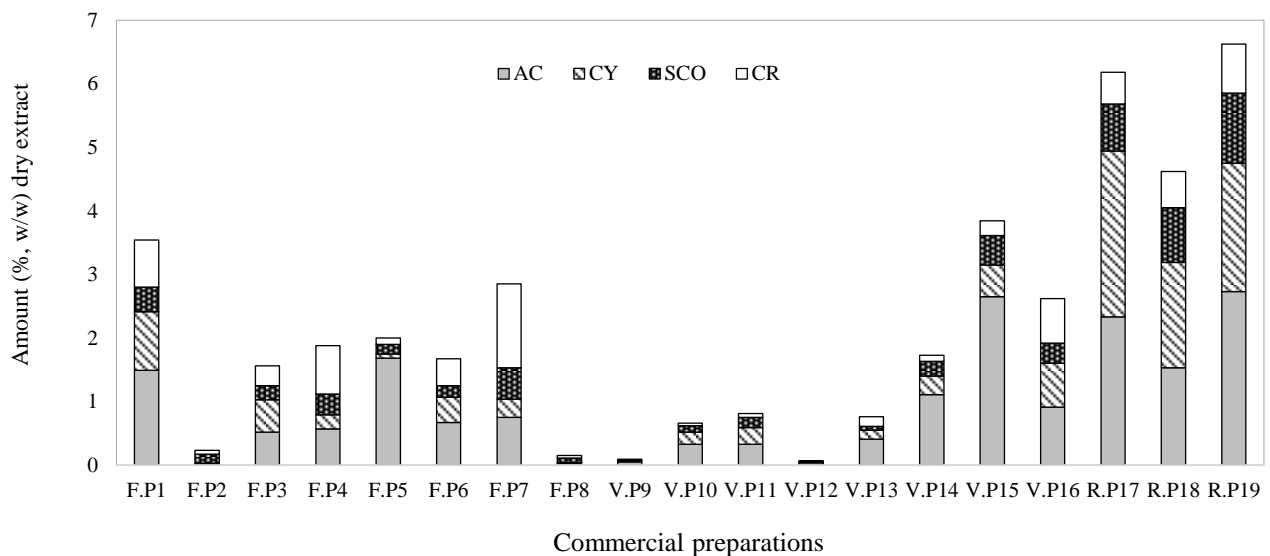
**Table 5:** Accuracy of the method

Amount spiked (%)	level	% Recovery (n = 9)							
		1-CQA	CF	AC	CY	CR	SCO	1,5-diCQA	4,5-diCQA
80 %		90.02	103.07	94.75	95.61	91.40	102.81	94.88	91.74
(RSD %)		(0.51)	(0.63)	(0.59)	(1.21)	(2.78)	(0.51)	(1.17)	(1.87)
100 %		95.22	106.34	91.94	96.12	94.40	103.57	97.90	95.26
(RSD %)		(0.67)	(0.46)	(0.88)	(1.10)	(1.83)	(0.87)	(0.68)	(1.33)
120 %		100.41	106.11	93.49	95.66	95.09	105.07	101.07	92.59
(RSD %)		(2.53)	(0.91)	(1.28)	(0.39)	(1.75)	(1.70)	(0.60)	(0.73)

CQA (caffeoylquinic acid); CF (caffeic acid); AC (chlorogenic acid); CY (cynarine); CR (cynaroside); SCO (scolymoside)

**Content of di-CQA****Figure 3:** Amounts of di-CQAs of commercial artichoke supplements

di-CQA: Dicafeoylquinic acid; F.P(1-9): Foreign products; V.P(9-16): Vietnamese products; R.P(17-19): Researched products

**Figure 4:** Amounts of major phenolic compounds of commercial artichoke supplements

AC: Chlorogenic acid; CY: Cynarine; SCO: Scolymoside; CR: Cynaroside; F.P(1-9): Foreign products; V.P(9-16): Vietnamese products; R.P(17-19): Researched products

From Figure 4, it can be observed that scolymoside content was generally higher than cynaroside in domestic preparations (V.P9-12 and V.P14-15). Conversely, cynaroside was usually found in greater amounts compared to scolymoside in foreign preparations (N.P1, N.P3-4, N.P6-7, and R.P17-19). This dissimilarity in flavonoid amounts among the preparations can be attributed to the variation in extraction solvents used. Cynaroside content was found to be more abundant in ethanol-water extracts, while scolymoside content tended to be predominant in artichoke dry extracts extracted with water. This difference can be attributed to the structural variation between the two compounds, with scolymoside possessing an additional glucosyl moiety. These findings highlight the variability in the content of flavonoids, specifically cynaroside and scolymoside, in different artichoke preparations, which can be attributed to the choice of extraction solvents and their impact on the extraction efficiency of specific compounds.

## Conclusion

In this study, the newly developed UPLC method has demonstrated its efficacy in concurrently quantifying a comprehensive range of 12 polyphenols. This includes 4 monocaffeoylquinic acids, 5 dicaffeoylquinic acids, 2 flavonoids, and caffeic acid, across diverse artichoke leaf dry extract preparations. The method has effectively met all essential criteria for system suitability, linear range, intra-day precision, inter-day precision, and accuracy, underscoring its reliability and robustness. The applicability of this UPLC method extends to quality control endeavors, making it a valuable tool for assessing the polyphenolic composition of commercial artichoke preparations. Furthermore, the study findings accentuated significant disparities in phenolic content among various commercial artichoke supplements, a variance attributed to distinct manufacturing procedures. This underscores the importance of standardized or quantified extracts within the production of artichoke preparations. By integrating standardized extracts, manufacturers can guarantee consistent and dependable levels of active constituents. This, in turn, facilitates enhanced quality control and ensures the reliable therapeutic effects of artichoke supplements.

## Conflict of Interest

The authors declare no conflict of interest.

## Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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