

EXTRACTION, FRACTIONATION, AND SEPERATION OF CYNAROSIDE AND SCOLYMOSIDE FROM *Cynara scolymus* L. GROWN IN IRAQ

Mohammed M. Farhan¹
Instructor

Dhia S. Hassawi^{2*}
Professor

Nabeel K. Ibraheem³
Professor

^{1,2} Biology Dept., Coll. of Sci., University of Anbar, Iraq

³Dept. of Plant Biotech., Coll. of Biotech., Al-Nahrain University, Iraq

*dhassawi@yahoo.com

ABSTRACT

Artichoke (*Cynara scolymus* L.) is a nutritious vegetable that grown all over the world. It is a promising herbal plant, rich in bioactive components. It is considered as medicinal plant due to its nutritional and phytochemical composition, especially high proportion of phenolic compounds. The primary aim of this study was to achieve chemical profile analyses of artichoke for different phytochemicals, especially Scolymoside and Cynaroside. Methanolic crude was extracted from Artichoke leaves by rotary evaporator and separated by column chromatography. The fractions monitored by Thin Layer Chromatography (TLC), and identified in High-Pressure Liquid Chromatography (HPLC). Two important compounds, Scolymoside and Cynaroside were separated and identified. In overall, Artichoke could be regarded as a rich source of biologically active compounds and considered as bio-functional with putative antioxidant effects.

Key words: *Cynara scolymus*, Artichoke, Flavonoids, Phytochemicals

فرحان وآخرون

مجلة العلوم الزراعية العراقية - 2023: 54(2): 421-430

إستخلاص وتجزئة وفصل مادتي سيناروسيد وسكوليموسيد من نبات الخرشوف المنزرع في العراق

نبيل خلف ابراهيم²

ضياء سعدالله حساوي¹

محمد مطلب فرحان¹

أستاذ

أستاذ

مدرس

¹ قسم علوم الحياة، كلية العلوم، جامعة الأنبار، مدينة الرمادي، العراق

² قسم التقنيات الحيوية الزراعية، كلية التقنيات الإحيائية، جامعة النهرين، مدينة بغداد، العراق

المستخلص

الخرشوف (*Cynara scolymus* L.) نبات غذائي يزرع في جميع أنحاء العالم، وهو نبات عشبي واعد غني بالمكونات النشطة بيولوجيًا. يعتبر من النباتات الطبية بسبب تركيبته الغذائية والكيميائية، وخاصة إحتوائه على نسبة عالية من المركبات الفينولية. كان الهدف الأساسي من هذه الدراسة هو إجراء بعض التحليلات الكيميائية للخرشوف لمختلف المواد النباتية وخاصة Scolymoside و Cynaroside. تم استخلاص المواد الميثانولية الخام من أوراق الخرشوف بواسطة المبخر الدوار وفصلها باستخدام العمود الكروماتوجرافي. تمت متابعة هذه المواد بواسطة تقنية كروماتوغرافيا الطبقة الرقيقة (TLC) و Thin Layer Chromatography، وتم تحديدها باستخدام جهاز الكروماتوغرافيا السائلة عالية الضغط (HPLC) - High-Pressure Liquid Chromatography. لقد تم فصل وتحديد مركبين مهمين هما Scolymoside و Cynaroside. وبشكل عام، يمكن اعتبار نبات الخرشوف مصدرًا غنيًا للمركبات النشطة بيولوجيًا، كما يمكن اعتباره عاملاً حيويًا لإحتوائه على مضادات الأكسدة.

الكلمات المفتاحية: *Cynara scolymus*، نبات الخرشوف، الفلافونويد، الفاييتوكيميكال

INTRODUCTION

Artichoke (*Cynara scolymus* L.) is a nutrition and medicinal plant; the curative possibility of which was known to the Greeks, Romans, and ancient (15). This medicinal herb has been appropriately used in conventional medicine for a long and helps cure various ailments (22). Its leaf extracts are used as traditional herbal products for pharmaceutical preparations (14). Artichokes contain phenolics compounds such as Cynarin, Chlorogenic, and caffeic acid (6); also, its different plant parts are considered potential sources of valuable phytoconstituents, mainly polysaccharides, and polyphenols (24). Flavonoids include Cynaroside (luteolin-7-O-glucoside) and Scolymoside (luteolin-7-O-rutinoside) (8). Flavonoids are secondary metabolites of plants with polyphenolic structure. The flavonoid groups of poly phenolic compounds have low toxicity in mammals and are widely dispense in plant kingdom (17). Main dietary sources of flavonoids in the form of flavones, flavonols, flavonones, and isoflavones are found in tea, apple, cherry, tomato, onion, thyme, parsley, grape fruit, lemon, orange, neem, and ginkgo (4). Flavonoids have gained attention because of their broad biological and pharmacological activities. In these order, flavonoids have been reported to exert multiple biological possession including cytotoxicity, antimicrobial, anti-inflammatory as well as antitumor activities. The best-described property of almost every group of flavonoids is their capacity to act as powerful antioxidants which can protect the human body from free radicals and reactive oxygen species (21). Scolymoside is a minor flavonoid found in the leaves and leaf extracts of artichoke (9), while Cynaroside is considered as essential artichoke compounds. Cynaroside is a flavone compound that can be found in artichoke (19). It is an abundant flavonoid produced in plants that has several health benefits, including neuroprotective properties (10). Cynaroside also possesses potential antibacterial, antifungal and antioxidant (11). The aim of this study was characterize the flavonoids and to separate and identify Scolymoside and Cynaroside from artichoke leaves by using

High-Pressure Liquid Chromatography (HPLC).

MATERIALS AND METHODS

Extraction and fractionation of flavonoids

Leaves powder (500 g) was free from fat (defatted) with hexane for 24 hours and then left to dry at room temperature. Defatted leaves were extracted with 3L of 80% methanol (CH_3OH) in soxhlet appliance until complete consumption. The methanolic extract was evaporated under reduced pressure at a temperature not overriding 40°C by using a rotary evaporator apparatus to deign a dark greenish-yellow sediments designated as a crude. The crude was acidified with 5% hydrochloric acid to pH 2 and divide with an egalitarian volume of ethyl acetate to get an aqueous acidic layer (AAL) and ethyl acetate layer (EAL). The AAL was then separated and basified with an equal volume of 5% NaOH to pH 10 and extracted with chloroform CHCl_3 in the separatory funnel to get a CHCl_3 layer and aqueous basic layer. The CHCl_3 layer evaporated under pressure at 40°C to produce dark residue was specified as fraction1 (F-1), while the aqueous basic layer was specified as fraction2 (F-2). The EAL of the methanolic crude extract was evaporated under pressure at 40°C and basified with 300ml of 5% NaOH to pH 10; then separated with CHCl_3 in the separatory funnel to get aqueous basic layer (ABL) and EAL. The ABL was separated, evaporated to dryness, and acidified with 5% HCl to pH 2; then extracted with ethyl acetate to get fraction designated as flavinoid fraction3 (F-3). Flavonoids extract was subjected to silica gel column chromatography (100 g, 4X60 cm), and eluted firstly with 100% hexane (C_6H_{14}), followed by increasing amounts of methylene chloride (CH_2Cl_2) in methanol (C_6H_{14}) as follows, C_6H_{14} : (CH_2Cl_2): (25:75), (50:50), (75:25), and (100%) CH_2Cl_2 , then increase polarity with methanol CH_3OH in CH_2Cl_2 with an increment of 10% up-to CH_3OH (100%). One hundred fractions (10 ml each) were collected and observed on TLC silica gel plates, the platessilica gel F₂₅₄ (Merck, Germany) were developed using solvents 1 to 5., and the spots were detected under UV light.

Solvent systems used for fractions

S_{1f}: Toluene C₆H₅CH₃: chloroform CHCl₃: acetone CH₃COCH₃ (40:25:35) (1).

S_{2f}: Ethylacetate CH₃COOC₂H₅: methanol CH₃OH: formic acid CH₂O₂ (50:50:1) (23).

S_{3f}: n-Butanol C₄H₉NO₃: glacial acetic acid CH₃COOH: water (40:10:50) (23).

S_{4f}: Hexane C₆H₁₄: Ethylacetate CH₃COOC₂H₅ (90:10) (7).

S_{5f}: Ethylacetate CH₃COOC₂H₅: glacial acetic acid CH₃COOH: formic acid CH₂O₂: water (30:0.8:1.2:8) (7).

The fraction was taken (1mg dissolved in 1ml solvent) and utilized with TLC plates, using pipette in form of spots and allowed to dry and then developed by ascending technique. After imbibition and ascension of the solvent system, the plates of Thin Layer Chromatography (TLC) were exposed to UV light at 254 and 366 nm.

Fractionation and identification of flavonoids

The polyphenolic compounds of artichoke extracts were fractionated and identified for flavonoids by HPLC, according to the method described by (2). The eluted peaks were monitored by UV-Vis 10A-SPD spectrophotometer. The main compounds were separated on FLC (Fast Liquid Chromatographic) column under the optimum conditions. Column: phenomenex C-18, 3µm using a hypersil C18 reversed- phase column with 5µm particle size (100 X 4.6 mm I.D) column. The mobile phase was linear gradient of solvent A (0.1% trifluoroacetic acid) in deionized water and solvent B (100% of acetonitrile). The program was started with elution with 95% A (0-1min); a linear gradient was used until 10 min, lowering A to 20%; from 17 min to 24 min, and A decreased to 0%. The flow rate was 1.3 ml/ min, and the runs were integrated at 280 nm for each phenolic derivative. Scanning was performed from 200 to 600 nm. Phenolic compounds were identified by comparing retention times and UV-VIS spectra with those of pure

standards and the range of calibration curves. The repeatability of the quantitative analysis was ±4%. The results were expressed as microgram/ml of each compound per total phenolic compounds. The phenolic compounds of extract calculated according to the following equation:====Concentration of sample (µg/ml) = $\frac{\text{area of sample}}{\text{area of standard}}$ X conc. of standard X dilution

RESULTS AND DISCUSSION**Fractionation and Separation of Flavonoids**

The flavonoid extract was produced from the extraction and fractionation process of the active compound ethyl acetate to get fraction3 (flavonoid). It was subjected to column chromatography on column No.1 Silica gel (100g, 60 X 3cm), using a C₆H₁₄ and CH₂Cl₂ increase polarity with CH₃OH. Sub-fractions (1-10) were collected and monitored by TLC silica gel plates using different solvent systems as mobile phases. Result of TLC Sub-fractions (8, 9, and 10) showed different spots pattern after the chromatoplates were air-dried and visualized under daylight and UV lights at 366 nm (Figure 1). Sub-fraction 8 was separated in C₆H₁₄-CH₂Cl₂ (20-80); the S1f solvent C₆H₅CH₃-CHCl₃-acetone (40-25-35) detected five spots. While Sub-fraction 9 was separated in C₆H₁₄- CH₂Cl₂ (10-90) and produced seven spots with S1f solvent. At the same time, Sub-fraction 10 was separated in C₆H₁₄- CH₂Cl₂ (0-100), and revealed six spots by S1f solvent. Retardation factor (R_f) of sub-fraction 8 ranged between (0.21-0.70), Sub-fraction 9 ranged between (0.21-0.78), as well sub-fraction 10 ranged between (0.15-0.74) (Table 1). Sub-fractions (1-7) did not give clear patterns through identification by TLC. Therefore, the powders of sub-fractions 8, 9, and 10 with 1.75, 1.63, and 1.30 g, respectively were used to complete the separation phases by column chromatography No.2 Silica gel and Sephadex G50 (30g, 40 X 1cm) and column chromatography No.3 Sephadex G50 (10g, 20 X 0.5cm).

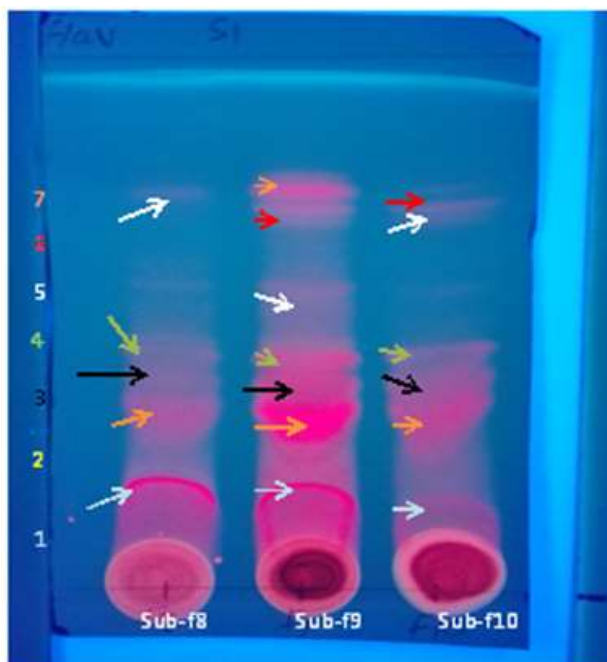


Figure 1. TLC of flavonoid extract subtractions (8, 9, 10), column No. 1 using S1f $C_6H_5CH_3$ - $CHCl_3$ – CH_3COCH_3 (40-25-35), UV light (366nm).

Table 1. TLC sub-fractions (8, 9, 10), R_f values, UV light (366nm).

Fractions	Spot No.	R_f values	Color UV light at 366nm
Sub-fraction 8 C_6H_{14} - CH_2Cl_2 (20-80)	1	0.21	Red
	2	0.35	Pink
	3	0.41	light pink
	4	0.47	light pink
	5	0.70	light pink
Sub-fraction 9 C_6H_{14} - CH_2Cl_2 (10-90)	1	0.21	Red
	2	0.31	Red
	3	0.38	Pink
	4	0.45	Pink
	5	0.58	light pink
	6	0.74	light pink
Sub-fraction 10 C_6H_{14} - CH_2Cl_2 (0-100)	7	0.78	Pink
	1	0.15	Pink
	2	0.31	Pink
	3	0.38	Pink
	4	0.45	light pink
	5	0.70	light pink
	6	0.74	light pink

Isolation of Fractions

Sub-fraction 8, column No.1, (1.75g), (C_6H_{14} : CH_2Cl_2 - CH_3OH) (20:80)

Sub-fraction 8 was subjected to column No.2 (phase-1) packed by C_6H_{14} only. Elution was started with C_6H_{14} and polarity was gradually increased with CH_2Cl_2 by CH_3OH . Ten ml of each fraction was collected. The collected fractions (100 fractions) were then concentrated by rotary evaporator, and the

powder was detected by TLC using solvent system. Fractions (10-20) gave 84mg powder that treated with solvent (C_6H_{14} : CH_2Cl_2 - CH_3OH) (90:10) and showed three spots by TLC using mobile phase S3_f ($C_9H_{19}NO_3$: CH_3COOH : water) (40:10:50). They visualized under UV lights at 366 nm (Figure 2); R_f of spots was 0.64, 0.84, and 0.90 (Table 2).

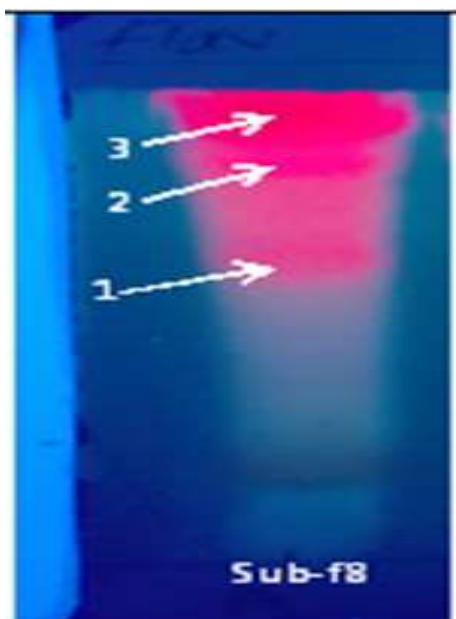


Figure 2. TLC of flavonoid extract sub-fraction 8, column No. 2, using S3f ($C_9H_{19}NO_3$: CH_3COOH : water) (40:10:50), UV light (366nm).

Table 2. TLC for sub-fraction No. 8, R_f values, color UV light (366 nm).

Spot No.	R_f values	Color UV light 366nm
1	0.64	Light pink
2	0.84	Pink
3	0.90	Pink

Sub-fraction 10, column No.1, (1.30g), (C_6H_{14} : CH_2Cl_2 - CH_3OH) (0:100)

Sub-fraction 10 was subjected to column No.2 (phase-1) packed by C_6H_{14} only. Elution was started with C_6H_{14} and polarity was gradually increased with CH_2Cl_2 by CH_3OH . Ten ml of each fraction was collected. The collected fractions (100 fractions) were then concentrated using rotary evaporator, and the powder was detected by TLC using solvent

system. Fractions (70-80) gave 83mg powder that treated with solvent (C_6H_{14} : CH_2Cl_2 - CH_3OH) (0:100) and showed four spots by TLC using mobile phase $S1fC_6H_5CH_3-CHCl_3-CH_3COCH_3$ (40-25-35), they visualized under UV lights at 366 nm (Figure 3); R_f spots range was 0.22- 0.58 (Table 3).

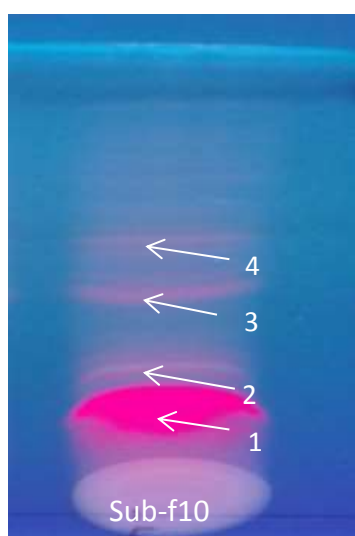


Figure 3. TLC of flavonoid extract sub-fraction 10, column No.2 (phase-1), using $S1fC_6H_5CH_3-CHCl_3-CH_3COCH_3$ (40-25-35), UV light (366nm).

Table 3. TLC for sub-fraction No. 10, R_f values, color UV light (366 nm).

Spot No.	R _f values	Color UV light 366nm
1	0.22	Pink
2	0.30	Light pink
3	0.47	Light pink
4	0.58	Light pink

Separation of Flavonoids**Flavonoid sub-fractions 8, column No.2 (C₆H₁₄: CH₂Cl₂- CH₃OH) (90:10)**

Sub-fractions 8, column No.2 was submitted through column chromatography No.3 Sephadex G50 (10g, 20 X 0.5cm) filled with 100% C₆H₁₄. Elution was started with C₆H₁₄ and polarity was gradually increased with CH₂Cl₂ by CH₃OH. Ten ml of each fraction was collected; the collected fractions (100

fractions) were then concentrated using rotary evaporator, and detected by TLC using solvent system. Fractions (80-90) produced 35mg powder; when treated with solvent (C₆H₁₄: CH₂Cl₂- CH₃OH) (20:80) gave one spot with TLC using S2f (CH₃COOC₂H₅: CH₃OH: CH₂O₂) (50:50:1). It visualized under UV lights at 366nm (Figure 4); R_f spot was 0.91 (Table 4).

**Figure 4. TLC of flavonoid fraction 8, column No.3 (phase-2), using S2f (CH₃COOC₂H₅: CH₃OH: CH₂O₂) (50:50:1), UV light (366nm).****Table 4. TLC for fraction No. 8, R_f values, color UV light (366 nm).**

Spot No.	R _f values	Color UV light 366nm
1	0.90	Pink

Flavonoid sub-fractions 10, column No.2 (phase-1) (C₆H₁₄: CH₂Cl₂- CH₃OH) (0:100)

Sub-fractions 10, column No.2 was submitted through column No.3 filled with 100% C₆H₁₄. Elution was started with C₆H₁₄ and polarity was gradually increased with CH₂Cl₂ by CH₃OH. Ten ml of each fraction was collected; the collected fractions (100

fractions) were then concentrated using rotary evaporator, and detected by TLC using solvent system. Fractions (100-110) produced 19mg powder; when treated with solvent (C₆H₁₄: CH₂Cl₂- CH₃OH) (10:90) it gave one spot with TLC using S3f (C₉H₁₉NO₃: CH₃COOH: water) (40:10:50). It visualized under UV lights at 366 nm (Figure 5); R_f spot was 0.71 (Table 5).

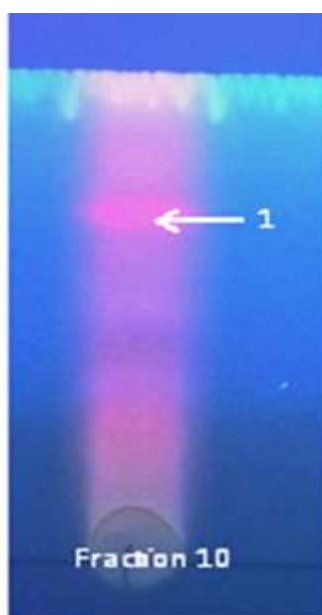


Figure 5. TLC of flavonoid fractions 10, column No.3 (phase-2), using S3f ($C_9H_{19}NO_3$: CH_3COOH : water) (40:10:50), UV light (366nm).

Table 5. TLC for fraction No. 10, *R_f* values, color UV light (366 nm).

Spot No.	<i>R_f</i> values	Color UV light 366nm
1	0.71	Pink

Identification of the flavonoid fractions by HPLC

The fractions from flavonoids extract of Artichoke leaves were identified by HPLC. The flavonoid compounds include glycosides luteolin-7- β -rutinoside

(Scolymoside), luteolin-7- β -D-glucoside, luteolin-4- β -D-glucoside, and luteolin 7-glucoside (Cynaroside). The results of the retention time and area (μ volt) are shown in (Table 6); the flavonoids compounds standard presented in (Figure 6).

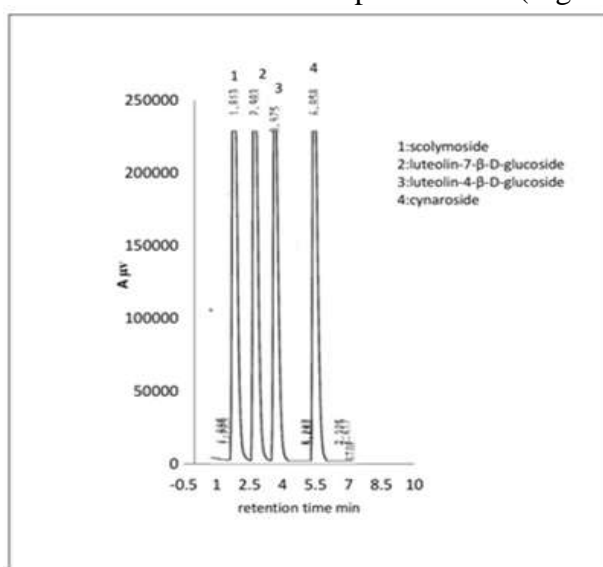


Figure 6. Flavonoid compounds, retention time (min), and area (μ volt) for the standard analysis by HPLC

Table 6. Flavonoid compounds, retention time (min), area (μ volt) for the standard

Flavonoids compounds	Standard	
	Retention time(min)	Area (μ volt)
glycosides luteolin-7- β -rutinoside (SC)	1.813	247519
luteolin-7- β -D-glucoside	2.903	232260
luteolin-4- β -D-glucoside	3.975	200789
Luteolin 7- glucoside (CN)	6.058	219838

Fraction 8 showed one peak for the retention time (1.808) and this was identical to the standard of Scolymoside (Table 7) and (Figure 7); the concentration for the fraction was separated by HPLC (Table 8). Fraction 10 showed one peak (6.062) and was identical to

the standard of Cynaroside (Table 9) and (Figure 8); the concentration for the fraction was separated by HPLC (Table 10). Sub-fraction 9 showed two unclear mixed spots that are difficult to be identified by HPLC; for this reason, this fraction was ignored.

Table 7. Comparison of retention times of fraction No. 8 with standard flavonoid after separation in HPLC

Peak No.	Retention time (min)	identical standard	Retention time (min)
1	0.068	Unknown	
2	1.338	Unknown	
3	1.808	glycosidesluteolin-7-β-rutinoside (SC)	1.813
4	3.827	Unknown	
5	3.258	Unknown	
6	3.457	Unknown	
7	8.437	Unknown	

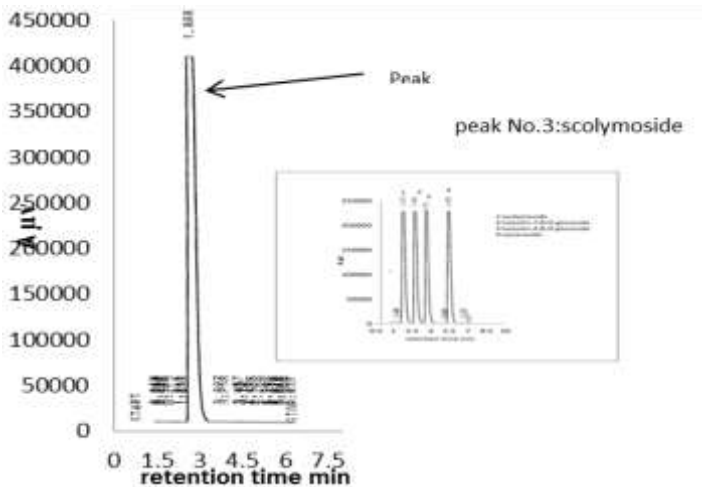


Figure 7. Glycosidesluteolin-7-β-rutinoside (Scolymoside), retention time (min), area (μvolt) for the fraction 8 separation in HPLC

Table 8. Glycosidesluteolin-7-β-rutinoside (Scolymoside), retention time (min), area (μvolt) and concentration for fraction No.8 after separation in HPLC

Fraction	Retention time(min)	Area (μvolt)	Concentration %
8	1.808	439175	100
Total	-	439175	100

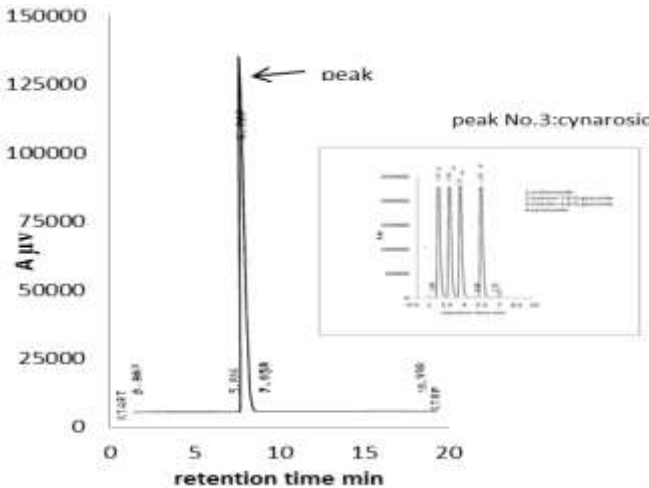


Figure 8. Luteolin 7- glucoside (Cynaroside), retention time (min), area (μvolt) for the fraction 10 separation in HPLC

Table 9. Comparison of retention times of fraction No. 10 with standard flavonoid after separation in HPLC

Peak No.	Retention time (min)	identical standard	Retention time (min)
1	0.382	Unknown	-
2	5.816	Unknown	-
3	6.062	Luteolin 7- glucoside (CN)	6.058
4	7.058	Unknown	-
5	18.998	Unknown	-

Table 10. Luteolin 7- glucoside (Cynaroside), retention time (min), area (μvolt) and concentration for fraction No. 10 after separation in HPLC

Fraction	Retention time(min)	Area (μvolt)	Concentration %
10	6.062	134907	100
Total		134907	100

Artichoke has been well known as a traditional medicine because of its phenolic compounds (18). The pharmaceutical properties of this plant are linked to their special chemical composition, which includes high levels of polyphenols. (The application of biotechnology tools such as in vitro culture techniques could be used for the controlled production of artichoke secondary metabolites (5). The results of this study revealed that Artichoke leaves possess important flavonoid compounds that may improve its quality as a functional food; this in agreement with (3). The process of separation and purification using the column chromatography method, which used the flavonoid extract of Artichoke proved efficient. The Scolymoside and Cynaroside were separated and identified by HPLC. This result is similar to (20) and (13) who mentioned that Artichoke is containing flavonoid compounds, including luteolin, luteolin-7-O glucoside, luteolin-7-rutinoside, and Cynaroside. (9) used liquid chromatographic isolation techniques (Sephadex LH-20) in combination with analytical methods (HPLC-DAD-MS and HPLC-DAD) to separate, characterize, and quantify minor Artichoke compounds. The most important flavonoid compounds were Scolymoside, Cynaroside, and cynaropicrin. Flavonoid compounds separated from Artichoke included glycosides luteolin-7- β -rutinoside (Scolymoside), and luteolin 7-glucoside (Cynaroside) their weight was 84 and 83 mg, respectively. The method of separation by column chromatography was efficient as indicated by the produced amount of Scolymoside and Cynaroside. The Scolymoside and Cynaroside were separated after the extraction of flavonoids from Artichoke. It is difficult to separate and purify

these materials directly from the extract by column chromatography because of the abundance of other secondary metabolic materials. All chromatography separation methods are mainly aimed at separating two or more of the material. The separation process depends on the reality that one of the materials in the stationary phase (Silica gel) is attached compared to other materials that tend to move with the mobile phase (solvent). Extended studies on the chemical components of the Artichoke detected that flavinod substances are considered to be as the active ingredients of the plant and included 0.1–1.0 % flavone glycosides (e.g. luteolin-7- β -rutinoside (Scolymoside), luteolin-7 β -glucoside, and luteolin-4 β -glucoside (16). Five compounds have been recognized in Artichoke as Cynaroside, apigenin-7-O-glucoside, Scolymoside, sesquiterpens viz. grosheimin, and solstitialin. Their structures were elucidated by spectral methods (7). Moreover, Ultrasound-Assisted Extraction technique proved to be more appropriate and effective for the extraction of antiradical and phenolic compounds (12). In conclusion, Scolymoside and Cynaroside can be isolated from Artichoke; they are futuristically favorable biologically active compounds. They are separated from flavonoid by column chromatography because it did not separate directly from methanolic crude of Artichoke.

REFERENCES

1. Abdul-Jalil, T. Z., K. Saour, and A. M. Nasser, 2010. Phytochemical study of some flavonoids present in the fruits of two Ammi L. species wildy grown in Iraq. Iraqi Journal of Pharmaceutical Sciences, 19(1): 48-57
2. Al-Farsi, M. A. and C.Y. Lee, , 2008. Optimization of phenolics and dietary fibre

- extraction from date seeds. Food Chemistry, 108(3): 977-985
3. Ali, M. A., *et al.*, 2021. Phenolic constituents, antioxidant, and antimicrobial activities of globe artichoke (*Cynara scolymus* L.) aqueous extracts. Trop J Nat Prod Res, 5(11):1986-1994
 4. Beecher, G. R., 2003. Overview of dietary flavonoids: nomenclature, occurrence and intake. The Journal of nutrition, 133(10): 3248S-3254S
 5. Bekheet, S. and V. Sotal, 2019. Biodiversity and medicinal uses of globe artichoke (*Cynara scolymus* L.) Plant. J. biodivers. conserv. bioresour. manag., 5(1): 39-54
 6. Christaki, E., E. Bonos, and P. Florou-Paneri, 2012. Nutritional and functional properties of cynara crops (globe artichoke and cardoon) and their potential application: a review. International Journal of Applied Science and Technology, 2(2) 64-70
 7. Elsayed, S., *et al.*, 2012. Chemical and biological constituents from the leaf extracts of the wild artichoke (*Cynara cornigera*). Int J Pharm Pharm Sci, 5: 396-400
 8. Fratianni, F., *et al.*, 2007. Polyphenolic composition in different parts of some cultivars of globe artichoke (*Cynara cardunculus* L. var. *scolymus* (L.) Fiori). Food chemistry, 104(3): 1282-1286
 9. Fritsche, J., *et al.*, 2002. Isolation, characterization and determination of minor artichoke (*Cynara scolymus* L.) leaf extract compounds. European food research and technology, 215(2): 149-157
 10. Iakovleva, I., *et al.*, 2015. The flavonoid luteolin, but not luteolin-7-O-glucoside, prevents a transthyretin mediated toxic response. PloS one, 10(5): 1-15
 11. Kishore, K., *et al.*, 2007. Phytochemical antimicrobial studies of methy angolensate and luteolin-7-O-glucoside isolated from callus cultures of *Soymida febrifuga*. Int. J. Biomed. Sci, 3: 269-278
 12. Kollia, E., *et al.*, 2017. Antioxidant activity of *Cynara scolymus* L. and *Cynara cardunculus* L. extracts obtained by different extraction techniques. Nat Prod Res., 31(10):1163-1167
 13. López-Lázaro, M., 2009. Distribution and biological activities of the flavonoid luteolin. Mini Reviews in Medicinal Chemistry, 9(1): 31-59
 14. Luca, S. V., *et al.*, 2022. Phytochemical and multi-biological characterization of two *Cynara scolymus* L. varieties: A glance into their potential large scale cultivation and valorization as bio-functional ingredients. Industrial Crops and Products, 178, April, 114623
 15. Magied, M. M. A., *et al.*, 2016. Artichoke (*Cynara scolymus* L.) leaves and heads extracts as hypoglycemic and hypocholesterolemic in rats. Journal of Food and Nutrition Research, 4(1): 60-68
 16. Malekshah, R., *et al.*, 2012. Effect of zeolite nano-materials and artichoke (*Cynara scolymus* L.) leaf extract on increase in urinary clearance of systematically absorbed nicotine. Arzneimittelforschung, 62(12): 650-654
 17. Manach, C., *et al.*, 2004. Polyphenols: food sources and bioavailability. The American journal of clinical nutrition, 79(5): 727-747
 18. Nguyen, N. Q., *et al.*, 2022. Extraction of cynarin and chlorogenic acid from Artichoke leaves (*Cynara scolymus* L.) and evaluation of antioxidant activity, antibacterial activity of extract. Vietnam J. Chem., 60: 571-577
 19. Nüßlein, B. and W. Kreis, 2005. Purification and characterization of a cynaroside 7-O- β -D-glucosidase from *Cynarae scolymi* folium. Acta Hort., 681: 413-420
 20. Pérez-García, F., T. Adzet, and S. Cañigueral, 2000. Activity of artichoke leaf extract on reactive oxygen species in human leukocytes. Free Radical Research, 33(5): 661-665
 21. Saxena, M., J. Saxena, and A. Pradhan, 2012. Flavonoids and phenolic acids as antioxidants in plants and human health. Int. J. Pharm. Sci. Rev. Res, 16(2): 130-134
 22. Sharma, P., *et al.*, 2021. The phytochemical ingredients and therapeutic potential of *Cynara scolymus* L. Pharmaceutical and Biomedical Research, 7(3): 141-160
 23. Wagner, H. and S. Bladt, 1996. Plant drug analysis: A thin layer chromatography atlas. 2nd Edition, Springer-Verlag, Berlin
 24. Zayed, A., A. Serag, and M. A. Farag, 2020. *Cynara cardunculus* L.: Outgoing and potential trends of phytochemical, industrial, nutritive and medicinal merits. Journal of Functional Foods, Volume 69, June, 103937.