

0002 - Artichoke for Phenolic Acids by HPLC

Botanical Name: *Cynara scolymus L.*

Common Names: Cynara, globe artichoke

Parts of Plant Used: Dried leaves

Uses: As a choleric

Modes of Action:

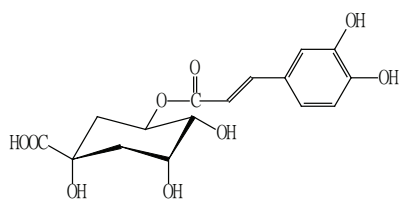
Long known as an herbal medicine, the dried leaves of artichoke have been used for their choleric properties since remote times.¹ In various pharmacological test systems, artichoke leaf extracts have shown antibacterial, antioxidative, anti-HIV, bile-expelling, and hepatoprotective properties; urinate activity; and inhibition of cholesterol biosynthesis and LDL oxidation.²⁻¹⁰ The mono- and dicaffeoylquinic acid derivatives and flavonoids are believed to be bioactive compounds in artichoke, although there are no supporting data.



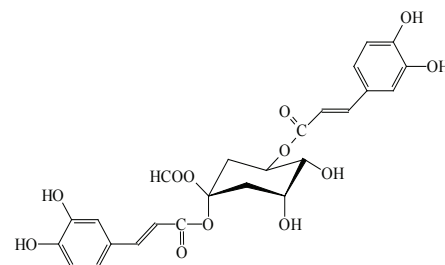
Chemistry and Chemical Markers for Quality Control:

Artichoke leaves have been studied extensively and found to be a rich source of polyphenolic compounds, with mono- and dicaffeoylquinic acids and flavonoids the major chemical components.¹¹ The main caffeoylquinic acid derivatives in artichoke leaves are chlorogenic acid and cynarin (1,5-dicaffeoylquinic acid). Other compounds identified in artichoke include sesquiterpenoid lactones and flavonoids (e.g., luteolin-7-O-glycoside, luteolin-7-O-rutinoside). The caffeoylquinic derivatives are used as marker compounds for quality control of artichoke leaf extracts.

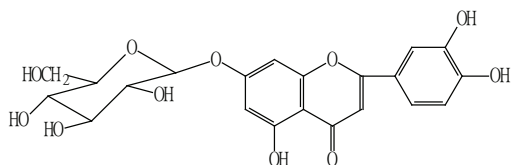
Major Phenolic Compounds Identified in Artichoke Leaves:



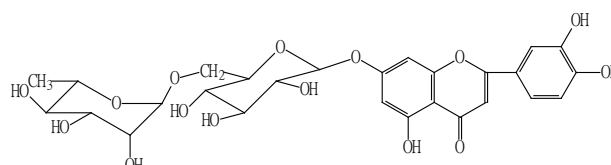
Chlorogenic acid



Cynarin (1,5-dicaffeoylquinic acid)



Cynaroside (luteolin-7-O-glucoside)



Luteolin-7-O-rutinoside

Methods of Analysis:

Various methods have been used to analyze the phenolic compounds in artichoke, including colorimetry, reversed-phase HPLC (RP-HPLC), micellar electrokinetic capillary chromatography, and TLC.¹²⁻¹⁸ HPLC is the most accepted analytical method for caffeoylquinic acid derivatives.

The extraction solvent is key in the analysis of caffeoylquinic acid derivatives. Various solvents have been tried for extracting compounds from artichoke leaves, with 60% methanol found to be best.¹¹

Method 1:

The method of Bilia et al.¹⁹ can be used to analyze chlorogenic acid, cynarin, isochlorogenic acid, luteolin-7-O-glucoside(cynaroside), and luteolin-7-O-rutinoside.

Sample Preparation:

Prepare samples in 60% and 40% methanol.

Chromatography:

Column: LiChrosorb RP-18, 5- μ m, 250mm x 4 mm with LiChrosorb RP-18 guard column, 5 μ m, 10 x 4 mm.

Mobile phase: Solvent A = water (adjusted to pH 3 with phosphoric acid), solvent B = acetonitrile.

Gradient:

Time (minutes)	%A	%B
0	88	12
10	82	18
15	82	18
30	55	45
35	0	100
42	0	100
50	88	12

Flow rate: 1.3 mL/minute
 Injection volume: 25 μ L
 Detection wavelength: 350 nm
 Column temperature: 26°C

Validation Data:

Linearity: 0.10 to 2.5 mcg with a correlation coefficient greater than 0.99.

Accuracy: Not specified

Precision: Each of the seven compounds had an RSD that was less than 3.7%.

Selectivity: Peak identification was determined against standards.

Ruggedness: Not specified

Robustness: Not specified

LOD/LOQ: Not specified

Method 2:

The method of Wang et al.¹¹ was used to analyze cynarin, 1-caffeoylquinic acid, chlorogenic acid, luteolin-7-O-glucoside, and luteolin-7-O-rutinoside in artichoke leaves and to analyze two additional compounds, narirutin(naringenin-7-O-rutinoside) and apigenin-7-O-rutinoside (isorhoifolin), in artichoke heads (flowers).

Sample Preparation:

Extract 500 mg of dried leaves with 70 mL of 60% methanol by sonicating for 25 minutes. Cool the sample to room temperature and dilute to 100 mL with 60% methanol.

Chromatography:

Column: Phenomenex Prodigy ODS(3), 5 μ m, 150mm x 3.2mm .

Mobile phase: Solvent A = water (with 0.2% phosphoric acid), solvent B = acetonitrile.

Gradient: 6%B linear to 30%B in 20 minutes, hold at 30%B for an additional 5 minutes.

Flow rate: 1.2 mL/minute

Injection volume: 10 μ L

Detection wavelength: 330 nm

Validation Data:

Linearity: 1.08 to 216 mcg/mL for chlorogenic acid, 1.1 to 220 mcg/mL for 1-caffeoylquinic acid, 0.92 to 184 mcg/mL for luteolin-7-O-rutinoside, 1.06 to 212 mcg/mL for luteolin-7-O-glycoside, and 1.24 to 248 mcg/mL for cynarin; the correlation coefficient for each compound was 1.

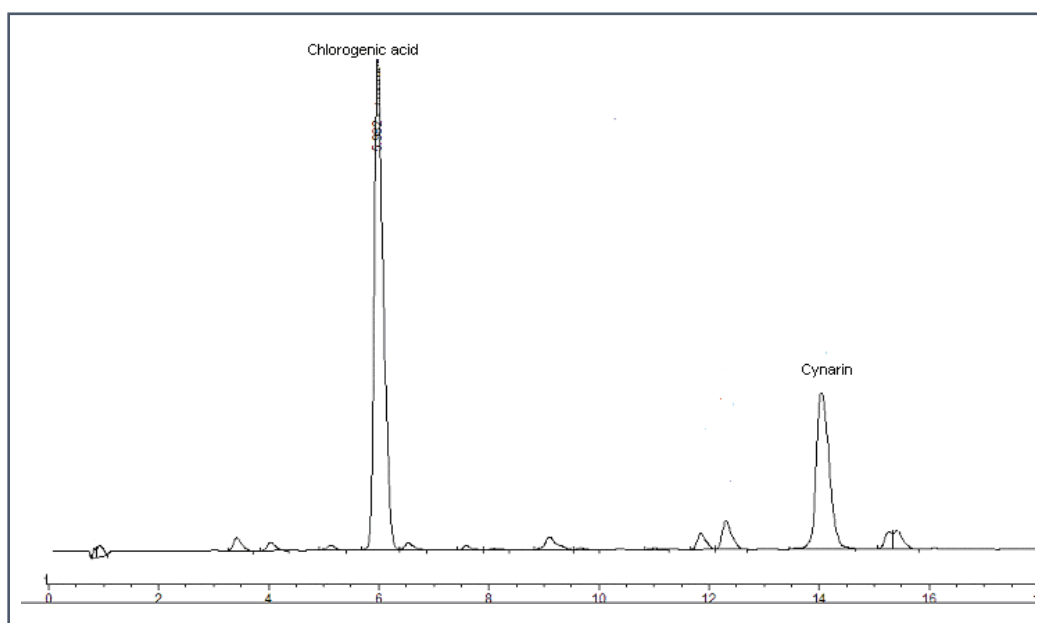
Accuracy: The percent recoveries were from 97.6 to 100.7 for these five compounds. Precision: Each compound had an RSD that was less than 2.33%.

Selectivity: Peak identification was determined against standards.

Ruggedness: Not specified

Robustness: Not specified

LOD/LOQ: Not specified



Representative HPLC chromatogram run by method 2.

References:

1. Bruneton J. Pharmacognosy, phytochemistry, medicinal plants. Secaucus, NY: Lavoisier Publishing; 1995:218–9.
2. Alamanni MC, Cossu M. Antioxidant activity of the extracts of the edible part of artichoke (*Cynara scolymus* L.) var spinoso sardo. Ital J Food Sci. 2003;15:187–95.
3. Dranik LI, Dolganenko LG, Slapke J, et al. Chemical composition and medical usage of *Cynara scolymus* L. Rastit Resur. 1996;32:98–104.
4. Martino V, Caffini N, Phillipson JD, et al. Identification and characterization of antimicrobial components in leaf extracts of globe artichoke (*Cynara scolymus* L.). Acta Horticulturae. 1999;501:111–4.
5. McDougall B, King PJ, Wu BW, et al. Dicafeoylquinic acid and dicafeoyltartaric acid are selective inhibitors of human immunodeficiency virus type 1 integrase. Antimicrob Agents Chemother. 1998;42:140–6.
6. Kraft K. Artichoke leaf extract—recent findings reflecting effects on lipid metabolism, liver and gastrointestinal tracts. Phytomedicine. 1997;4:369–78.
7. Brown JE, Rice-Evans CA. Luteolin-rich artichoke extract protects low density lipoprotein from oxidation in vitro. Free Radic Res. 1998;29:247–55.
8. Gebhardt R. Prevention of tauroolithocholate-induced hepatic bile canalicular distortions by HPLC-characterized extracts of artichoke (*Cynara scolymus*) leaves. Planta Med. 2002;68:776–9.
9. Gebhardt R. Antioxidative and protective properties of extracts from leaves of artichoke (*Cynara scolymus* L.) against hydroperoxide-induced oxidative stress in cultured rat hepatocytes. Toxicol Appl Pharmacol. 1997;144:279–86.
10. Perez-Garcia F, Adzet T, Canigual S. Activity of artichoke leaf extract on reactive oxygen species in human leukocytes. Free Radic Res. 2000;33:661–5.
11. Wang M, Simon JE, Aviles IF, et al. Analysis of antioxidative phenolic compounds in artichoke (*Cynara scolymus* L.). J Agric Food Chem. 2003;51:601–8.
12. Sevcikova P, Glatz Z, Slanina J. Analysis of artichoke (*Cynara cardunculus* L.) extract by means of micellar electrokinetic capillary chromatography. Electrophoresis. 2002;23:249–52.
13. Krawczyk A. Standardization of raw material and extract of artichoke (*Cynara scolymus* L.). Herba Pol. 2001;47:130–6.
14. Hammouda FM, Self El-Nasr MM, Ismail SI, et al. Quantitative determination of the active constituents in Egyptian cultivated *Cynara scolymus*. Int J Pharmacogn. 1993;31:299–304.
15. Debenedetti SL, Palacios PS, Wilson EG, et al. HPLC analysis of caffeoylquinic acids content in Argentine medicinal plants. Acta Horticulturae. 1993;333:191–9.
16. Slanina J, Taborska E, Musil P. Determination of cynarine in the decoctions of the artichoke (*Cynara cardunculus* L.) by the HPLC method. Cesko-Slov Farm. 1993;42:265–8.
17. Ben-Hod G, Basnizki Y, Zohary D, et al. Cynarin and chlorogenic acid content in germinating seeds of globe artichoke (*Cynara scolymus* L.). J Genet Breed. 1992;46:63–9.
18. Adzet T, Puigmacia M. High-performance liquid chromatography of caffeoylquinic acid derivatives of *Cynara scolymus* L. leaves. J Chromatogr. 1985;348:447–52.
19. Bilia AR, Bergonzi MC, Mazzi G, et al. Analysis and stability of the constituents of artichoke and St. John's wort tinctures by HPLC-DAD and HPLC-MS. Drug Dev Ind Pharm. 2002;28:609–19.