Eur Food Res Technol (2002) 215:149–157
DOI 10.1007/s00217-002-0507-0

ORIGINAL PAPER

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Isolation, characterization and determination of minor artichoke (Cynara scolymus L.) leaf extract compounds

Received: 12 November 2001 / Revised: 28 January 2002 / Published online: 3 May 2002

Abstract Artichoke (*Cynara scolymus L.*) and artichoke leaf extracts (ALE) have a long history as a traditional part of the Mediterranean diet as well as in folk medicine for the treatment of dyspeptic disorders. Although several biological mechanisms of action have been suggested, e.g. increased biliary secretion leading to an increased cholesterol elimination and/or inhibition of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase activity resulting in a decreased cholesterol biosynthesis, convincing and conclusive human studies investigating the blood cholesterol lowering properties of artichoke or ALE are currently limited. The aim of the present study was to isolate, characterize and determine minor artichoke compounds with regard to their blood cholesterol lowering potential, bitter taste sensation and antioxidant potential.

Liquid chromatographic isolation techniques (Sephadex LH-20) in combination with analytical methods (HPLC-DAD-MS and HPLC-DAD) were successfully employed to separate, characterize, and quantify minor artichoke compounds. In commercially available ALE the following compounds were identified: 8-deoxy-11-hydroxy-13-chlorogrosheimin, cryptochlorogenic acid, chlorogenic acid, neochlorogenic acid, cynarin, cynaratriol (tentatively), grosheimin, 8-deoxy-11,13-dihydroxygrosheimin, luteolin-7-O-rutinoside, luteolin-7-O-glucoside, and cynaropicrin. Most of these compounds were liquid chromatographically described for the first time. The concentration of essential ALE compounds, namely chlorogenic acid, cynarin, and luteolin-7-O-glucoside, was in the range of 0.35–18.34, n.d.-1.02, 0.04 – 10.65 mg/g ALE, respectively. Cynaropicrin, the predominant bitter ALE principle, was present at concentrations between <0.06 and 22.6 mg/g ALE. Rancimat assay revealed an equivocal picture with respect to the antioxidant (AOX) properties of ALE dissolved in sunflower oil: four extracts showed a effect, while three ALE had an antioxidative effect and one extract showed no effect at all at concentration between 500 and 4000 mg/kg (compared to refined sunflower oil). However, all investigated individual artichoke compounds – chlorogenic acid, cynarin, luteolin, luteolin-7-O-glucoside – showed a remarkable antioxidative effect, chlorogenic acid being the strongest AOX compound. Moreover, chlorogenic acid showed a strong, dose-dependent linear HMG-CoA reductase inhibitory effect at concentrations between 5 and 50 mg/ml. Luteolin and luteolin-7-O-glucoside showed an even stronger inhibitory effect at 10 mg/ml (relative HMG-CoA reductase activity: 2.4% and 6.5%, respectively compared to 26.5% activity after chlorogenic acid treatment). All studied commercially available ALE showed a moderate inhibitory effect at 10 mg/ml.

Keywords Artichoke · HMG-CoA reductase inhibition · Antioxidant · HPLC-MS · Liquid chromatography

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Introduction

Artichokes, in particular artichoke (*Cynara scolymus L.*) bloom heads, have a well-recognized history of consumption as food, especially as part of the Mediterranean diet. In addition, artichoke leaf extracts (ALE) have been documented since ancient times as a traditional folk medicine mainly attributed to its choleretic, diuretic and hypocholesterolemic activities. Artichokes are cultivated worldwide on an area of about 125.000 ha resulting in an annual production of 1.42 million ton (t). Spain (25.000 ha), Italy (50.000 ha), and France (13.000 ha) contributed to approximately 75% of the entire cultivation area [1].

Chlorogenic acid, cynarin (1,5-di-caffeoyl quinic acid), luteolin, and luteolin glycosides (e.g. scolymoside and cyanoroside) are considered as essential artichoke com-

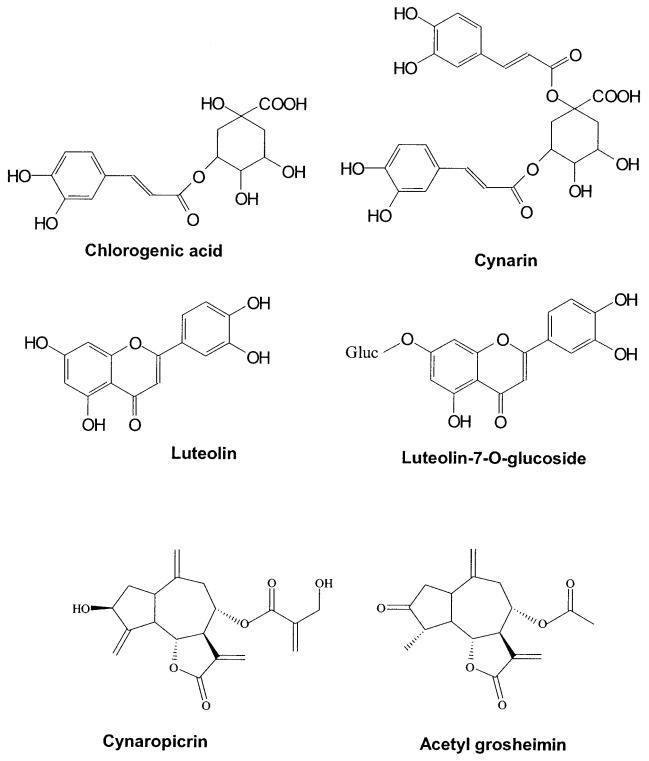

Fig. 1 The chemical structures of artichoke compounds. Acetyl grosheimin was used for quantification of cynaropicrin, the major bitter principle of artichoke leaf extracts

pounds (Fig. 1), while sesquiterpene lactones, such as cynaropicrin, are the predominant bitter principles of ALE. Nowadays, ALE are still widely used for therapeutic purposes and are produced by highly standardized procedures [2]. However, controlled, well-designed clinical trials that prove an unequivocal hypocholesterolemic action are currently limited.

Petrowicz et al. [3] found no significant effect on serum cholesterol levels in a randomized, placebo-controlled, double-blind study on 44 healthy volunteers after a daily 640 mg administration of an ALE (Hepar-SL forte) over a 12-week period. However, subgroup analyses revealed a cholesterol lowering effect of ALE in subjects with baseline total cholesterol levels higher than 210 mg/dl. Englisch et al. [4] reported a 18.5% decrease of total cholesterol after daily consumption of 450 mg dry artichoke extract (CY450) compared to a 8.6% decrease of total cholesterol of placebo group. LDL-cholesterol decrease in the treatment group was 22.9% and 7.2% in the placebo group.

Moreover, ALE have been long used against liver complaints due to the hepatoprotective effects of these extracts [5,6].The mechanism of action of the hepaprotective effect was investigated by Gebhardt [7,8]who investigated the formation of malondialdehyde (MDA) in cultured rat hepatocytes after ALE treatment. Gebhardt [8] showed that ALE did not affect basal MDA production, but prevented the hydroperoxide-induced increase of MDA formation in a dose-dependent manner.

Table 1 Commercially available artichoke extracts investigated in present study. Artichoke leaf extracts (ALE), artichoke heads (AH)

Country of distributor	Sample description
Netherlands	Capsules, ALE 1
Netherlands	Capsules, ALE 2
Germany	Capsules, ALE 3
USA	Capsules, ALE 4
USA	Powdered extract, ALE 5
France	Powdered extract, ALE 6
Italy	Powdered extract, ALE 7
France	Liquid extract, ALE[a] 8
Germany	Juice, AH

[a] contained also artichoke heads and roots

Gebhardt and Fausel [9] concluded that ALE have a marked antioxidative potential which may be attributed to some extent to polyphenols and flavonoids present in ALE.

3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase is the key enzyme in the biosynthesis of cholesterol [10] which catalyses the conversion of HMG-CoA to mevalonic acid in the presence of NADPH [11]. HMG-CoA reductase inhibition leads ultimately to a decrease of the plasma cholesterol concentration in-vivo and is therefore considered to be an important mechanism of action for the evaluation of cholesterol lowering food ingredients since increased plasma cholesterol level is a well-known risk factor for coronary vascular diseases [12].

Therefore, the scope of the present study was threefold: (1) to isolate, characterize and determine biologically active compounds present in commercially available ALE, (2) to explore the antioxidative potential of individual ALE compounds and total extracts and (3) to investigate the capability of selected artichoke compounds as well as total ALE on the HMG-CoA reductase inhibition.

Material and methods

Artichoke samples

Commercially available artichoke products (dietary supplements, juices, and powders) were obtained from local drug stores and super markets. The investigated artichoke products are shown in Table 1.

Chemicals

Chlorogenic acid [3-O-caffeoyl quinic acid; >99% purity] was obtained from Acros Organics (Geel, Belgium), cynarin [1,5-dicaffeoyl quinic acid; 90% purity] was purchased from Apin Chemicals Limited (Abingdon, UK), acetylgrosheimin [>90% purity, determined by HPLC] was obtained from Chemdiv (San Diego, USA), luteoline [3',4',5,7-tetrahydroxyflavone; >90% purity] and luteoline-7-O-glucoside (>90% purity) were obtained from Extrasynhese (Genay, France). Rosmarinic acid [97%] was purchased from Sigma-Aldrich (Zwijndrecht, The Netherlands). Isopropanol, acetonitril, methanol, formic acid, acetic acid, dichloromethane, acetone for high performance liquid chromatography (HPLC) and high performance thin layer chromatography (HPTLC) analysis

were all analytical grade quality and were obtained from Across (Geel, Belgium). For HMG-CoA reductase inhibition assay the following reagents were used: acetronitril (Merck, Darmstadt, Germany), phosphoric acid (Merck), pipes buffer (1,4 piperazine-bis-ethanesulfonic acid) (Sigma-Aldrich), ENP buffer (50 mM PIPES, 10 mM EDTA, 100 mM NaCl adjusted to pH 7 with NaOH), dimethyl sulfoxide (Sigma-Aldrich), EDTA (Boehringer Mannheim, Germany), sodium chloride (Merck), mevastatin (Sigma-Aldrich), dl-Dithiothreitol (Sigma), NADPH (Boehringer Mannheim), [^{14}C] HMG-CoA reductase (370 kBq/500 µl) (0.345 mM) (Amersham, UK), HMG-CoA reductase microsome suspension (TNO, Leiden, The Netherlands), 6 N HCl (Merck), [^{3}H]-mevalonic acid ammonium salt (9.25 MBq/500 µl) (DuPont, USA), cartridges Sep-Pak plus aminopropyl (NH2) Waters (Milford, USA), emulsifier Scintillator Plus Packard (Packard Bioscience, Groningen, The Netherlands).

Sample preparation

Artichoke capsules were carefully opened and approximately 1.0 g of artichoke powder was dissolved in 10 ml of ethanol/water (20/80 v/v) and stirred at ambient temperature for 30 min.

Isolation of minor artichoke compounds

Sephadex LH 20 (Pharmacia, Sweden) was swollen overnight in ethanol/water (20:80 v/v) containing 1.0 vol. % acetic acid (equilibration solution). The swollen gel material was carefully transferred into an XK 26/70 column (Pharmacia). The column was equilibrated at a flow rate of 2.0 ml/min (P-500 pump controlled with a liquid chromatography controller LCC-500 both of Pharmacia) until a constant bed height was achieved. The column was then connected to a variable wavelength detector (VWM 2141, Pharmacia) and a fraction collector (Frac 100, Pharmacia). The absorption was on-line measured at 254 and 320 nm.

For isolation of individual artichoke compounds, 1.0 g of artichoke extract was dissolved in 10 ml equilibration solution and stirred. The extracts were filtered before liquid chromatography (LC) analysis (0.22-µm millipore filter). The Sephadex LH 20 column was run with equilibration solution for 5 h at a constant flow of 2.0 ml/min. After 5 h, the eluent was switched to 99-vol.% of ethanol and 1.0 vol. % acetic acid and the elution was continued for another 3 h. The individual fraction volume was 10 ml. The collected fractions were screened with UV detection and pooled in four different fractions. The pooled fractions were evaporated with a rotary evaporator until constant weight and redissolved in 5.0 ml ethanol (20 vol. %). Each fraction was analysed by means of HPLC and HPTLC for the presence of cynaropicrin. In addition, the organoleptic sensation of the pooled fractions was assessed by a taste panel.

HPTLC screening of bitter artichoke compounds

HPTLC was performed according to Wiedenfeld [13] using Kiesgel 60 F_{254} plates (Merck, Germany) in order to screen for bitter principles obtained by Sephadex LH 20 fractionation as described earlier. The mobile phase consisted of dichloromethane and acetone (75:25 v/v). About 2 µl of each sample was applied onto the HPTLC plates. Visualization of the spots was performed using anisaldehyde spray reagent (Sigma-Aldrich, Zwijndrecht, NL). At the position of sesquiterpene lactones light violet spots were visible. The detection limit of acetyl grosheimin was estimated to be approximately 0.05 mg/g.

HPLC analysis of minor artichoke compounds

Qualitative HPLC analysis

Analytical separations were carried out on a 250×4.6 mm stainless steel LiChrospher C_{18} reversed phase column (Merck, Darmstadt, Germany) with a particle size of 5 µm and an average pore diameter of 100 Å. The analytical chromatographic separations were performed on an HP 1100 HPLC system equipped with a quaternary pump G1311A and a UV/DAD detector G 1315A and controlled by a ChemStation software system (Agilent Technologies, Waldbronn, Germany). Column temperature was set to 25°C. The injection volume was 20 µl. Chromatograms were recorded at 254 nm (cynaropicrin) and 320 nm (flavonoids). The artichoke leaf extracts were chromatographed using a binary gradient consisting of solvent A: isopropanol/acetonitrile/methanol/0.3 vol. % aqueous formic acid 18:30:12:40 (v/v) and solvent B: 0.3% aqueous formic acid. A linear gradient from 8% (A) (0 min) to 48% (A) (35 min) at a flow rate of 1 ml/min was applied.

Isomerization of chlorogenic acid

In order to obtain reference compounds for the identification of chlorogenic acid isomers, namely neochlorogenic acid (3-O-caffeoylquinic acid) and cryptochlorogenic acid (4-O-caffeoylquinic acid), pure chlorogenic acid (5-O-caffeoylquinic acid) was dissolved in saturated aqueous $NaHCO_3$ solution and heated at 60°C for 3 h. The obtained solution contained neochlorogenic acid (29.6%), chlorogenic acid (31.6%), and cryptochlorogenic acid (33.3%) as determined by HPLC analysis.

HPLC-ESI-MS coupling

The HPLC-MS experiments were performed on a Hewlett Packard LC-MSD instrument system equipped with a quaternary pump G1311A, UV/DAD detector G1315A, and a mass spectrometer G1946A with an ESI interface (Agilent Technologies, Waldbronn, Germany). Mass spectra were acquired in positive ion mode in a mass range of m/z 100–600 amu at a fragmentor voltage of 70 V. Nitrogen was used as drying gas at a flow rate of 10 l/min and 300° C. Nebulizer pressure was set to 60 psi. The capillary voltage was optimized to 3500 V. For all spectra manual baseline subtraction was performed.

Quantitative HPLC analysis

Quantification of individual artichoke compounds was performed on a Shimadzu HPLC-DAD system (Shimadzu, Den Bosch, The Netherlands) which was equipped with: two LC-10AD pumps, SIL-10 AD autosampler, CTO-10 AC column oven, SPD-M10A diode-array detector, and a SCL-10A controller. CLASS-VP 5.0 software (Shimadzu) was employed for data analysis. Chromatographic conditions were similar to those described above for qualitative analysis. The UV spectra were recorded from 210 to 340 nm with 0.64 s/scan. The amounts of cynaropicrin in ALE were calculated by means of external standard calibration (acetyl grossheimin) recorded at 254 nm (operational linearity range for external standard quantification: 0.06–1.0 mg/g; calibration line $y = 317091x + 8259.2$; $r=0.999$; detection limit acetyl grosheimin (3 x δ noise level): 0.06 mg/g).

Quantification of chlorogenic acids, cynarin luteoline and luteoline-7-O-glucoside was performed with rosmarinic acid as internal standard as previously described by Brand [14]. Briefly, rosmarinic acid (0.10 mg/ml) was dissolved in 20% aqueous methanol (stock solution). Approximately 100 mg ALE (exactly weighed) was dissolved in 10 ml stock solution and mixed thoroughly. In case of artichoke juices, 2.0 g and 1.0 g of the juice was mixed with 8.0 ml and 9.0 ml internal standard stock solution, respectively and mixed thoroughly. The extracts were filtered (0.22-µm millipore filter) before HPLC analysis. All quantitative analyses were performed as duplicates. The response factor of chlorogenic acid, cynarin and luteolin-7-glucoside was determined as 0.98, 1.15 and 1.77, respectively. Based on the structure similarity of the chlorogenic acid isomers, the response factor determined for chlorogenic acid was also employed for the quantification of neo- and cryptochlorogenic acid. The detection limits for the determination of chlorogenic acid, cynarin, and luteolin-7-glucoside was about 30 µ g/g, respectively.

Antioxidant activity

A Metrohm 679 Rancimat instrument (Herisau, Switserland) was used to assess the antioxidant activity of individual artichoke compounds as well as of total ALE. Approximately 300 mg ALE powder was mixed with 3.0 g of fresh refined sunflower oil in a dedicated glass cylinder. The oxidation experiments were carried out at 100°C, and air was blown through the mixtures at a flow rate of 20 l/h. For the control experiment, refined sunflower oil (α-tocopherol: 772 mg/kg, β-tocopherol: 23 mg/kg, γ-tocopherol: 6 mg/kg, δ-tocopherol: 1 mg/kg; PO value: 1.5 mmol O_2/2 kg) was used under the same conditions as described above. All tests were performed in triplicates and results are provided as mean values (SD).

HMG-CoA reductase inhibition

The assessment of HMG-CoA reductase inhibition by ALE and individual artichoke compounds was performed according Alberts et al.[11]. HMG-CoA reductase was obtained from a microsomal fraction of rat liver (TNO Leiden, The Netherlands). Relative HMG-CoA reductase activity (compared to positive controls) was calculated as:

$$\text{Relative HMG} - \text{CoA reductase activity} = \frac{\text{HMG} - \text{CoA activity of sample}}{\text{HMG} - \text{CoA activity of positive control}} \times 100$$

Sample preparation

ALE were dissolved in ENP buffer containing 10% ethanol. The final concentration of the samples was 10 mg/ml. The artichoke juice was diluted twice. Individual ALE compounds such as chlorogenic acid, cynarin, luteolin, and luteolin-7-O-glucoside were dissolved in 10% ethanol-ENP buffer and dimethyl sulfoxide and tested at various concentrations: 1, 10, 25, 50 mg/ml, respectively. PH of all samples was adjusted between 6.5 and 7 by addition of diluted NaOH solution. For all samples duplicate analyses were performed.

Statistical Analysis

Significant differences (P <0.05) and highly significant differences (P <0.01) between the samples and the references were determined by using Student's *t*-tests. Analysis of variances was performed by F-tests.

Results and discussion

Isolation of artichoke compounds

Sephadex LH 20 chromatography was successfully employed to isolate minor artichoke compounds from commercially available artichoke preparations. Table 2 shows the elution sequence of selected minor artichoke compounds (ex ALE) as determined by HPTLC and HPLC analysis. The colored complex (HPTLC) was visible down to approximately 0.05 mg/g as determined for acetyl grosheimin. This result is comparable with findings of Schneider and Thiele [15] who used Dragendorffs reagent for TLC detection of cynaropicrin (0.025 mg/cm^2). Organoleptic evaluation of the collected fractions by a taste panel revealed fraction D as the most bitter fraction. HPTLC, HPLC and organoleptic analysis confirmed the presence of cynaropicrin, the predominant bitter principle of ALE, in fraction D.

Analytical characterization of minor compounds

Conventional analytical characterization techniques, e.g., ^{13}C-NMR, ^{1}H-NMR and GC-MS, have been applied to characterize bitter artichoke principles [16,17]. In this study, HPLC-ESI-MS coupling was performed for the identification of cynaropicrin and other constituents present in ALE.

Figure 2 shows the chromatographic separation of an ALE. The chromatographic run was monitored by absorbance detection at 254 nm (Fig. 2a). Figure 2b depicts the total ion chromatogram (TIC). Cynaropicrin showed an $[M+H]^+$-peak at m/z 347.2 corresponding to the molecular formula $C_{19}H_{22}O_6$. From a comparison of the selected ion chromatogram (SIC) of m/z 347 with the TIC and UV chromatogram, the peak at a retention time of 37.3 min can be assigned to cynaropicrin (Fig. 2c).

SIC was also successfully applied to identify additional minor artichoke compounds such as those listed in Table 3. Additionally, authentic standards and isomerized cholorgenic acid references have been injected to compare retention times and UV-spectra. Most of the mentioned compounds have been fully spectroscopically characterized by Bernhard et al. [16] and Barbetti et al.[17]. However, in the present study, the liquid chromatographic behavior of a comprehensive number of minor ALE compounds is presented in more detail for the first time. The MS-spectrum of cynaropicrin extracted from the peak maximum is shown in Fig. 3. In addition to the $[M+H]^+$-peak at m/z 347.2, the sodium adduct $[M+Na]^+$ m/z 369.2 and potassium adduct $[M+K]^+$ m/z 385.2 can be assigned. Dominant is the fragment ion $[M^*+H]^+$ m/z 245 due to the loss of

Table 2 Elution profile of minor artichoke compounds (detectable +; not detectable –) of the artichoke fractions by means of Sephadex LH 20 chromatography

	Fraction A	Fraction B	Fraction C	Fraction D
Chlorogenic acid	–	[a]	+	–
Cynarin	–	–	+	–
Luteolin	–	–	–	–
Luteolin-7-gluc	–	–	[a]	+
Acetyl grosheimin[b]	–	–	–	+
Cynaropicrin	–	–	–	+

[a] trace amounts detectable
[b] probably only in trace amounts present in ALE; used as model bitter compound for optimization of liquid chromatography (LC) fractionation

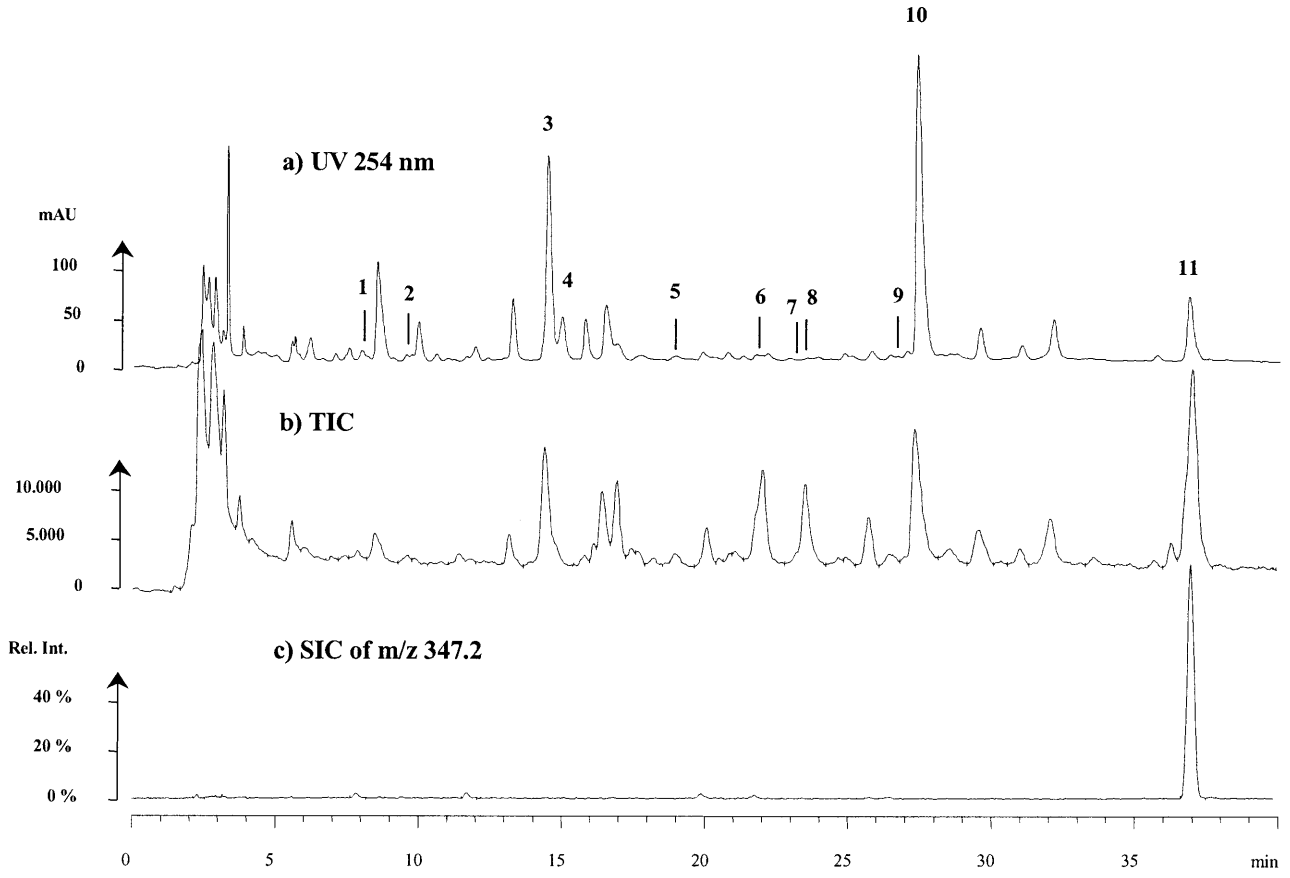

Fig. 2a–c HPLC-ESI-MS coupling of an artichoke extract using a Merck LiChrospher RP-C_{18} column (5 µm, 100 A). **a** UV chromatogram recorded at 254 nm. **b** Total Ion Chromatogram (TIC). **c** Selected Ion Chromatogram (SIC) of m/z 347: **1** 8-deoxy-11-hydroxy-13-chlorogrosheimin (?); **2** cryptochlorogenic acid; **3** chlorogenic acid; **4** neochlorogenic acid; **5** cynarin; **6** cynaratriol (?); **7** grosheimin; **8** 8-deoxy-11,13-dihydroxygrosheimin; **9** luteoline-7-O-rutinoside; **10** luteoline-7-O-glucoside; **11** cynaropicrin

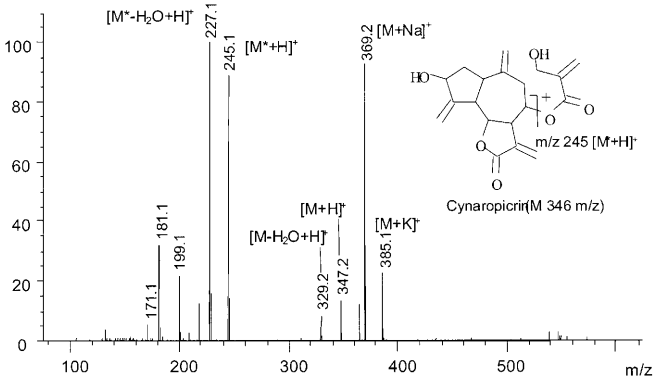

Fig. 3 ESI-MS spectrum of cynaropicrin extracted at peak maximum (see Fig. 2)

the ester unit $C_4O_3H_5$, as well as the fragment ion $[M^*\text{-}H_2O\text{+}H]^+$ m/z 227. Fragments with lower mass m/z 199.1 and m/z 181 can be explained as fragments of the lactone ring. Other fragment ions are only detectable in low abundance (below 10%).

Table 3 Minor artichoke compounds identified by HPLC-MS SIC mode

Compound	Retention time (min)	Monitored m/z
8-deoxy-11-hydroxy-13-chlorogrosheimin[a]	7.9	298.2
Cryptochlorogenic acid	9.2	354.3
Chlorogenic acid	14.4	354.1
Neochlorogenic acid	15.0	354.2
Cynarin	18.9	516.4
Cynaratriol[a]	21.9	282.2
Grosheimin	23.3	262.2
8-deoxy-11,13-dihydroxygrosheimin	23.7	280.1
Luteoline-7-*O*-rutinoside	26.3	594.3
Luteoline-7-*O*-glucoside	27.6	448.2
Cynaropicrin	37.0	346.2

[a] tentatively identified

Cynaropicrin content

The major bitter principles of artichoke belong to the group of sesquiterpene lactones (guaianolides). Dehydrocynaropicrin, grosheimin, cynaratriol, and cynaropicrin are known artichoke bitter compounds, while the latter is considered as the predominant bitter principle (Fig. 1) contributing approximately 80% of the total bitter artichoke taste [16,18]. Schneider and Thiele [15] reported that the bitter artichoke principles are only located in the green part of the plant (e.g. axis of the sprouts and leaf laminas), while no bitter substances were found in the roots, completely developed blossoms and fruits.

In the present study, four artichoke extracts, one artichoke juice and four artichoke capsules were analyzed by HPLC-DAD for their cynaropicrin contents. The content of other sesquiterpene lactones was below the detection limit of the employed HPLC-DAD method (0.06 mg/g). As can be seen in Table 4, the cynaropicrin content of the analyzed artichoke products showed a considerable variation (<0.06 – 22.6 mg/g) and may be explained by seasonal influences as well as variety differences of the individual ALE. Schneider and Thiele [19] investigated the seasonal variation of the bitter artichoke compounds and found that the content of bitter principles of the leaves passes two maxima during a vegetation period. The first maximum in early summer (beginning in July) is higher than the second from September till October. The maximum amount of cynaropicrin observed in the present study is comparable with values reported by Thiele and Schneider [14] who found an average of total bitter compound content of 2.55% (dry matter) in five ALE of the same cultivar (*Cynara scolymus var. inermis*). The total bitter content was calculated as cynaropicrin as determined by titration after lactone cleavage. Moreover, the drying conditions may also alter the bitter compound content of ALE as suggested by Schneider and Thiele [14] who found a 25% decrease of the bitter artichoke compounds after 24 h drying at 80°C.

Table 4 Mean cynaropicrin content (mg/g) of commercially available artichoke products determined by HPLC-DAD: n.d. not detectable (<0.06 mg/g), AH artichoke heads, ALE artichoke leaf extracts

Sample	Cynaropicrin content [mg/g]
Juice, AH	n.d.
Liquid extract, ALE[a]	0.4
Capsules, ALE 1	n.d.
Capsules, ALE 2	1.3
Capsules, ALE 3	n.d.
Capsules, ALE 4	0.3
Powdered extract, ALE 5	n.d.
Powdered extract, ALE 6	22.6
Powdered extract, ALE 7	n.d.

[a] contained also artichoke heads and roots

Minor compound composition

Wide variation in the content of *o*-diphenols in ALE depends mainly on the age of the artichoke leaves (vegetation cyclus) and the climatic conditions both before and during harvest [20]. As one may expect wide variations also exists between different artichoke cultivars [21]. The composition of commercially available artichoke products with regard to ortho-diphenolic compounds is presented in Table 5.

Processing of artichoke material, especially the applied drying temperature, the extraction solvent, and the solvent temperature, has also a significant impact on the content of the pharmacologically active compounds as well as on the composition of the artichoke extracts. Brand [14] investigated the loss of caffeoyl quinic acids (CQA) and flavonoids during the drying process. Drying at ambient temperature (25°C) decreased the CQA and total flavonoid content by 48% and 38%, respectively (CQA and flavonoid content before drying: 4.0% and 0.92% (dry matter), respectively). Higher drying temperatures, e.g., 60°C, lead to a 97% CQA loss, while the flavonoid content decreased by 84%. These data are in accordance with CQA losses reported by Nichiforesco and Coucou [22].

Cynarin is another pharmacologically active artichoke compound [6].Since studies of Panizzi et al. [23] it is known that aqueous extraction increase the formation of cynarin (1,5-di-O-CQA) by intraesterification of 1,3-di-O-CQA. Alcoholic extraction, however, prohibits the cynarin formation. Therefore, cynarin is not considered to be an essential genuine artichoke compound. High cynarin contents in artichoke products are therefore mainly due to processing. The influence of solvent temperature on the cynarin content in ALE was studied by Brand [14]. Whilst a 2-h aqueous extraction at 60°C did

Table 5 Amount of cryptochlorogenic acid, chlorogenic acid, neochlorogenic acid, cynarin, and luteolin-7-glucoside in commercially available artichoke products determined by HPLC-DAD: ALE artichoke leaf extract, AH artichoke heads

	Cryptochlorogenic acid [mg/g extract]		Chlorogenic acid [mg/g extract]		Neochlorogenic acid [mg/g extract]		Cynarin [mg/g extract]		Luteolin-7-gluc [mg/g extract]	
Artichoke product	*mean*	*SD*	*mean*	*SD*	*mean*	*SD*	*mean*	*SD*	*mean*	*SD*
Powdered extract, ALE 5	0.80	0.01	0.79	0.004	0.80	0.037	0.00	0.000	0.20	0.001
Powdered extract, ALE 6	0.24	0.00	10.48	0.035	0.00	0.000	0.17	0.024	10.65	0.043
Powdered extract, ALE 7	1.49	0.48	18.34	0.117	1.27	0.017	0.60	0.002	3.46	0.003
Juice, AH	0.35	0.00	0.30	0.003	0.25	0.002	1.02	0.010	0.29	0.003
Liquid extract, ALE[a]	0.03	0.00	0.35	0.001	0.75	0.003	0.28	0.000	0.04	0.001
Capsules, ALE 1	1.15	0.01	4.44	0.013	1.43	0.100	0.81	0.010	2.19	0.025
Capsules, ALE 2	0.11	0.00	7.12	0.883	0.00	0.000	0.31	0.059	0.43	0.083
Capsules, ALE 3	0.52	0.02	1.57	0.046	0.71	0.020	0.00	0.000	3.12	0.039
Capsules, ALE 4	2.43	0.02	3.95	0.016	2.32	0.018	0.00	0.000	1.85	0.009

[a] contained also artichoke heads and roots

Table 6 Antioxidant potential of individual artichoke compounds as determined by measurement of the induction time (Rancimat assay) in sunflower oil

Artichoke compound (8–33 mg/ml)	Induction time [h] at 100° C (mean n=3 (SD))
Refined sunflower oil (reference)	8.7 (0.1)
Chlorogenic acid	11.0 (0.3)[a]
Cynarin	10.0 (0.5)[b]
Luteolin-7-glucoside	10.2 (0.2)[a]
Luteolin	11.0 (0.6)[b]
Acetyl groshemin	9.5 (0.1)[b]
γ-tocopherol (1 mg/g)	12.1 (0.1)[a]

[a] $P < 0.01$
[b] $P < 0.05$

Table 7 Antioxidant potential of artichoke products as determined by measurement of the induction time (Rancimat assay) in sunflower oil

Artichoke extracts [300 mg/g oil]	Induction time [h] at 100°C (mean n=3 (SD))
Refined sunflower oil (reference)	8.6 (0.1)
Capsules, ALE 1	7.2 (0.3)
Capsules, ALE 2	11.0 (0.1)[a]
Capsules, ALE 3	13.3 (0.3)[a]
Capsules, ALE 4	7.8 (0.1)
Liquid extract, ALE*	7.1 (0.4)
Powdered extract, ALE 5	7.8 (0.1)
Powdered extract, ALE 6	9.3 (0.2)
Powdered extract, ALE 7	11.3 (1.1)
γ-tocopherol (1 mg/g)	12.1 (0.1)[b]

[a] $P < 0.05$
[b] $P < 0.01$
*contained also artichoke heads and roots

not lead to a significant formation of cynarin, extraction at 100°C for 2 h did significantly increase the formation of cynarin (no quantitative data provided).

Antioxidant activity

Over the past few years, a number of medicinal plants have been investigated for their quenching activity of specific reactive oxygen species (ROS), such as the hydroxy radical, the superoxide anion, singlet oxygen, and lipid peroxides [24,25]. The AOX potential was assessed by means of spectrophotometry [26], electron paramagnetic resonance [27], and chemiluminescence [28]. However, information on the total AOX activity (= capacity to scavenge all species of free radicals) of medicinal plants is still scare. Total AOX activity may be measured either by the oxygen radical absorbance capacity (ORAC) [29], by the Trolox equivalent antioxidant capacity (TEAC) method [30], or by the Rancimat method [31,32]. In the present study, the AOX activity of several pure ALE compounds at various concentrations (8, 17, 33 mg/ml, respectively) as well as total ALE (300 mg/ml) were studied by means of the Rancimat assay (Table 6). Luteolin and chlorogenic acid showed the strongest AOX effect, followed by luteolin-7-glucoside and cynarin. Acetly grosheimin showed the lowest AOX potential under the applied test conditions. Compared to γ-tocopherol, all tested ALE compounds had a lower AOX effect.

In contrast, chlorogenic acid, 3,5-dicaffeoyl quinic acid, caffeic acid and protocatechuic acid showed more radical scavenging activities on DPPH (1,1-diphenyl-2-picrylhydrazyl) at room temperature than dl-α-tocopherol or ascorbic acid at concentrations ranging from 1 to 50 µM [33]. Moreover, in another model system, chlorogenic acid, 3,5-dicaffeoyl quinic acid and caffeic acid inhibited the formation of conjugated dienes from linoleic acid, whilst 3,5-dicaffeoyl quinic acid showed the strongest inhibitory effect. However, under the investigated assay conditions, dl-α-tocopherol showed the strongest inhibitory activity at 10 µM level [33].

In Table 7 the induction time of sunflower oil enriched with various ALE is presented. Surprisingly, four out of eight total ALE showed a pro-oxidative effect under the tested conditions (shorter induction time than reference), whereas three extracts revealed a remarkable AOX effect at 300 mg/g. This incongruent picture can currently not be explained and needs further investigations.

Moreover, ALE retarded LDL oxidation in a dose dependent manner as measured by prolongation of the lag phase to conjugated diene formation, a decrease in the rate of propagation and a sparing of endogenous LDL α-tocopherol during oxidation according to Brown and Rice-Evans [34]. Luteolin (1µ M) demonstrated an efficacy similar to that of 20 µg/ml ALE in inhibiting lipid peroxidation. Luteolin-7-glucoside also demonstrated a dose dependent reduction of LDL oxidation that was less pronounced than that of luteolin. Mechanistic AOX studies suggested that both compounds act as metal ion chelators. Moreover, Brown & Rice-Evans [34] reported that artichoke flavonoids may also act as hydrogen donors supporting the AOX potential of ALE.

Overall, the AOX data of total ALE and individual ALE compounds obtained from the present study is of interest for a comparative in vitro evaluation of the total antioxidant activity of artichoke extracts. However, these values need to be combined with in vivo data to assess the antioxidant efficacy of artichoke extracts more completely.

HMG-CoA reductase inhibition

3-hydroxy-3-methylglutaryl coenzyme A reductase (HMG-CoA; EC1.1.1.34) is the key enzyme in the biosynthesis of cholesterol [10]. The enzyme is bound to the endoplasmatic reticulum and exhibits a very short half-life of 2 to 4 h [35, 36, 37]. HMG-CoA reductase catalyses the conversion of HMG-CoA to mevalonic acid in the presence of NADPH [11]. HMG-CoA reductase inhibition leads ultimately to a decrease of the plasma cholesterol concentration in-vivo and is therefore an impor-

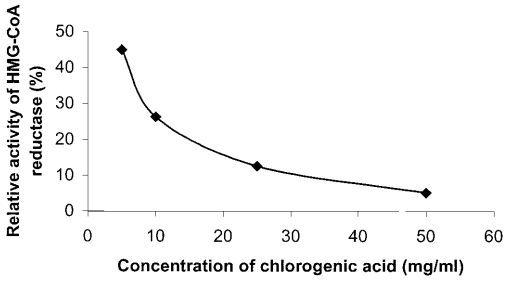

Fig. 4 Inhibitory effect of chlorogenic acid on the activity of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase between 5 and 50 mg/ml

Table 8 Relative activity of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase (%) after incubation with selected artichoke compounds

Artichoke compound	Concentration	HMG-CoA reductase activity (%) (n=2)
None (positive control)		10
Chlorogenic acid	10 mg/ml	26.5
Luteolin	10 mg/ml	2.4
Luteolin-7-glucoside	10 mg/ml	6.5
Mevastatin (*Comparison*)	10×10^{-6} mg/ml	60

tant mechanism of action for the evaluation of the biologically functionality of dietary ingredients. Among others, statins are known HMG-CoA reductase inhibitors due to the similarity to their HMG-CoA structure. In this study, mevastatin was used as a comparison for the HMG-CoA assay. Several individual artichoke compounds – cholorgenic acid, cynarin, luteolin, and luteolin-7-glucoside – as well as total ALE were tested for their HMG-CoA reductase inhibition potential. Chlorogenic acid showed a strong dose-response effect at concentration between 5 and 50 mg/ml (Fig. 4). Luteolin and luteolin-7-glucoside showed an even stronger inhibitory effect under the applied experimental conditions (Table 8). Cynarin was not soluble under the employed solvent conditions, thus the HMG-CoA inhibitory effect could not be assessed appropriately. However, Cynarin and caffeic acid showed negligible HMG-CoA inhibitory effects as reported by Gebhardt [38]. However, it should be noted that cynarin is almost insoluble in aqueous systems and therefore an appropriate evaluation may be difficult under these experimental conditions. This is also partially true for luteolin and luteolin-7-O-glucoside. Therefore, we added DMSO to the buffer in order to enhance the solubility of those compounds. However, it should be noted that DMSO by itself also has a weak HMG-CoA reductase inhibitory effect.

Commercially available ALE were also tested for their HMG-CoA reductase inhibition effect. The results are shown in Table 9. ALE2 and ALE7 showed the strongest inhibitory effect, while the other ALE revealed a moderate inhibitory effect. These results are in accordance with Gebhardt [38] who reported luteolin as strongest inhibitory compound followed by luteolin-7-O-glucoside (62.8 ± 5.2% inhibition by luteolin compared to 22.2 ± 4.2% inhibition by luteolin-7-O-glucoside at 100 µ g/ml). It should be noted that under physiological conditions, additional luteolin may be liberated from luteolin-7-glucoside by β-glucosidase activity which may boost the inhibitory effect in vivo. Compared to mevastatin, however, the inhibitory effect of ALE is approximately a magnitude of 10^5 lower.

Table 9 Inhibition of HMG-CoA reductase by artichoke extracts (10 mg/ml)

Supplier	HMG-CoA reductase activity (%)
None (positive control)	100
Juice, AH	19.2
Liquid extract, ALE[a]	48.7
Capsules, ALE 1	56.0
Capsules, ALE 2	27.2
Capsules, ALE 3	34.1
Capsules, ALE 4	59.2
Powdered extract, ALE 5	37.4
Powdered extract, ALE 6	46.7
Powdered extract, ALE 7	16.5
Mevastatin (10×10^{-6} mg/ml) *Comparison*	60

[a] contained also artichoke heads and roots

Since ALE have a diverse composition and not all individual compounds are known, it is hard to conclude which compound(s) play a key role in the HMG-CoA reductase inhibition. Moreover, further studies are necessary to investigate the potential synergistic effects of ALE compounds on HMG-CoA reductase inhibition as well as the effect of individual artichoke compounds on other proposed mechanism of action, e.g. biliary secretion.

Conclusions

LC was successfully applied to isolate, characterize and quantify minor ALE compounds. The AOX effect of individual artichoke compounds as well as total ALE was less pronounced than γ-tocopherol as analyzed by the rancimat assay. The potential of HMG-CoA reductase inhibition is low, compared to well-known inhibitors such as statins (e.g., mevastatin is approximately $5x10^5$ times stronger than the most potent ALE). Therefore, extreme high ALE intake will be necessary in order to achieve a significant blood cholesterol lowering effect. Therefore, a substantial blood cholesterol lowering effect achieved by an artichoke containing food product will only be feasible in combination with other blood cholesterol lowering ingredient(s). Finally, high intake of ALE will always be accompanied by remarkable bitter taste which hampers a potential functional food application.

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