JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Quantitative Determination of Phenolic Compounds in Artichoke-Based Dietary Supplements and Pharmaceuticals by High-Performance Liquid Chromatography

KATRIN SCHÜTZ, ERNA MUKS, REINHOLD CARLE, AND ANDREAS SCHIEBER*

Institute of Food Technology, Section Plant Foodstuff Technology, Hohenheim University, August-von-Hartmann-Strasse 3, D-70599 Stuttgart, Germany

Dietary supplements are among the most rapidly growing products in the food and personal care market with an estimated worldwide volume exceeding $60 billion. The main problem associated with dietary supplements is their legal classification. Being neither food nor medicine, they often inhabit a gray area between the two, which makes legal regulatory extremely difficult. Thus, a coexistence of products processed from the same botanical source on the same market as dietary supplement or pharmaceutical is possible. In the present study, various artichoke-based dietary supplements were investigated for their phenolic profile and compared to artichoke phytopharmaceuticals. Quantification of individual hydroxycinnamic acids and flavonoids was carried out by external calibration. For the first time, determination of several apigenin derivatives was included. Chlorogenic acid represented the major constituent in all samples investigated with the exception of juice derived from fresh flower heads, which exhibited a higher cynarin content. Furthermore, a distinction between products made from artichoke leaves or flower heads was possible. The results obtained revealed great diversity of pharmaceuticals and dietary supplements, highlighting the need of standardized quality requirements.

KEYWORDS: Artichoke; *Cynara scolymus* L.; dietary supplements; pharmaceuticals; flavonoids; caffeoylquinic acids

INTRODUCTION

Artichoke (*Cynara scolymus* L.) is a herbaceous perennial plant belonging to the Compositae. Apart from being consumed as a fresh or canned vegetable, artichoke is a well-known herbal remedy with a long history. According to the Commission E (*1*) and ESCOP monographs (*2*), the fresh or dried, cut leaves, pressed juice of the fresh plant, and other galenical preparations are internally used to treat digestive complaints and hepatobiliary disturbance. In various studies, including in vitro, in vivo, and human trials, the pharmacological activities, such as choleretic (*3*, *4*), lipid-lowering and anti-atherogenic (*5*−*7*), hepatoprotective (*8*, *9*), antioxidative effects (*10*, *11*), and inhibition of cholesterol biosynthesis (*12*, *13*), were well documented. These broad therapeutic indications cannot be ascribed to a single but to several active compounds generating additive or synergistic pharmacologic effects, including monocaffeoylquinic and dicaffeoylquinic acids, and flavonoids such as luteolin and its 7-*O*-glucoside (*14*−*18*).

Besides these artichoke pharmaceuticals, various dietary supplements containing artichoke extracts are available on the market in dosage forms such as dragées, capsules, and effervescent tablets. Dietary supplements together with functional foods are among the most rapidly growing sectors in the food and personal care product industry. This development is due to the loss of consumer confidence in the modern diet, the aging population, the increased trend toward self-medication, and finally an overall enhancement in health awareness and disease prevention among customers (*19*, *20*). According to the FDA, the worldwide market for dietary supplements is estimated at more than $60 billion (*21*).

The legal classification of those products still proves to be difficult because they do not easily fall into the legal categories of food or drug but rather often inhabit a gray area between the two. In the U.S., the Dietary Supplement Health and Education Act (DSHEA) of 1994 regulates and specifies labeling requirements of dietary supplements. Corresponding to its definition, "herbs or other botanicals" are included in dietary supplements (*22*). Because of the marginal qualification made by the DSHEA, a broad diversity of botanicals has been sold as dietary supplement ingredients, including many that are considered medicinal preparations under most regulatory regimes in EU countries (*23*, *24*). In contrast to the U.S., the European regulatory status of dietary supplements is diversified due to the difference in tradition, historical, and cultural background, and different legislation and enforcement practices at national level. Therefore, in some EU countries, botanical products are sold as food, implying renunciation of medicinal claims, whereas

* Author to whom correspondence should be addressed [telephone ++49-711-459-23125; fax ++49-711-459-24110; e-mail schieber@uni-hohenheim.de].

10.1021/jf062009b CCC: $33.50
Published on Web 10/18/2006

in other EU countries these preparations are regarded as herbal medicines registered by full or simplified registration procedures (*24*). As a result, some herbal products are sold at identical dosage in the same member state as medicine and food supplement. When sold as herbal medicinal product, they are governed by drug law, and risk/benefit considerations are essential in the evaluation of safety and efficacy, whereas dietary supplements are subjected to food law and must be absolutely safe (*24*, *25*).

However, although the majority of consumers trust in the safety and efficacy of these products (*21*), a legal regulation, allowing a clear classification of dietary supplements and pharmaceuticals, is required. With respect to artichoke-based dietary supplements and artichoke pharmaceuticals, the establishment of a suitable analytical method for the determination of phenolics representing the active principles of such products would be a first step. Therefore, the aim of the present study was to prove the suitability of an established method for the determination of polyphenolics in artichokes for the analysis of artichoke-based dietary supplements and pharmaceuticals.

MATERIALS AND METHODS

Materials. Reagents and solvents were purchased from VWR (Darmstadt, Germany) and were of analytical or HPLC grade. Deionized water was used throughout. Chromabond, 1000 mg, solid-phase extraction cartridges were obtained from Macherey & Nagel (Düren, Germany). Six pharmaceuticals and seven commercial dietary supplements were obtained from local drugstores and supermarkets.

Sample Preparation. The determination of individual phenolic compounds was carried out according to a previously established method (*26*) with slight modifications of the extraction procedure. Briefly, dragées and effervescent tablets were gently ground in a mortar, and hard gelatin capsules were opened manually. Aliquots (1.5 g for dragées, 0.7 g for capsules, and 4.2 g for effervescent tablets) were extracted by stirring with 100 mL of aqueous methanol (60%, v/v) for 1 h at ambient temperature. Exhaustive extraction was ensured by extraction of the residue. After filtration through a filter paper, the extracts were evaporated to dryness in vacuo at 30 °C, and the residue was dissolved in water. After the solution was adjusted to pH 7.0, the volume was made up to 25 mL with deionized water. The juice samples were used without former preparation after adjustment to pH 7.0 for solid-phase extraction. After activation of the cartridges, aliquots of 2 or 4 mL of the extract or juice were applied to the sorbent. Hydroxycinnamic acid derivatives were subsequently eluted with 100 mL of 10% aqueous methanol (fraction I). Rinsing with 50 mL of pure methanol eluted neutral compounds (fraction II). The eluates were evaporated to dryness in vacuo, and the residues obtained were dissolved appropriately (0.5−2.0 mL) with 50% aqueous methanol. Polyphenol analyses were carried out using a series 1100 HPLC (Hewlett-Packard, Waldbronn, Germany) equipped with ChemStation software, a model G1322A degasser, a model G1312A binary gradient pump, a model G1329/1330A thermoautosampler, a model G1316A column oven, and a model G1315A diode array detection system. The column used was a 150 × 3.0 mm i.d., 4 μm particle size C_{18} Hydro-Synergi from Phenomenex (Torrance, CA), with a security guard 4 × 3.0 mm i.d. C_{18} ODS, operated at a temperature of 25 °C. The mobile phase consisted of 2% (v/v) acetic acid in water (eluent A) and of 0.5% acetic acid in water and acetonitrile (50:50, v/v; eluent B). The gradient program was as follows: 10% B to 18% B (20 min), 18% B to 24% B (10 min), 24% B to 30% B (15 min), 30% B isocratic (20 min), 30% B to 55% B (5 min), 55% B to 100% B (5 min), 100% B isocratic (8 min), 100% B to 10% B (2 min). Total run time was 90 min. The injection volume for all samples was 5 μL. Simultaneous monitoring was performed at 280 nm (narirutin, naringenin 7-*O*-glucoside), 320 nm (hydroxycinnamic acids), 330 nm (apigenin derivatives), and 350 nm (luteolin derivatives) at a flow-rate of 0.4 mL/min. Spectra were recorded from 200 to 600 nm. All data given represent mean values ± standard deviation of two independent experiments ($n = 2$).

RESULTS AND DISCUSSION

Samples. A total of 13 commercial preparations were investigated for their polyphenolic profile, including six medicinal preparations with five dragées (preparations **1**−**5**) and one fresh plant juice (preparation **6**), and seven dietary supplements. The dietary supplements were comprised of two hard gelatin capsules (preparations **8**, **9**), two effervescent tablets (preparations **10**, **11**), and three dragée formulations (preparations **7**, **12**, **13**). Of the latter, two were combination preparations containing gentian or the enzymes bromelain and papain (preparations **12**, **13**). According to the labeled specification, the predominant excipient of the dragées and hard gelatin capsules was maltodextrin, whereas the matrix of the effervescent tablets mainly consisted of citric acid and sodium hydrogen carbonate. The content of artichoke extract ranged from 20% to 46% for the dragée formulation. For the two hard gelatin capsules, 31% and 54%, and for the two effervescent tablets, 4% and 5% of artichoke extract were specified. Furthermore, according to their specification, five products contained a dried aqueous artichoke leaf extract (preparations **1**−**5**), and two were composed of a dried artichoke juice (preparations **10**, **12**). The extracts used for the remaining dragées and capsules were not further specified (preparations **7**−**9**, **11**, **13**). The fresh plant juice was produced from artichoke heads.

Methodology. The previously established sample preparation and liquid chromatographic method (*26*) proved to be suitable also for the determination of phenolic compounds in artichoke-based dietary supplements and pharmaceuticals. Because of the solid-phase extraction applied, problems resulting from large ranges in the contents of hydroxycinnamic acids and flavonoids could be overcome by dissolving the evaporated fractions in appropriate volumes (0.5−2.0 mL). Furthermore, coelution of luteolin 7-*O*-glucoside with luteolin 7-*O*-glucuronide and apigenin 7-*O*-glucoside with apigenin 7-*O*-glucuronide could be avoided. Because of the limited availability of reference substances, HPLC coupled to mass spectrometry proved to be extremely helpful for peak assignment. Among the caffeoylquinic acid derivatives, four monocaffeoylquinic acids (1-*O*-caffeoylquinic, 3-*O*-caffeoylquinic, 4-*O*-caffeoylquinic, and 5-*O*-caffeoylquinic acids) and five dicaffeoylquinic acids (1,3-di-*O*-caffeoylquinic, 3,4-di-*O*-caffeoylquinic, 3,5-di-*O*-caffeoylquinic, 1,5-di-*O*-caffeoylquinic, and 4,5-di-*O*-caffeoylquinic acids) were quantified. Flavonoid contents comprising luteolin 7-*O*-glucoside, luteolin 7-*O*-glucuronide, luteolin 7-*O*-rutinoside, apigenin 7-*O*-glucoside, apigenin 7-*O*-glucuronide, apigenin 7-*O*-rutinoside, naringenin 7-*O*-glucoside, and narirutin were also determined (**Figure 1**).

Phenolic Profile and Content of Artichoke Pharmaceuticals and Dietary Supplements. As compared to the great number of investigations dealing with the determination of pharmaceutically relevant compounds and their quantification in artichoke leaves and heads (*27*−*29*), little is known about the composition of artichoke pharmaceuticals and dietary supplements (*30*−*32*). As can be seen in **Figure 2**, the monocaffeoylquinic acids were the major compounds (from 38.6% to 78.8%) based on total phenolic contents in all samples, and, interestingly, the amounts were slightly higher in dietary supplements (from 54.1% to 78.8%) than in pharmaceuticals (from 38.6% to 67.1%). At the same time, higher caffeic acid contents, which would be the result of dicaffeoylquinic acid hydrolysis, could not be observed (**Table 1**). Within the

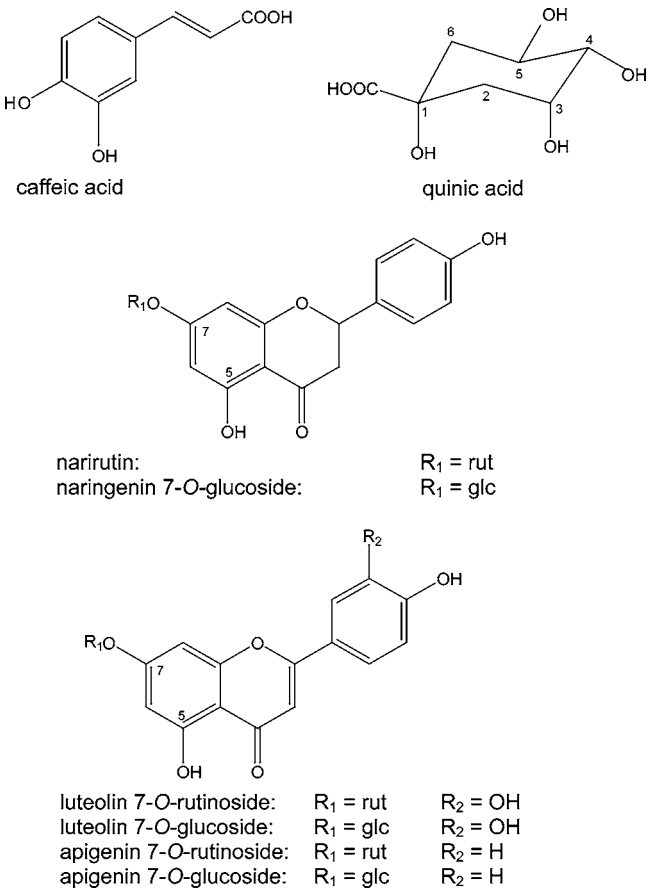

Figure 1. General structures and substitution patterns of caffeoylquinic acids and flavonoids detected in artichoke pharmaceuticals and artichoke-based dietary supplements.

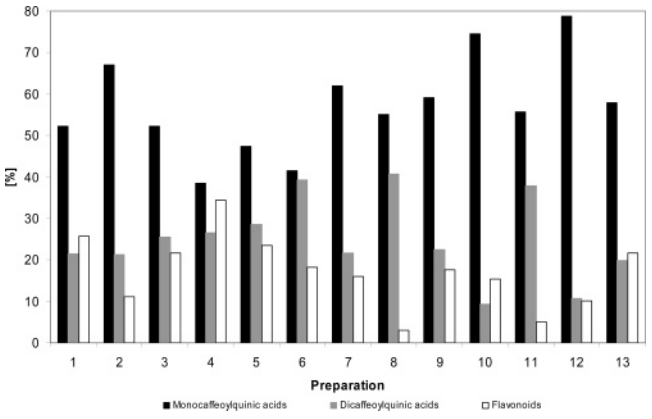

Figure 2. Contents of monocaffeoylquinic acids, dicaffeoylquinic acids, and flavonoids [%] based on total phenolic content in artichoke pharmaceuticals (preparations **1**–**6**) and artichoke-based dietary supplements (preparations **7**–**13**).

monocaffeoylquinic acids (**Figure 3**), chlorogenic acid (5-*O*-caffeoylquinic acid) was predominant (36.7−67.8%), followed by 3-*O*- and 4-*O*-caffeoylquinic acids (10.1−30.4% and 17.2−28.1%, respectively), and 1-*O*-caffeoylquinic acid (1.6−15.2%). According to a previous report dealing with the isomerization behavior of caffeoylquinic acid derivatives in various solvents, these findings are typical of an aqueous extraction procedure (*33*). Exceptions are the fresh plant juice (product **6**) and the effervescent tablet (product **10**), which were made with a pressed juice of artichoke leaves pursuant to the labeled specification. Both exhibited almost equal quantities of chlorogenic acid and

Table 1. Phenolic Contents of Artichoke Pharmaceuticals (Products **1–6**) and Dietary Supplements (Products **7–13**) (Mean ± Standard Deviation, $n = 2$)[a]

compound	1[b]	2[b]	3[b]	4[b]	5[b]	6[c]	7[b]	8[d]	9[d]	10[e]	11[e]	12[b]	13[b]
caffeic acid	0.06 ± 0.00	0.04 ± 0.00	0.08 ± 0.00	0.06 ± 0.00	0.06 ± 0.00	0.23 ± 0.00	0.02 ± 0.00	0.06 ± 0.00	0.01 ± 0.00	0.10 ± 0.00	0.05 ± 0.01	0.02 ± 0.00	0.08 ± 0.00
1-*O*-caffeoylquinic acid	0.34 ± 0.02	0.21 ± 0.00	0.60 ± 0.01	0.46 ± 0.02	0.41 ± 0.01	1.58 ± 0.03	0.14 ± 0.00	0.17 ± 0.00	0.07 ± 0.00	0.03 ± 0.01	nd	0.07 ± 0.00	0.39 ± 0.00
3-*O*-caffeoylquinic acid	1.40 ± 0.06	0.92 ± 0.05	1.69 ± 0.01	1.62 ± 0.07	1.15 ± 0.04	2.98 ± 0.06	0.22 ± 0.00	1.06 ± 0.01	0.16 ± 0.00	3.32 ± 0.06	0.64 ± 0.07	0.61 ± 0.02	1.38 ± 0.01
5-*O*-caffeoylquinic acid	2.95 ± 0.04	2.83 ± 0.06	3.47 ± 0.01	3.28 ± 0.29	2.73 ± 0.02	3.23 ± 0.01	1.24 ± 0.01	1.28 ± 0.01	0.70 ± 0.00	3.08 ± 0.30	0.82 ± 0.07	3.08 ± 0.09	4.11 ± 0.00
4-*O*-caffeoylquinic acid	1.42 ± 0.03	1.03 ± 0.01	1.88 ± 0.00	1.66 ± 0.03	1.29 ± 0.01	2.21 ± 0.05	0.40 ± 0.00	0.98 ± 0.01	0.22 ± 0.00	2.34 ± 0.04	0.55 ± 0.06	0.78 ± 0.01	1.56 ± 0.01
1,3-di-*O*-caffeoylquinic acid	1.23 ± 0.03	0.54 ± 0.00	1.79 ± 0.00	2.50 ± 0.09	1.42 ± 0.03	6.01 ± 0.16	0.23 ± 0.00	1.20 ± 0.00	0.13 ± 0.00	0.82 ± 0.01	0.61 ± 0.06	0.13 ± 0.01	0.75 ± 0.00
3,4-di-*O*-caffeoylquinic acid	0.21 ± 0.01	0.11 ± 0.00	0.32 ± 0.00	0.41 ± 0.03	0.28 ± 0.00	0.88 ± 0.03	0.04 ± 0.00	0.44 ± 0.00	0.01 ± 0.00	0.08 ± 0.01	0.21 ± 0.02	0.07 ± 0.00	0.24 ± 0.00
3,5-di-*O*-caffeoylquinic acid	0.12 ± 0.01	0.10 ± 0.00	0.19 ± 0.00	0.20 ± 0.02	nd	0.55 ± 0.01	0.04 ± 0.00	0.26 ± 0.00	0.01 ± 0.00	0.08 ± 0.00	0.18 ± 0.02	0.06 ± 0.00	0.25 ± 0.00
1,5-di-*O*-caffeoylquinic acid	0.67 ± 0.00	0.57 ± 0.02	1.01 ± 0.01	1.21 ± 0.06	1.27 ± 0.01	1.12 ± 0.02	0.26 ± 0.00	0.25 ± 0.01	0.19 ± 0.01	0.04 ± 0.00	0.17 ± 0.01	0.27 ± 0.01	0.94 ± 0.01
4,5-di-*O*-caffeoylquinic acid	0.29 ± 0.01	0.26 ± 0.00	0.42 ± 0.00	0.51 ± 0.01	0.40 ± 0.01	0.89 ± 0.04	0.15 ± 0.00	0.43 ± 0.00	0.10 ± 0.00	0.10 ± 0.01	0.20 ± 0.01	0.09 ± 0.00	0.38 ± 0.01
luteolin 7-*O*-glucoside	1.50 ± 0.01	0.47 ± 0.04	1.90 ± 0.02	3.13 ± 0.10	1.62 ± 0.05	0.51 ± 0.02	0.37 ± 0.00	0.05 ± 0.00	0.20 ± 0.01	1.43 ± 0.04	0.05 ± 0.00	0.29 ± 0.03	1.63 ± 0.09
luteolin 7-*O*-glucuronide	1.23 ± 0.13	0.19 ± 0.01	0.90 ± 0.00	2.43 ± 0.09	0.86 ± 0.03	1.09 ± 0.03	0.14 ± 0.00	nd	0.09 ± 0.00	0.09 ± 0.01	0.03 ± 0.00	nd	0.72 ± 0.01
apigenin 7-*O*-glucoside	0.07 ± 0.00	0.04 ± 0.00	0.10 ± 0.00	0.15 ± 0.01	0.09 ± 0.00	0.50 ± 0.01	nd	nd	0.01 ± 0.00	0.06 ± 0.00	nd	0.02 ± 0.00	0.08 ± 0.00
apigenin 7-*O*-glucuronide	0.16 ± 0.01	0.05 ± 0.00	0.16 ± 0.00	0.47 ± 0.02	0.11 ± 0.01	1.33 ± 0.05	0.05 ± 0.00	0.07 ± 0.00	0.02 ± 0.00	nd	0.06 ± 0.00	nd	0.16 ± 0.00
naringenin 7-*O*-glucoside	nd	nd	nd	nd	nd	0.06 ± 0.00	nd	nd	nd	nd	nd	nd	nd
luteolin 7-*O*-rutinoside	0.05 ± 0.00	0.05 ± 0.00	0.08 ± 0.00	0.07 ± 0.01	0.05 ± 0.00	0.23 ± 0.01	0.02 ± 0.00	0.05 ± 0.00	0.01 ± 0.00	0.25 ± 0.00	0.04 ± 0.00	0.25 ± 0.00	0.17 ± 0.01
apigenin 7-*O*-rutinoside	0.02 ± 0.00	0.03 ± 0.00	0.03 ± 0.00	0.03 ± 0.00	nd	0.52 ± 0.01	nd	0.02 ± 0.00	0.01 ± 0.00	0.02 ± 0.00	nd	0.02 ± 0.00	0.03 ± 0.00
narirutin	nd	nd	nd	nd	nd	0.10 ± 0.00	nd	nd	nd	nd	nd	nd	nd
total caffeoylquinic acids	8.63 ± 0.09	6.56 ± 0.08	11.37 ± 0.02	11.87 ± 0.32	8.95 ± 0.06	19.44 ± 0.19	2.82 ± 0.01	6.08 ± 0.02	1.59 ± 0.01	10.16 ± 0.31	3.37 ± 0.14	5.17 ± 0.09	9.99 ± 0.02
total flavonoids	3.02 ± 0.13	0.82 ± 0.04	3.16 ± 0.02	6.28 ± 0.14	2.74 ± 0.06	4.36 ± 0.06	0.54 ± 0.00	0.20 ± 0.00	0.35 ± 0.01	1.86 ± 0.04	0.18 ± 0.00	0.58 ± 0.03	2.79 ± 0.09
total polyphenolics	11.71 ± 0.16	7.43 ± 0.09	14.62 ± 0.02	18.22 ± 0.35	11.75 ± 0.08	24.03 ± 0.20	3.38 ± 0.01	6.33 ± 0.02	1.95 ± 0.01	12.12 ± 0.31	3.60 ± 0.14	5.78 ± 0.09	12.86 ± 0.10
recommended daily allowance	1–2[b]	1–2[b]	1–2[b]	1–2[b]	1–2[b]	3[c]	1[b]	3[d]	3[d]	1–2[e]	1[e]	1–2[b]	3[b]

[a] nd, not detected. Contents: milligrams per capsule, dragée, effervescent tablet, or per 10 mL of juice. [b] Dragée. [c] Juice. [d] Capsule. [e] Effervescent tablet.

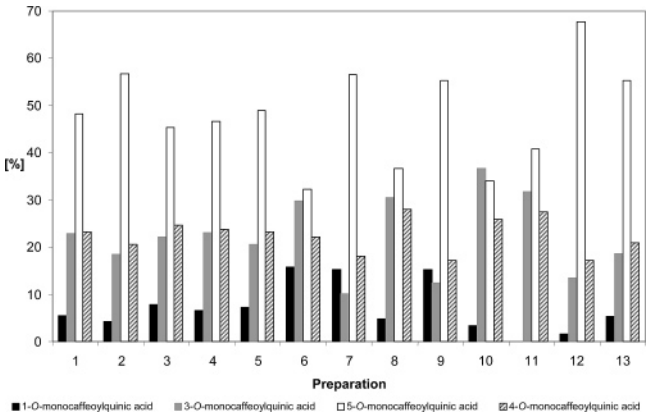

Figure 3. Content of individual monocaffeoylquinic acids relative to total monocaffeoylquinic acid in artichoke pharmaceuticals (preparations **1**–**6**) and artichoke-based dietary supplements (preparations **7**–**13**).

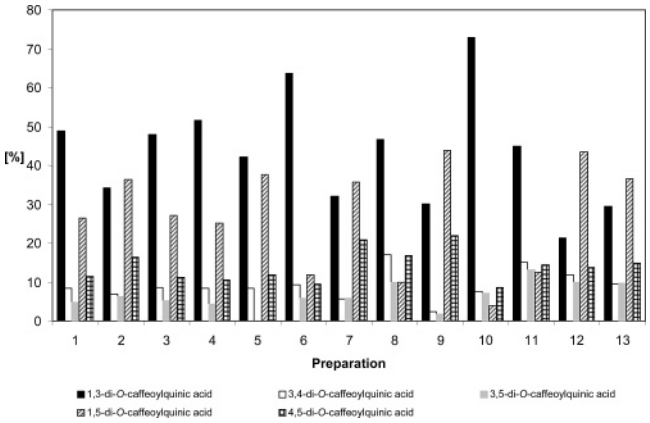

Figure 4. Content of individual dicaffeoylquinic acids relative to total dicaffeoylquinic acid in artichoke pharmaceuticals (preparations **1**–**6**) and artichoke-based dietary supplements (preparations **7**–**13**).

3-*O*-caffeoylquinic acid relative to the total monocaffeoylquinic acid content, which may be ascribed to the utilization of fresh raw material and hence to differences in processing. 1-*O*-Caffeoylquinic acid was not detected in preparation **11** and was only found in smaller amounts as compared to the other monocaffeoylquinic acids. Only in two products (**7** and **9**) did its content exceed that of 3-*O*-caffeoylquinic acid.

In addition to the monocaffeoylquinic acids, the dicaffeoylquinic acids are also important for the pharmacological activities of artichoke preparations. From **Figure 4**, it becomes obvious that 1,3-di-*O*-caffeoylquinic acid (cynarin) was generally the major compound (34.2−51.6%) of the dicaffeoylquinic acid fraction based on the total dicaffeoylquinic acid content. Cynarin is not a genuine constituent of artichoke but an artifact attributed to the isomerization of 1,5-di-*O*-caffeoylquinic acid during aqueous extraction. Consequently, the predominant contents of the cynarin precursor, 1,5-di-*O*-caffeoylquinic acid, in products **2**, **7**, **9**, **12**, and **13** were the result of incomplete isomerization during processing. The preparations **6** and **10** were again remarkable due to their high cynarin contents of 63.6% and 72.8%, respectively. By comparison, 1,5-di-*O*-caffeoylquinic acid only accounted for 11.8% and 3.8% in these products. In all other samples, the relative contents of 1,5-di-*O*-caffeoylquinic acid ranged between 10.0% and 37.6%. Further compounds detected were 3,4-di-*O*-caffeoylquinic acid, 3,5-di-*O*-caffeoylquinic acid, and 4,5-di-*O*-caffeoylquinic acid, reaching relative amounts of 16.9%, 13.2%, and 21.9%, respectively.

Besides the hydroxycinnamic acids, the flavonoid contents of artichoke pharmaceuticals and dietary supplements were determined. For the first time, not only the luteolin derivatives but also the apigenin derivatives were included in the study. Moreover, differentiation between flavonoid glucosides and glucuronides was achieved. In four preparations (**1**, **3**, **12**, and **13**), the flavonoid contents were comparable to those of the dicaffeoylquinic acid fraction, and in one (**4**) even higher. Nevertheless, in most samples maximum contents could be determined for the monocaffeoylquinic acids, followed by the dicaffeoylquinic acids and flavonoids (**Figure 2**). In contrast to the dietary supplements, a descending order concerning the individual flavonoid contents could be observed for the pharmaceutical dragée preparations. Thus, luteolin 7-*O*-glucoside was followed by luteolin 7-*O*-glucuronide, apigenin 7-*O*-glucuronide, apigenin 7-*O*-glucoside, luteolin 7-*O*-rutinoside, and apigenin 7-*O*-rutinoside (**Table 1**). The detection of apigenin 7-*O*-rutinoside in products made from artichoke leaves was surprising insofar as this compound should be unique to artichoke heads (*29*). In the juice made from fresh artichoke heads, apigenin 7-*O*-glucuronide was the predominant compound of the flavonoid fraction, and two further minor compounds, narirutin and naringenin 7-*O*-glucoside, were identified and quantified. These findings confirmed that narirutin and naringenin 7-*O*-glucoside are typical of artichoke heads (*29*, *34*). Generally, the flavonoid contents of the dietary supplements were lower than those of the pharmaceuticals.

Phenolic Content and Possible Pharmacological Activities of Artichoke Pharmaceuticals and Dietary Supplements. Even though numerous studies including human trials (*3*, *6*, *7*, *35*) were conducted to elucidate the pharmacological activities of artichoke preparations, little is known regarding the required concentration of individual compounds. The Commission E proposes an average daily dosage of 6 g of drug or equivalent preparations (*1*). Because the drug should meet a minimum requirement of 0.2% of total caffeoylquinic acid, the daily dosage corresponds to approximately 12 mg of caffeoylquinic acids (*30*, *32*). According to the present results, all pharmaceuticals investigated met this minimum requirement. The juice especially, produced from the fresh plant material (flower heads), was characterized by a high polyphenol content relating to recommended daily allowance (**Table 1**, product **6**). This may be attributed to the fact that fresh instead of dried material was used. Even drying at ambient temperature of artichoke leaves resulted in a loss of 48% dicaffeoylquinic acids and 38% flavonoids, respectively (*31*). In contrast to the pharmaceuticals, only three of the seven dietary supplements (preparations **8**, **10**, and **13**) included in this study contained the minimum amount of caffeoylquinic acids required for pharmaceuticals. This is of particular interest, because the choleretic activity was found to be dose-dependent (*36*). Furthermore, in a very recent investigation, luteolin 7-*O*-glucoside (1.1 mg), chlorogenic acid (13.9 mg), and cynarin (5.7 mg) were tested in the isolated perfused rat liver model for their choleretic activity. While luteolin 7-*O*-glucoside had no marked influence on the bile flow, chlorogenic acid as well as cynarin increased bile flow by 10% and 20%, respectively. The greater effect of cynarin as compared to that of chlorogenic acid suggested that the caffeic acid moiety might be the pharmacologically relevant constituent (*4*). Thus, a high content of dicaffeoylquinic acids would be desirable. With respect to the flavonoid fraction, inhibition of cholesterol biosynthesis and a dose-dependent reduction of low-density lipoprotein oxidation were observed for luteolin and, to a lesser

extent, for its 7-*O*-glucoside (*13*, *37*). Therefore, a high content of these compounds would also be recommendable.

Besides luteolin derivatives, apigenin 7-*O*-glucoside, apigenin 7-*O*-glucuronide, and apigenin 7-*O*-rutinoside were determined in artichoke pharmaceuticals and dietary supplements for the first time. As can be seen from **Table 1**, the contents of apigenin 7-*O*-glucoside and its glucuronide were higher than those of luteolin 7-*O*-rutinoside in all pharmaceuticals investigated. Thus, considering those apigenin derivatives, among others, are responsible for the antispasmodic and anti-inflammatory activity of chamomile preparations (*38*), studies confirming similar effects in artichoke may be of interest.

The present results clearly demonstrate the great diversity of phenolic contents in dietary supplements and pharmaceuticals, particularly among dietary supplements. Dietary supplements had generally lower dosage with the exception of products **8**, **10**, and **13**, which came close to pharmaceutical preparations. Hence, comparable pharmaceutical effects might be assumed, and the respective products should be specified and authorized as medicine rather than as dietary supplements. For the rest of the dietary supplements, the question arises which physiological properties they should have. A nutritional value that herbal products should have when sold as food supplements could be excluded because of relatively low mineral and vitamin contents (*25*, *39*, *40*). Therefore, from the present results it is evident that the problem of borderline botanical-sourced products remains to be solved. The answer to this problem lies in a clear distinction between the products regarded as herbal or traditional herbal medicinal products and food supplements as well as in an efficient quality control of the products (*24*, *41*). For this purpose, the method applied in this study may be useful.

LITERATURE CITED

(1) Blumenthal, M., Busse, W. R., Goldberg, A., Gruenwald, J., Hall, T., Riggins, C. W., Rister, R. S., Eds. Artichoke Leaf. *The Complete German Commission E Monographs. Therapeutic guide to herbal medicines*; American Botanical Council: Austin, TX, 1998; pp 84−85.

(2) ESCOP. Cynarae Folium. *Monographs on the Medicinal Uses of Plant Drugs*, 2nd ed.; European Scientific Cooperative on Phytotherapy; Thieme: Stuttgart, 2003; pp 118−125.

(3) Kirchhoff, R.; Beckers, C.; Kirchhoff, G.; Trinczek-Gärtner, H.; Petrowicz, O.; Reimann, H. J. Increase in choleresis by means of artichoke extract. Results of a randomised placebo-controlled double-blind study. *Phytomedicine* **1994**, *1*, 107−115.

(4) Matuschowski, P.; Nahrstedt, A.; Winterhoff, H. Pharmakologische Untersuchungen eines Frischpflanzenpresssaftes aus *Cynara scolymus* auf choleretische. Wirkung. *Z. Phytother.* **2005**, *26*, 14−19.

(5) Dorn, M. Besserung erhöhter Lipidwerte durch Artischocken-Preβsaft. *Aerztez. Naturheilverfahren* **1994**, *35*, 796−802.

(6) Fintelmann, V. Antidyspeptische und lipidsenkende Wirkungen von Artischockenextrakt. *Z. Allg. Med.* **1996**, *72*, 48−57.

(7) Englisch, W.; Beckers, C.; Unkauf, M.; Ruepp, M.; Zinserling, V. Efficacy of artichoke dry extract in patients with hyperlipoproteinemia. *Arzneim.-Forsch./Drug Res.* **2000**, *50*, 260−265.

(8) Adzet, T.; Camarasa, J.; Laguna, J. C. Hepatoprotective activity of polyphenolic compounds from *Cynara scolymus* against CCl_4 toxicity in isolated rat hepatocytes. *J. Nat. Prod.* **1987**, *50*, 612−617.

(9) Gebhardt, R.; Fausel, M. Antioxidant and hepatoprotective effects of artichoke extracts and constituents in cultured rat hepatocytes. *Toxicol. in Vitro* **1997**, *11*, 669−672.

(10) Zapolska-Downar, D.; Zapolski-Downar, A.; Naruszewicz, M.; Siennicka, A.; Krasnodebska, B.; Kolodziej, B. Protective properties of artichoke (*Cynara scolymus)* against oxidative stress induced in cultured endothelial cells and monocytes. *Life Sci.* **2002**, *71*, 2897−2908.

(11) Jiménez-Escrig, A.; Dragsted, L. O.; Daneshvar, B.; Pulido, R.; Saura-Calixto, F. In vitro antioxidant activities of edible artichoke *(Cynara scolymus* L.) and effect on biomarkers of antioxidants in rats. *J. Agric. Food Chem.* **2003**, *51*, 5540−5545.

(12) Gebhardt, R. Artischockenextrakt - In-vitro-Nachweis einer Hemmwirkung auf die Cholesterinbiosynthese. *Med. Welt.* **1995**, *46*, 348−350.

(13) Gebhardt, R. Inhibition of cholesterol biosynthesis in primary cultured rat hepatocytes by artichoke (*Cynara scolymus* L.) *extracts. J. Pharmacol. Exp. Ther.* **1998**, *286*, 1122−1128.

(14) Eich, J.; Paper, D. H.; Grün, M. Luteolin 7-*O*-*β*-glucuronide from artichoke leaves. *Pharm. Pharmacol. Lett.* **2001**, *1*, 9−10.

(15) Häusler, M.; Ganzera, M.; Abel, G.; Popp, M.; Stuppner, H. Determination of caffeoylquinic acids and flavonoids in *Cynara scolymus* L. by high performance liquid chromatography. *Chromatographia* **2002**, *56*, 407−411.

(16) Zhu, X.; Zhang, H.; Lo, R. Phenolic compounds from the leaf extract of artichoke (*Cynara scolymus* L.) and their antimicrobial activities. *J. Agric. Food Chem.* **2004**, *52*, 7272−7278.

(17) Wagenbreth, D.; Eich, J. Pharmaceutically relevant phenolic constituents in artichoke leaves are useful for chemical classification of accessions. *Acta Hortic.* **2005**, *681*, 469−474.

(18) Romani, A.; Pinelli, P.; Cantini, C.; Cimato, A.; Heimler, D. Characterization of Violetto di Toscana, a typical Italian variety of artichoke (*Cynara scolymus* L.). *Food Chem.* **2005**, *95*, 221−225.

(19) Ransley, J. K. The rise and rise of food and nutritional supplements - an overview of the market. In *Food and Nutritional Supplements*; Ransley, J. K., Donnelly, J. K., Read, N. W., Eds.; Springer-Verlag: Berlin, Germany, 2001; pp 1−16.

(20) Wang, M.; Liang, C. P.; Wu, Q. L.; Simon, J. E.; Ho, C. T. Instrumental analysis of popular botanical products in the U.S. market. *ACS Symp. Ser.* **2006**, *925*, 39−54.

(21) Crowley, R.; FitzGerald, L. H. The impact of cGMP compliance on consumer confidence in dietary supplement products. *Toxicology* **2006**, *221*, 9−16.

(22) Marriott, B. M. An introduction to dietary supplements of plant origin. In *Dietary Supplements of Plant Origin*; Maffei, M., Ed.; Taylor & Francis Inc.: New York, 2003; pp 1−17.

(23) Viell, B. Funktionelle Lebensmittel und Nahrungsergänzungsmittel. *Bundesgesundheitsbl-Gesundheitsforsch-Gesundheitsschutz* **2001**, *44*, 193−204.

(24) Gulati, O. P.; Ottaway, P. B. Legislation relating to nutraceuticals in the European Union with a particular focus on botanical-sourced products. *Toxicology* **2006**, *221*, 75−87.

(25) Bombardelli, V. Herbal dietary supplements in the European market. In *Dietary Supplements of Plant Origin*; Maffei, M., Ed.; Taylor & Francis Inc.: New York, 2003; pp 18−24.

(26) Schütz, K.; Kammerer, D.; Carle, R.; Schieber, A. Identification and quantification of caffeoylquinic acids and flavonoids from artichoke (*Cynara scolymus* L.) heads, juice, and pomace by HPLC-DAD-ESI/MS^n. *J. Agric. Food Chem.* **2004**, *52*, 4090−4096.

(27) Hammouda, F. M.; Seif El-Nasr, M. M.; Shahat, A. A. Flavonoids of *Cynara scolymus* L. cultivated in Egypt. *Plant Foods Hum. Nutr.* **1993**, *44*, 163−169.

(28) Ševčiková, P.; Glatz, Z.; Slanina, J. Analysis of artichoke (*Cynara cardunculus* L.) extract by means of micellar electrokinetic capillary chromatography. *Electrophoresis* **2002**, *23*, 249−252.

(29) Wang, M.; Simon, J. E.; Aviles, I. F.; He, K.; Zheng, Q. Y.; Tadmor, Y. Analysis of antioxidative phenolic compounds in artichoke (*Cynara scolymus* L.). *J. Agric. Food Chem.* **2003**, *51*, 601−608.

(30) Brand, N.; Weschta, H. Die analytische Bewertung der Artischocke und ihrer Präparate. *Z. Phytother.* **1991**, *12*, 15−21.

(31) Brand, N. Der Extrakt in Artischockenpräparaten. *Dtsch. Apoth. Ztg.* **1997**, *137*, 60−76.
(32) Häusler, M.; Ganzera, M.; Stuppner, H.; Abel, G.; Popp, M. Artischocken-Präparate. *Dtsch. Apoth. Ztg.* **2003**, *143*, 64−73.
(33) Adzet, T.; Puigmacia, M. High-performance liquid chromatography of caffeoylquinic acid derivatives of *Cynara scolymus* L. leaves. *J. Chromatogr.* **1985**, *348*, 447−453.
(34) Sánchez-Rabaneda, F.; Jáuregiu, O.; Lamuela-Raventós, R. M.; Bastida, J.; Viladomat, F.; Codina, C. Identification of phenolic compounds in artichoke waste by high-performance liquid chromatography-tandem mass spectrometry. *J. Chromatogr. A* **2003**, *1008*, 57−72.
(35) Holtmann, G.; Adam, B.; Haag, S.; Collet, W.; Grünewald, E.; Windeck, T. Efficacy of artichoke leaf extract in the treatment of patients with functional dyspepsia: a six-week placebo-controlled, double-blind, multicentre trial. *Aliment. Pharmacol. Ther.* **2003**, *18*, 1099−1105.
(36) Saénz Rodriguez, T.; García Giménez, D.; de la Puerta Vázquez, R. Choleretic activity and biliary elimination of lipids and bile acids induced by an artichoke leaf extract in rats. *Phytomedicine* **2002**, *9*, 687−693.
(37) Brown, J. E.; Rice-Evans, C. Luteolin-rich artichoke extract protects low density lipoprotein from oxidation *in vitro*. *Free Radical Res.* **1998**, *29*, 247−255.
(38) Carle, R.; Gomaa, K. Chamomile: A pharmacological and clinical profile. *Drugs Today* **1992**, *28*, 559−565.
(39) Herrmann, K. Inhaltsstoffe der Artischocke. *Ind. Obst.-Gemueseverwert.* **1997**, *4*, 125−129.
(40) Erbersdobler, H. E., Meyer, A. H., Eds. Beispiele für Arzneimittel. *Praxishandbuch Functional Food*; Behrs Verlag: 2003; Vol. 2, p 2.
(41) Schilter, B.; Andersson, C.; Anton, R.; Constable, A.; Kleiner, J.; O'Brien, J.; Renwick, A. G.; Korver, O.; Smit, F.; Walker, R. Guidance for the safety assessment of botanicals and botanical preparations for use in food and food supplements. *Food Chem. Toxicol.* **2003**, *41*, 1625−1649.

Received for review July 18, 2006. Revised manuscript received September 5, 2006. Accepted September 6, 2006.

JF062009B