In Vitro Phytoequivalency of Artichoke Extracts (Cynara scolymus L.) and Their Drug Products

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In this study, the quantitative amounts of chlorogenic acid (CA) in spray dried artichoke (Cynara scolymus L.) leaf extract (SD) and standardized artichoke commercial products (CP1, CP2) were calculated by using HPLC. The amounts of CA were 4.66±2.96x10⁻³ mg/capsule and 27.9±9.98x10⁻³ mg/capsule for CP1 and CP2 respectively. The in vitro phytoequivalence of SD, CP1 and CP2 were evaluated by using the content uniformity, in vitro dissolution and disintegration tests. A complete CA release was observed in all formulations which correspond to almost 25 mg for CP2 and 5 mg for SD and CP1. CA was released in a delayed manner from CP2 formulation. This result is compatible with the disintegration results indicating the slow disruption of the gelatin capsule in CP2 formulation. The time recorded for the disintegration of CP1, CP2 and SD were 10.97±0.64, 57.25±1.56 and 6±0.30 minute respectively. The results of the present work indicate that CA in commercial products was present but with amounts less than required to be contained in a capsule. Also these commercial products have different chromatographic profiles. So this study revealed that some artichoke extracts which are standardized as CA, were not phytoequivalent.

Key words: Artichoke, Chlorogenic acid, Spray drying, Dissolution test, Disintegration test, Phytoequivalency.

Enginar Ekstreleri ve İlaç Formlarının In vitro Fitoeqivalansı

Bu çalışmada, püskürterek kurutulmuş enginar (Cynara scolymus L.) yaprak ekstresi (SD) ve standartize enginar yaprak ekstresi içeren preparatlardaki (CP1, CP2) klorojenik asit (CA) miktarı HPLC kullanılarak hesaplanmıştır. Klorojenik asit miktarı CP1 ve CP2 için sırasıyla 4.66±2.96x10⁻³ mg/kapsül ve 27.9±9.98x10⁻³ mg/kapsül olarak hesaplanmıştır. SD, CP1 ve CP2 nin in vitro fitoçeşitlerleri içeren tekduzeli, in vitro çözünme testi ve dağılma testleri kullanılarak araştırılmıştır. Tüm formülasyonlarda CA salımı neredeyse tamamen gerçekleşmiş, CP2 de 25 mg, SD ve CP1 de 5 mg olduğu görülmüştür. CP2 formülasyonunda CA salımı geçikmeli olarak gerçekleşmiştir. Bu sonuç dağılma testinde CP2 formülasyonunun geç parçalanması ile de uyuşuluk göstermektedir. CP1, CP2 ve SD formülasyonları için kaydedilen dağılma süreleri sırasıyla 10.97±0.64 dk, 57.25±1.56 dk ve 6±0.30 dk’dir. Çalışmanın sonuçları CA’nın bir kapsülde olması gerekende daha az bulunduğu göstermemiş, ayrıca piyasa preparatlarının kromatografik profilleri farklıdır. Bu çalışma klorojenik asit üzerinden standartize edilen piyasa preparatlarının fitoçeşitler olmalıdırını göstermiştir.

Anahtar kelimeler:Enginar, Klorojenik asit, Püskürterek kurutma, Çözünme testi, Dağılma testi, Fitoeqivalans.

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INTRODUCTION

Cynara scolymus L. belonging to the Compositae is one of the oldest cultivated edible plants. The plant and the leaf extracts have a long history of traditional usage in the Mediterranean diet as well as in folk medicine for the treatment of dyspeptic disorders. Artichoke (C. scolymus) leaf extracts have been documented since ancient times as a traditional folk medicine mainly attributed to their choleretic, diuretic and hypocholesterolemic activities (1). C. scolymus has also been used in Turkish folk medicine as a choleretic, diuretic and digestive agent. The leaf extracts of the plant have been used for the treatment of liver and bile disorders (2). Artichoke is a well-known herbal remedy with a long history according to the Commission E and ESCOP monographs (3).

Artichoke is originating from the Mediterranean area, which today is widely cultivated all over the world. Its flower head is eaten as a vegetable and prepared for different value-added products (4). In various studies, including in vitro, in vivo and human trials, the pharmacological activities of artichoke, such as choleretic, lipid-lowering and anti-atherogenic, hepatoprotective, antioxidative effects and inhibition of cholesterol biosynthesis were well documented. These therapeutic indications are due to the phytochemical compounds in artichoke (3).

C. scolymus leaves and extracts contain a complex of substances. The most important chemical components are caffeoylquinic acids, flavonoids and sesquiterpenes. Chlorogenic acid (5-O-caffeoylquinic acid), cynarin (1,3-O-dicaffeoylquinic acid), luteolin and luteolin glycosides (e.g. scolymoside and cynaroside) are considered as essential artichoke compounds (1). The choleretic and hepatostimulating effects of extracts of C. scolymus have been reported to depend on their caffeoylquinic acid (chlorogenic acid, cynarin (1,5-O-dicaffeoylquinic acid), cryptochlorogenic acid, neochlorogenic acid etc.) contents (5).

Natural products are important for pharmaceutical research and for drug development as a source of therapeutic agents. At present, the demand for herbal or medicinal plant products has increasing significantly. Nowadays, the development of new technologies to obtained standardized dried natural extracts is an important subject of the herbal processing industries (6).

There are many ways to prepare dry plant extracts that are used in medicine. Several techniques are implemented to obtain powder, including freeze-drying, spouted bed drying and spray drying. However, spray dryers are the most frequently used instruments in herbal processing industries (6,7). The spray drying process has already been widely used in pharmaceutical, chemical, food, and cosmetic industries (8). The process consists of several steps including the atomization of the liquid stream, contact of the liquid droplets with the drying air, dry spherical particle formation via the evaporation of the solvent and collection of the dry product (9). Spray drying is applicable even for heat-labile materials without significant alteration of the structures and activities by virtue of its quick processing (10). The demand for dry extract powders prepared by spray drying is recently increasing because obtained powders are less hygroscopic, exhibit better quality in terms of batch to batch variation, possess improved flowability and dissolution properties (11). In order to prepare an adequate solid dosage form (capsule, tablet) of a herbal extract powder and introduce to drug market, it is essential to stabilize their powder properties. By adjusting the spray dryer parameters such as inlet temperature, pump capacity and feed pump solution rate it is possible to obtain dry powders with desired properties (12).

According to “WHO (World Health Organization) guidelines on GMP (Good Manufacturing Practices) for herbal medicines”, the uniformity of weight, disintegration time and dissolution properties should be documented for finished herbal products (13). Among these controls dissolution test is a very important quality control test used in formulation development and manufacturing process monitoring in pharmaceutical industry. It is also an important tool for the prediction of in vivo performance of drugs (14).

In the present study dry extracts of aqueous artichoke leaves were prepared by using spray
dryer. Spray drying method was chosen due to the advantages mentioned above. The quantitative amounts of chlorogenic acid in dry extract and commercial products with standardized artichoke extracts available in Turkey were measured comparatively. The in vitro phytoequivalence of the dry extract and two herbal preparations which were stated to include 10 % chlorogenic acid, marketed in Turkey was evaluated. The content uniformity, in vitro dissolution tests and disintegration tests were performed for this evaluation.

EXPERIMENTAL

Artichoke samples

C. scolymus was collected from Konya, Turkey. Voucher specimens were deposited at the Herbarium of Ankara University, Faculty of Pharmacy (AEF 24775).

Two different commercial products (CP) containing C. scolymus leaf extract and standardized 10 % as chlorogenic acid were purchased from pharmacy in Turkey:

CP 1: Cynara scolymus (300 mg dried Artichoke leaf extract - 10 % chlorogenic acid) + Silybum marianum (200 mg Milk Thistle extract – 80 % silymarin)
CP 2: Cynara scolymus (300 mg dried Artichoke leaf extract - 10 % chlorogenic acid)

Chemicals

Chlorogenic acid (CA) was purchased from Sigma, Germany. HPLC methanol, HPLC acetonitrile, orthophosphoric acid and sodium hydroxide were purchased from Merck (Darmstadt, Germany). Hydrochloric acid and tribasic sodium phosphate were obtained from Riedel de Haén (Seelze, Germany). Purified water was generated by using Milli-QPlus System (Millipore Corp., Molsheim, France).

Spray dryer method

Büchi B 290 Mini Spray Dryer (Germany) system was used in this study. Spray drying method was applied to prepare dry extract from plant sample by modifying previously published method (7,8). According to these methods and experimental trials, the analysis were performed at 100 °C inlet temperature, 100 % aspirator, 12 mL/min (40 %) pump rate and 25 nozzle flow that read from system nozzle scale (15,16).

10 g of artichoke leaves were extracted with petroleum ether by using Soxhlet extractor for 8 hours and the process was followed by water extraction in magnetic stirrer for 3 hours. After the preliminary extraction, 2 g lactose was added to 100 mL of aqueous extract as a carrier to obtain an adequate bulk material with good flowability in dry state. The resulting aqueous extract was dried in spray dryer by using the optimized dryer parameters mentioned above thus dry powder extract (SD) was obtained. The production yield was calculated by using Equation 1.

\[
\text{Yield} (\%) = \left( \frac{a}{b} \right) \times 100 \quad (\text{Equation 1})
\]

a: the amount of dry extract yielded by using spray dryer
b: the amount of total plant material extracted with water

Content uniformity

The content uniformity test for capsules was based on the assay of the individual contents of active substance in capsules as stated in EP 6.0 (17). For this purpose the commercial products containing C. scolymus leaf extract (CP1, CP2) and the dry extract prepared by using spray dryer (SD) were evaluated by measuring the CA amount using the suitable HPLC technique described below. The content of each capsules were dissolved in 100 mL of 0.1 N HCl pH=1.2 (acidic medium) and filtered through 0.45 µm membrane filters before analysis and directly injected to HPLC.

HPLC Analysis

Preparation of samples

Dried artichoke leaf (300 mg) was accurately weighed into a 100 mL volumetric flask. Seventy milliliters of 70 % methanol solution was added and sample was sonicated for 30 minutes. The flasks were then filled up to volume with 70 % methanol. Commercial products (300 mg) and spray dryer extract (300 mg) were prepared in the same manner with the dried artichoke leaf but dilutions were made with acidic (pH 1.2) and phosphate buffer (pH 6.8) solutions which were used as in vitro dissolution media. The samples were
filtered into HPLC vials by using a disposable syringe and a 0.45 µm filter, for HPLC analysis.

Preparation of standards

Ten milligrams of CA was accurately weighed and placed into a 10 mL volumetric flask. Five milliliters of acidic/buffer medium was added, and the solutions were sonicated for 15 min. The flasks were filled up to the volume with the same solutions. Several dilutions were made to obtain standard solutions. Calibration curves were established on fifteen data points covering concentration ranges of 0.125-40 µg/mL for CA. The calibration curve for CA was obtained by plotting the peak area versus the CA concentration. The conditions of HPLC system and validation parameters used for CA assay in different mediums are given in Table 1.

The quantitative analysis of CA was performed with a Agilent Series 1100 chromatograph equipped with a diode-array detector (G1315-DAD), vacuum degasser and column heater (Agilent, USA). A Supelcosil C18 column (250 mm x 4.6 mm, 5 µm particle size) was used for all separations. The mobile phase was made up of 0.2 % phosphoric acid in water (A) and acetonitrile (B) in a two-step linear gradient elution: the initial composition at the time 0 was A:B 94:6 (v/v), at the 15th min it was changed to A:B 82:18 (v/v). The flow rate was 0.5 mL/min. The column temperature was maintained at 40 °C, the sample injection volume was 50 µL and the wavelength was 330 nm.

Table 1. Conditions of HPLC system and validation parameters used for CA assay in different mediums.

<table>
<thead>
<tr>
<th>Calibration medium</th>
<th>Acidic medium</th>
<th>Buffer medium</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wavelength</td>
<td>330 nm</td>
<td>330 nm</td>
</tr>
<tr>
<td>Mobile phase A</td>
<td>0.2 % Phosphoric acid in water</td>
<td>0.2 % Phosphoric acid in water</td>
</tr>
<tr>
<td>Mobile phase B</td>
<td>Acetonitrile</td>
<td>Acetonitrile</td>
</tr>
<tr>
<td>Flow rate</td>
<td>0.5 mL/min</td>
<td>0.5 mL/min</td>
</tr>
<tr>
<td>Injection volume</td>
<td>50 µL</td>
<td>50 µL</td>
</tr>
<tr>
<td>Linearity and range</td>
<td>0.125-40 µg/mL</td>
<td>0.125-40 µg/mL</td>
</tr>
<tr>
<td>Slope</td>
<td>28.83</td>
<td>25.55</td>
</tr>
<tr>
<td>Intercept</td>
<td>-1.791</td>
<td>1.423</td>
</tr>
<tr>
<td>Determination coefficient (r²)</td>
<td>0.9999</td>
<td>0.9995</td>
</tr>
<tr>
<td>Limit of Quantification (LOQ)</td>
<td>2.445 ng/mL</td>
<td>7.479 ng/mL</td>
</tr>
<tr>
<td>Limit of Determination (LOD)</td>
<td>0.8069 ng/mL</td>
<td>2.468 ng/mL</td>
</tr>
</tbody>
</table>

In vitro dissolution test

For in vitro dissolution tests, the dry extract powder obtained by spray dryer method was placed in hard gelatin capsules at a quantity of 300 mg to obtain the same leaf extract amount with the commercial products. The in vitro dissolution test was performed by the paddle method (USP apparatus II) at 37±0.5°C using SOTAX AT7 Smart, Sotax AG (Basel, Switzerland). The dissolution tests were performed in two different dissolution mediums given in dissolution general chapter in USP (711) (18). The dissolution mediums were 0.1 N HCl pH 1.2 (acidic medium) and 0.1 N HCl solution: 0.2 N tribasic sodium phosphate (1.5:7.5) pH 6.8 (buffer medium). The paddle speed was adjusted to 75 rpm and the capsules (CP1, CP2 and SD) were introduced into 900 mL dissolution medium (acidic medium or buffer medium) at 37 °C. One milliliter of samples were taken from the release medium at 5th, 15th, 30th, 45th, 60th, 90th and 120th minutes. The aliquots withdrawn for analysis were replaced with equal volumes of fresh dissolution medium at 37 °C. The collected samples were filtered through 0.45 µm membrane filter and directly analyzed by HPLC as described above. Each dissolution
test was performed in triplicate for each medium. The cumulative percentages of the drug released from capsules were calculated.

**Disintegration test**

The *in vitro* disintegration test was performed for CP1, CP2 and SD samples by using Aymes disintegration apparatus (Istanbul, Turkey). One capsule was introduced into one tube of the basket rack. The rack was immersed into 0.1 N HCl pH 1.2 (acidic medium) at 37 °C and apparatus was operated at a frequency rate of 30 cycles/min. The time (min) for the capsule disintegration was recorded and test was performed in triplicate.

**RESULTS AND DISCUSSION**

Herbal substances are a diverse range of botanical materials including leaves, herbs, roots, flowers, seeds, bark etc. A comprehensive specification must be developed for each herbal substance even if the starting material for the manufacture of the herbal medicinal product is a herbal preparation. The specification should be established on the basis of recent scientific data and should be set out in the same way as the European Pharmacopoeia monographs. Assay is one of the most important specification about herbal remedies (19). The European Pharmacopoeia monograph (*Cynarae Folium*) states that CA content of artichoke leaves for officinal use must be at least 0.8 % (17). The calculated CA content of artichoke leaves used in the research was 1.06 %. This data demonstrate that the herbal material meets this requirement.

When developing a formulation and identifying the best process conditions for the production of a spray-dried powder it is necessary to make preliminary studies on small quantities of the plant extracts with an appropriate equipment (15). The preliminary studies were conducted to choose the best solvent for the highest yield. The methanolic and aqueous extracts were prepared and dried by spray dryer. The yields of the aqueous extracts were higher than the methanolic extracts, but the powder derived from the aqueous extract could not be sufficiently dried and not satisfactory because of oily particles in the leaves. This was attributed to the presence of several compounds in the extract composition that are not suitable for drying. In order to eliminate the oily non-polar compounds preliminary extraction techniques were applied by using Soxhlet extractor.

Some difficulties were experienced during experiments for obtaining a dry extract from the herb using the spray dryer. For example, the powder derived from the aqueous extract could not be sufficiently dried, clinging on glass parts of the apparatus, making it impossible to obtain a yield. To overcome this issue, various techniques were used, including preliminary extraction by using non-polar solvent (petroleum ether) and carrier (lactose) addition. Lactose is a commonly used carrier due to its low stickiness and non-toxic properties. Lactose has a high glass transition temperature of at 101°C which leads the improvement of flow properties of the dried material (20).

After the preliminary extraction dry *C. scolymus* leaf extract was successfully obtained by using a validated spray dryer method. The amount of 3.23 g dry *C. scolymus* leaf extract was obtained per 100 mL aqueous extract containing 2 g lactose by spray drying, in which 1.23 g leaf extract was present. The yield was calculated as 12.3 %.

In HPLC method, CA was used as an external standard in this study. Samples were prepared with known concentrations of CA. A calibration curve was obtained by plotting the peak area ratio of CA versus concentrations and became linear over the range of 0.125-40 µg/mL with the determination of high coefficient ($r^2$) 0.9999 in acidic medium and 0.9995 in buffer medium. The limits of quantification were 2.445 ng/mL and 7.479 ng/mL; limits of detection were 0.8069 ng/mL and 2.468 ng/mL for CA assay in acidic and buffer medium. HPLC chromatograms of commercial products and UV-DAD spectrum of chlorogenic acid are given in Figure 1. The CA content in two commercial products and standard errors (SE) are given in Table 2.
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Figure 1. HPLC chromatograms of commercial products (CP1, CP2), spray dryer extract (SD) and UV-DAD spectrum of chlorogenic acid.

Table 2. Content uniformity test results and label content of commercial products.

<table>
<thead>
<tr>
<th>Herbal Preparation</th>
<th>CA assay mg/capsule ± SE</th>
<th>CA assay % ± SE</th>
<th>Labelled amount of herbal extract mg</th>
<th>Labelled amount of CA assay %</th>
</tr>
</thead>
<tbody>
<tr>
<td>CP1</td>
<td>4.66 ± 2.96x10^{-3}</td>
<td>1.55 ± 9.81x10^{-4}</td>
<td>300</td>
<td>10</td>
</tr>
<tr>
<td>CP2</td>
<td>27.9 ± 9.98x10^{-3}</td>
<td>7.97 ± 2.83x10^{-3}</td>
<td>300</td>
<td>10</td>
</tr>
<tr>
<td>SD</td>
<td>3.61 ± 2.66x10^{-3}</td>
<td>1.20 ± 8.66x10^{-4}</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

It was reported on the labels that commercial products contain 10 % CA in 300 mg dried herbal extract. It is indicated that during the shelf life the variation in the content of a herbal medicinal drug should not exceed 5 % of the declared assay value (21). Therefore the commercial products (CP1 and CP2) have to contain 30 mg ± 1.5 mg CA. As shown in Table 2 the measured amounts of CA were 4.66 ± 2.96x10^{-3} mg and 27.9 ± 9.98x10^{-3} mg for CP1 and CP2 respectively. These values are lower than they should be. CP2 can be considered as a better formulation than CP1 with respect to the CA content. SD contains lower CA than commercial products as expected since the only aim about spray dryer study was to obtain dry extract from aqueous artichoke leaf extract. CA content in SD was calculated for comparing with commercial products as an additional data.

According to the “Guidance on equivalence of herbal extracts in Complementary Medicines” in order to determine whether or not a herbal preparation is ‘not significantly different’, or ‘essentially the same’, it is first necessary to establish quantitative assessment. If it is not possible to establish equivalence of an extract or preparation via process controls, qualitative and quantitative comparison of the original extract, and the intended replacement, may be made using profile chromatograms of the native extract. Comparison of two extracts for the purposes of determining the degree of similarity or difference must include a
quantitative assessment of the chromatographic profiles (22). Several experiments were performed to evaluate the phytoequivalence of commercial products containing artichoke extracts and SD to predict their efficacy. The results of the present work indicated that in commercial products, CA was present but less than the declared amount in their labels (10 % CA on the label) in a capsule (Table 2).

The release profiles in different dissolution mediums at pH 1.2 and pH 6.8 reveal that the release was not pH dependent. Almost 100 % of CA was released within 2 hours both in acidic and buffer medium. Figure 2 shows how CA release profile and release times were different in two commercial product containing artichoke extracts. In Figure 3 these results are given as the percentage. The results were presented both as the percentage and milligram amount of the released CA in order stress the content differences. While 100 % of CA was released in all formulations this amount corresponds to almost 25 mg for CP2 and 5 mg for SD and CP1. As it is seen from these data CA was released in a delayed manner from CP2 formulation. This result is compatible with the disintegration results indicating the slow disruption of the gelatin capsule in CP2 formulation (Table 3). According to the in vitro disintegration test performed by Aymes disintegration apparatus the time recorded for the disintegration of CP1, CP2 and SD were 10.97 ± 0.64, 57.25 ± 1.56 and 6 ± 0.30 min respectively. This slow disintegration of CP2 may be related to inappropriate storage conditions such as relative humidity and storage temperature. The hard gelatin capsules have a moisture content of 14.5-16.5 %. In order to preserve this it is necessary to provide appropriate temperature and humidity conditions during both storage and filling. If the capsules are stored at bad conditions they may become brittle, their shapes get distorted or the capsule cap may stick to body thus resist separation and result in delayed disintegration. Besides, a possible interaction of the capsule material with the capsule content might prolong the disintegration time. One proposed mechanism is crosslinking which occurs between the amino acid groups within the gelatin shell and the aldehyde groups of the content inside the gelatin capsule. As a result the thin, tough, and water-insoluble film formed around the capsule content limits the dissolution of the capsule content (23).

**Figure 2.** CA release profiles from the capsules as mg amounts.
In literature, biological activities of CA at different doses were proved by *in vivo* and *in vitro* studies. Also in many clinical studies, CA doses were ranged between 10-60 mg. The biological activities were also determined at nearly these doses (24, 25). Since the real CA amount in CP1 was less than the declared dosage, its biological effect is expected to be lower.

For the case of artichoke, the dry extract prepared by our spray drying technique can be a good product to use it in standardized extracts. The herbal drug manufacturers must focus on producing more standardized extracts by using techniques such as spray drying. This type of a technique may provide standardization on drug release and therapeutic effect. In other drying techniques, the size and the shapes of the particulates are not homogenous. So this may cause some problems to prepare pharmaceutical formulations especially in tablets and capsules.

Besides artichoke pharmaceuticals, various dietary supplements containing artichoke extracts are available on the market in dosage forms such as coated tablets, 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. However, although the majority of consumers trust in the safety and efficacy of these products, a legal regulation is required (26).

As the global use of herbal medicinal products continues to grow and many more new products are introduced into the market, public health issues, and concerns surrounding their safety are also increasingly recognized. Although some herbal medicines have promising potential and are widely used, many of them remain untested and their use also not monitored. This makes knowledge of their potential adverse effects very limited and identification of the safest and most effective therapies as well as the promotion of their rational use more difficult (22).

Pyhtoequivalence and quality control analysis have been performed to judge the efficacy and safety of artichoke extracts which are obtained from Turkish market. With respect to artichoke-based dietary supplements and artichoke pharmaceuticals, a suitable analytical method for the determination of phenolics has been developed. The results of the present work indicate that CA in commercial products was present but with amounts less than required to

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**Table 3.** The results of dissolution and disintegration tests.

<table>
<thead>
<tr>
<th></th>
<th>Time for disintegration ± SE (min)</th>
<th>Time required for 50 % CA release in acidic medium (min)</th>
<th>Time required for 50 % CA release in buffer medium (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CP1</td>
<td>10.97 ± 0.64</td>
<td>17.43±3.611</td>
<td>12.66±0.133</td>
</tr>
<tr>
<td>CP2</td>
<td>57.25 ± 1.56</td>
<td>73.00±8.127</td>
<td>65.47±4.900</td>
</tr>
<tr>
<td>SD</td>
<td>6 ± 0.30</td>
<td>8.993±1.321</td>
<td>11.82±2.28</td>
</tr>
</tbody>
</table>

**Figure 3.** CA release profiles from the capsules as percent amounts.
be contained in a capsule. Also these commercial products have different chromatographic profiles (Figure 1).

So this study revealed that some artichoke extracts which are standardized as CA, marketed in Turkey were not phytoequivalent. This clearly demonstrates the problem of food supplement botanical-sourced products and this problem still remains to be unsolved. The answer to this problem lies in a implementation of a legal regulation, allowing a clear classification of dietary supplements and pharmaceuticals.

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