# COMMERCIAL AND LABORATORY EXTRACTS FROM ARTICHOKE LEAVES: ESTIMATION OF CAFFEOYL ESTERS AND FLAVONOIDIC

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# Commercial and laboratory extracts from artichoke leaves: estimation of caffeoyl esters and flavonoidic compounds content

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**Abstract**

Artichoke leaf extracts are widely used alone or in association with other herbs for embittering alcoholic and soft drinks and to prepare herbal teas or herbal medicinal products. Despite this wide diffusion, the European Pharmacopoeia does not report an official method for the determination of the active principles of artichoke leaf extracts.

This work reports a quali-quantitative determination by HPLC/DAD and HPLC/MS techniques of both cynnamic acids and flavonoids present in some artichoke leaf commercial extracts (Com) compared with two different laboratory extracts (Lab). Most of the commercial extracts showed a similar quali-quantitative pattern with a single exception having five–six times higher value. The quantitative data from the Italian Pharmacopoeia(IP) official method does not evaluate the flavonoidic fraction and showed an overestimation of the caffeoyl esters with respect to the HPLC/DAD results. The proposed HPLC/DAD method was able to completely characterize and quantify this matrix and represents a contribution to better quality control of these herbal extracts.


*Keywords:* Artichoke leaf extract; HPLC/DAD; HPLC/MS; Caffeic acids; Flavonoids

## 1. Introduction

The artichoke (*Cynara scolymus* L.) is not only a good food, known for its pleasant bitter taste, but also an interesting and widespread herbal drug. Artichoke leaf extracts are widely used alone or in association with other herbs for embittering alcoholic and soft drinks and to prepare herbal teas or herbal medicinal products. In addition, the use of the dehydrated leaf meal in the feeding of young bulls is described [1]. Polyphenolic compounds, present mainly in the leaves rather than in the artichoke heads, have been documented as the active principles of this plant. The results from several clinical investigations showed the efficacy and safety of artichoke extracts in the treatment of hepato-biliary dysfunction and digestive complaints, such as loss of appetite, nausea, and abdominal pain [2].

Leaf extracts are reported to enhance detoxification reactions of the liver and have shown cholagogue and choleretic activity [3–5]. The ability to decrease cholesterol levels in the blood by reducing, in

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the liver, the rate of cholesterol synthesis and by removing fat accumulation from the liver and other tissues is also described [3,6,8,9]. Findings from clinical trials have confirmed the anti-hyperlipidemic effects of artichoke extracts, determining a decrease of both total cholesterol and LDL-cholesterol levels [7]. Recently, there is also a growing body of research which indicates therapeutic properties for artichoke leaf extract against irritable bowel syndrome (IBS) [10]. Moreover, a concentration-dependent inhibitory activity against oxidative stress in human leukocytes, when these cells are stimulated with agents that generate reactive oxygen species (ROS), has been observed [11].

The biological activities of artichoke, mainly the marked antioxidative effects [5,8,12], are attributed to caffeoylquinic acid derivatives, and flavonoids such as luteolin glycosides. Caffeoylquinic acids are present in artichoke as mono and dicaffeoyl esters and chlorogenic acid (5-caffeoylquinic acid) with cynarin (1,3-dicaffeoylquinic acid) and its isomer (1,5-dicaffeoylquinic acid) are described as the most abundant molecules [13]. Regarding bioavailability data, in vivo studies have recently demonstrated that the caffeoyl esters are adsorbed, metabolized, and excreted as methylated phenolic acids [14]. Finally, it is worth noting that scanty data are available in literature on the chemical characterization of the artichoke extracts used to carry out biological experiments, not only for the qualitative aspects but mainly for the quantitative ones.

Despite the wide diffusion of herbal extracts from *C. scolymus* L. leaves, the European Pharmacopoeia does not report an official method for determination of the active principles of artichoke till now. An official determination is described in the tenth edition of the Italian Pharmacopoeia (IP) where this determination is made by a spectrophotometric assay at 325 nm after caffeoylquinic acids precipitation [15].

This work reports a quali-quantitative determination by HPLC/DAD and HPLC/MS techniques, both of phenolic acids and flavonoids present in some artichoke leaf commercial extracts (Com) compared with laboratory extracts (Lab) prepared with two solvents at different polarity. The quantitative data from HPLC/DAD and from the Italian Pharmacopoeia official method are also compared and discussed.

## 2. Experimental

### 2.1. Chemicals and reagents

Methanol and water (HPLC grade) were purchased from Redel de Haën (Germany); formic acid (ACS reagent) was from Aldrich Company Inc. (Milwaukee, Wisconsin); caffeic and chlorogenic acid were obtained from Fluka Chemie AG (Switzerland). Cynarin (1,3-dicaffeoylquinic acid) was supplied by Roth (Germany). Caffeoylquinic esters (3-caffeoylquinic acid, 4-caffeoylquinic acid and an unidentified dicaffeoylquinic acid derivative, compounds **3**, **4,** and **10**) were kindly supplied by Professor Corrado Trogolo, Università la Sapienza, Roma. The pure flavonoid compounds, luteolin 7-O-glucoside and luteolin, compounds **8** and **15**, were purchased from Extrasynthèse (Lyon, Nord-Genay, France).

The commercial dry extracts of *C. scolymus* L. leaves were purchased from the Italian market and the dried leaves used for preparation of the laboratory extracts (Lab1 and Lab2) were obtained from Ulrich (Nichelino, Torino, Italy).

### 2.2. Sample preparation

#### 2.2.1. Dried commercial extracts

Four commercial samples were considered (Com1–4), all purchased from Italian factories working with herbal products. The samples for analyses were prepared by dissolving about 3 g of powdered extract in 100 ml of MeOH/$H_2O$ 1:1, this solution guaranteed the maximum solubility for all the samples. The solutions were filtered (0.45 μm) to obtain Com1, Com2, Com3, and Com4, and were directly analyzed by HPLC/DAD and HPLC/MS.

#### 2.2.2. Laboratory extracts

The same batch of dried leaves, purchased from Ulrich, was used to prepare two different laboratory extracts. Lab1 was obtained performing an extraction with water at room temperature, stirring for 10 min, and applying a laboratory ultrasound bath for 30 min. The solution was collected after filtration and squeezing of the solid residue, and then lyophilized. Lab2 was obtained mixing the ground dried leaves with EtOH/$H_2O$ 1:1 at 70 °C for 10 min under magnetic stirring, then filtered, concentrated to dryness and then

dissolved in MeOH/$H_2O$ 1:1 with a final concentration of 3% w/v. These samples were filtered (0.45 μm) and directly analyzed by HPLC/DAD and HPLC/MS.

### 2.3. HPLC/DAD analysis

HPLC/DAD analysis was performed on a Solvent Delivery System P4000 equipped with a UV600LP DAD detector and auto sampler AS3000, managed by a ChromQuest Chromatography data system (all from ThermoFinnigan, San Jose, CA, USA). The column was a 3.0 mm × 150 mm (3 μm) Inertsil ODS-3 (Chrompack, Netherland) equipped with a pre-column (2.0 mm × 10 mm, 5 μm) of the same phase; the oven temperature was 30 °C. The eluents were (A): $H_2O$ adjusted to pH 2.4 by HCOOH and (B): $CH_3CN$. The following concave (type 2) solvent gradient was applied: from 92% A and 8% B to 30% A and 70% B within 50 min. Flow elution was 0.4 ml $min^{-1}$ and 2 μl were injected for all the samples. Chromatograms were acquired at 326 nm and UV-Vis spectra were recorded in the range 200–450 nm.

### 2.4. HPLC/MS analysis

The spectra were registered in negative and positive ion mode, using two different instrumentations. The positive MS spectra were performed by an LCQ electrospray (ThermoFinnigan, San Jose, CA, USA) directly coupled with the HPLC-DAD (Agilent Technologies, Palo Alto, CA, USA) applying the same chromatographic conditions previously described but with a linear gradient shape. Capillary temperature was 220 °C, capillary voltage 3.0 V, source voltage 4.2 kV, tube lens voltage 30 V and collision energy 35%. Negative MS spectra were also performed by an API-Electrospray (Agilent Technologies, Palo Alto, CA, USA) with capillary temperature 350 °C and capillary voltage 3500 V; fragmentors applied were in the range 80–180 V.

### 2.5. HPLC/DAD quantitative procedure

The method of external standard was applied to quantify each compound. Quantification of individual phenols was performed using a five-point regression curve, each point in duplicate, developed through the use of authentic standards, operating in the range 0–40 μg. Measurements were performed at 326 nm for the caffeoylquinic acid and at 350 nm for the luteolin derivatives. The following $r^2$ values were obtained: 5-caffeoylquinic acid (chlorogenic acid) and cynarinm, $r^2 = 0.9999$; 1,5-dicaffeoylquinic acid, $r^2 = 0.9998$; caffeic acid and luteolin 7-O-glucoside, $r^2 = 0.9997$; luteolin, $r^2 = 0.9996$. The HPLC-DAD quantitative data were expressed as milligram per kilogram for both caffeoylquinic acids and flavonoids derivatives, as average of three determinations.

### 2.6. Italian Pharmacopoeia method

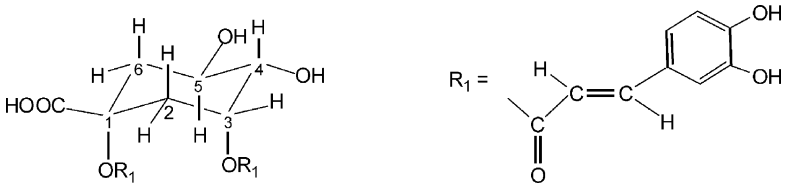
The procedure described in the Pharmacopoeia X edition was precisely applied. This determination was performed by a spectrophotometric assay at 325 nm after caffeoylquinic acids precipitation with lead acetate and resolubilization with acid methanol. Finally, the percentages of caffeoylquinic acids are expressed as chlorogenic acid [15].

## 3. Results and discussion

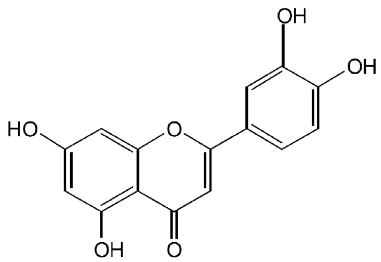
This work reports a quali-quantitative evaluation of the phenolic content of commercial (Com1–4) and experimental (Lab1–2) artichoke leaf extracts, performed applying both a HPLC/DAD determination on reverse phase and the official method indicated by the X edition of the Italian Pharmacopoeia [15].

The identification of each compound was performed by a comparison with available standards and by UV and MS spectra evaluation. This approach made it possible to rapidly discriminate between caffeoyl derivatives and flavonoidic compounds. The MS spectra in API/ES negative ionization mode, due to the low fragmentation level, was strongly diagnostic for the detection of both molecular weight and the characteristic fragmentation of the cynnamoyl and flavonoidic molecules [16]. Such results are in accordance with the findings obtained from the determination of a fingerprint of a total artichoke extract by direct infusion in ESI/MS [17].

The main chemical structures of the identified compounds are depicted in Fig. 1, and in Table 1 are listed the 15 compounds separated with the proposed HPLC method: four monocaffeoylquinic acid esters, five dicaffeoylquinic acid esters and five luteolin derivatives.




Cynarin




Luteolin

Fig. 1. Reference chemical structures of the main compounds: cynarin and luteolin.

A recent study, conducted on artichoke by-products to recover phenolic fractions with antioxidant activities, proposed an HPLC method on reverse phase able to detect only eleven caffeoyl derivatives [18]. Therefore, no data have been reported about the flavonoidic content, mainly constituted by luteolin glycosides [8,19]. Our HPLC method shows the advantages of a shorter elution procedure, the use of a more efficient stationary phase (particle diameter 3 μm) and the determination of the flavonoidic content.

The HPLC profiles of three representative extracts are shown in Fig. 2 with a profile of the caffeoyl derivatives at 326 nm for the richest extract and profiles at 350 nm, respectively, for Com2, Com4, and Lab2.

Table 1
List of the identified compounds with their relative retention time (Rt) values

| | Abbreviations | Rt values (min) | Compound |
|---|---|---|---|
| **1** | 2 CQ ac | 6.67 | 2-O-Caffeoylquinic acid |
| **2** | 5 CQ ac | 7.97 | 3-O-Caffeoylquinic acid |
| **3** | 3 CQ ac | 14.65 | 5-O-Caffeoylquinic acid |
| **4** | 4 CQ ac | 15.5 | 4-O-Caffeoylquinic acid |
| **5** | Caf ac | 17.26 | Caffeic acid |
| **6** | 1,5 di CQ ac | 22.16 | 1,5-O-Dicaffeoylquinic acid |
| **7** | Lut O-glu | 27.09 | Luteolin O-monoglucoside |
| **8** | Lut7-O-glu | 27.98 | Luteolin 7-O-glucoside or cynaroside |
| **9** | Lut-7-O-rut | 28.5 | Luteolin 7-O-rutinoside |
| **10** | A | 29.36 | Dicaffeoylquinic acid derivative |
| **11** | 1,3 di CQ ac | 30.18 | 1,3-O-Dicaffeoylquinic acid |
| **12** | Lut 7-O-glr | | Luteolin 7-O-glucuronide |
| **13** | B | 29.36 | Dicaffeoylquinic acid derivative |
| **14** | C | 31.36 | Dicaffeoylquinic acid derivative |
| **15** | Lut | 36.67 | Luteolin |

For the all considered extracts, the quantitative HPLC/DAD findings related to the distribution of each caffeoylquinic ester and flavonoid are summarized in Tables 2 and 3, respectively.

Among all the commercial extracts, only Com2 differs significantly, appearing largely to be richest in caffeoyl derivatives, with a total polyphenolic content around 10% (Fig. 3) and a percent lower amount of flavonoid with respect to the total dried weight of the extract. The other three commercial samples, all collected from the Italian market as well, showed a very similar pattern both in caffeoyl and luteolin derivatives. It can be concluded that the Com2 sample is obtained by a dedicated multisteps extraction procedure mainly focused to the enrichment in caffeoyl derivatives, while the other extracts were presumably prepared with similar extractive processes.

To evaluate the influence of the solvent on the total amount of extracted phenols, two laboratory extracts were prepared working on the same batch of dried leaves and applying two different extraction procedures: (1) water at room temperature; (2) EtOH/$H_2O$ 1:1 at 70 °C. The first procedure was applied because a recent paper reports data on the biological activity

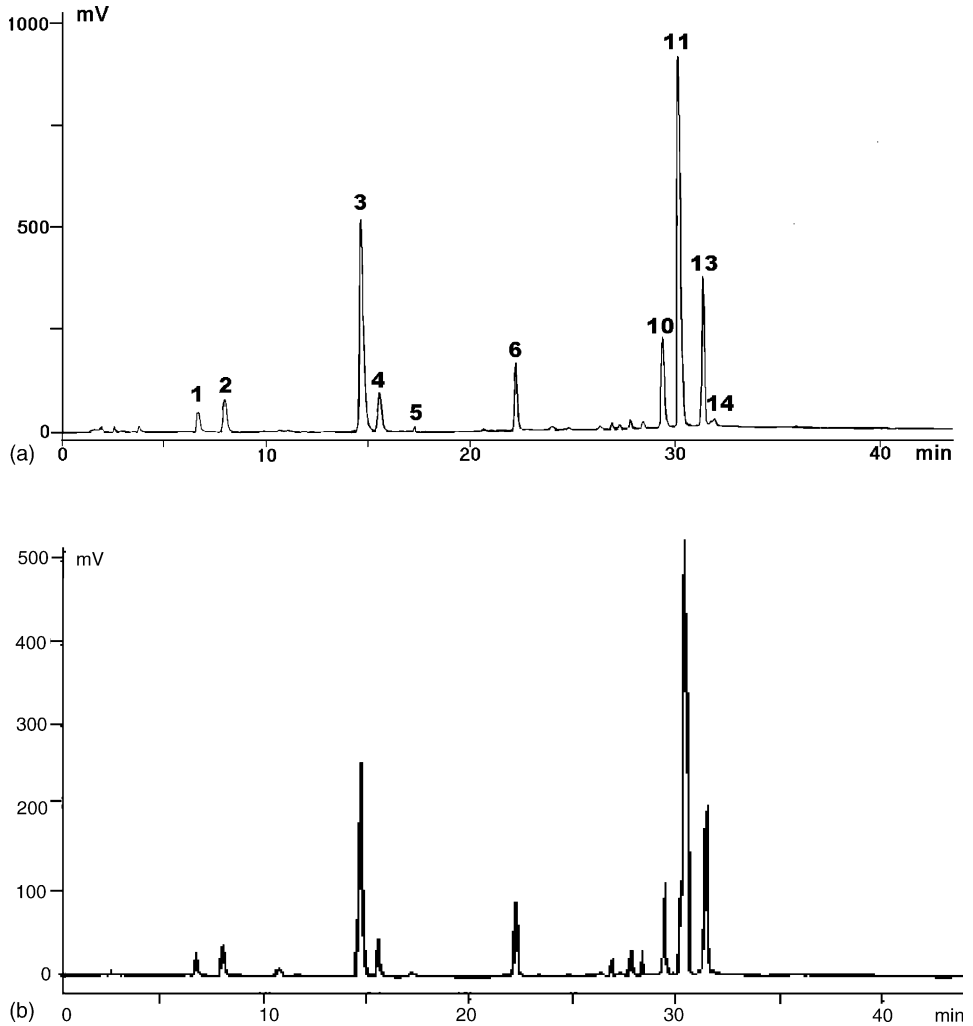



Fig. 2. HPLC/DAD profiles at (a) 326 nm of Com3 and at 350 nm for (b) Com3, (c) Com4, and (d) Lab2, respectively. The list of numbered peaks is in Table 1.

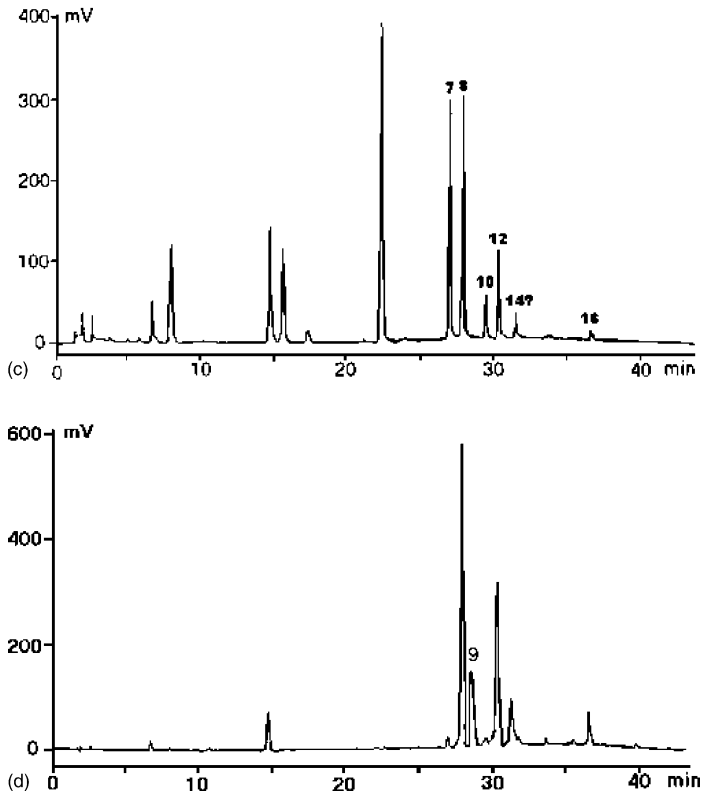



Fig. 2. (*Continued*).

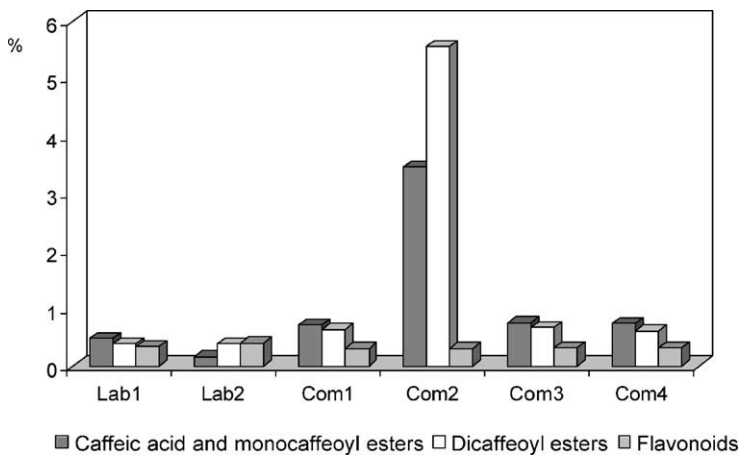



Fig. 3. Comparison between the total contents of monocaffeoyl derivatives, dicaffeoyl esters, and flavonoids both in Com and Lab extracts.

Table 2
Amounts of caffeic acid derivatives expressed as milligram per kilogram of dried extracts

| | 1 | 2 | 3 | 4 | 5 | 6 | 11 | 10 | 13 | 14 |
|---|---|---|---|---|---|---|---|---|---|---|
| Lab1 | 753 ± 5.68 | 444 ± 0.94 | 2359 ± 12.36 | 613 ± 1.34 | 685 ± 4.49 | 770 ± 6.91 | 2425 ± 38.79 | 355 ± 6.52 | 217 ± 4.92 | 230 ± 4.99 |
| Lab2 | 216 ± 0.99 | 97 ± 0.46 | 1235 ± 2.92 | 100 ± 0.68 | n.d. | n.d. | 3331 ± 25.32 | 194 ± 1.92 | 276 ± 1.25 | 100 ± 2.29 |
| Com1 | 829 ± 6.80 | 1725 ± 16.45 | 2783 ± 49.51 | 1743 ± 23.09 | 157 ± 2.09 | 3375 ± 49.53 | 2025 ± 24.01 | 596 ± 1.51 | 350 ± 4.35 | 11 ± 0.04 |
| Com2 | 2021 ± 8.77 | 3528 ± 15.77 | 24088 ± 136.08 | 4776 ± 38.89 | 166 ± 0.48 | 4811 ± 42.22 | 30985 ± 506.99 | 7045 ± 60.82 | 11578 ± 39.05 | 1067 ± 26.92 |
| Com3 | 844 ± 1.39 | 1828 ± 0.41 | 2921 ± 25.04 | 1794 ± 16.61 | 172 ± 2.11 | 3571 ± 31.63 | 2114 ± 26.34 | 633 ± 7.09 | 362 ± 0.63 | 12 ± 0.01 |
| Com4 | 643 ± 12.06 | 2040 ± 11.88 | 2559 ± 18.12 | 2011 ± 20.13 | 171 ± 1.56 | 3945 ± 19.57 | 1292 ± 6.91 | 582 ± 9.80 | 348 ± 6.94 | n.d. |

Data are the mean of three determinations ±S.D. The following calibration curves were applied: chlorogenic acid for the mono caffeoylesters; cynarin (1,3 DCQ) for the dicaffeoylesters; caffeic acid and 1,5 DCQ were calculated with the respective standards. The data are the average of three determinations. n.d.: not determined.

of aqueous artichoke leaf extracts, often with no or scanty information regarding their composition [18].

Moreover, hot extraction (70 °C) with aqueous ethanolic solution represents a common approach to obtain an exhaustive extraction of the phenolic fraction from raw vegetal material. For both Lab1 and Lab2 extracts, the sums of caffeoyl and luteolin derivatives show comparable values to those of Com1, Com3, and Com4, and remained below 1.3%. The highest amounts of free caffeic acid and monocaffeoyl quinic esters were obtained for the sample prepared only with water at room temperature (Lab1). The use of water, compared to EtOH/$H_2O$ 1:1 (Lab2), increases the hydrolysis of dicaffeoyl derivatives while the aqueous/ethanolic solution, even if working at 70 °C, does not modify the content of dicaffeoyl derivatives, but, as expected, increases the amount of flavonoids.

The total amounts of the three main classes of compounds, both in commercial and laboratory extracts expressed as per cent values, are summarized and compared in the histogram of Fig. 3. With the unique exception of Com2, the obtained results did not exceed 1.74%. Observing the flavonoidic content, it appeared mainly constituted by luteolin glycosides with lower values for the aglycone, which was abundant only in the Lab1 sample (Table 3).

Finally, a comparison of the findings obtained from the IP method and HPLC/DAD analysis was carried out. The percent of caffeoylquinic acids with IP method is derived from the caffeoylquinic acid precipitation with lead acetate, dissolution in acid methanol and, finally, a spectrophotometric determination at 325 nm, with data expressed as chlorogenic acid.

As shown in Fig. 4, this complex procedure resulted unable to discriminate among the samples, in fact, almost the same values were obtained for Lab1, Lab2, Com1, Com3, and Com4. Moreover, by applying this procedure, an over-estimation of the results with respect to the more accurate HPLC/DAD analysis (more then 50%) were obtained for all the extracts. Finally it does not supply any information about the flavonoidic content, while this class of compounds can easily be determined by HPLC/DAD and can also modulate the biological activity of the artichoke extracts.

Table 3
Amounts of flavonoid glycosides in both Com and Lab extracts

| | 7 | 8 | 9 | 15 |
|---|---|---|---|---|
| Lab1 | n.d. | 1002 ± 21.78 | 1255 ± 5.29 | 1170 ± 3.44 |
| Lab2 | n.d. | 2917 ± 10.57 | 775 ± 11.00 | 340 ± 1.08 |
| Com1 | 1511 ± 17.05 | 1357 ± 16.43 | 42 ± 2.12 | 181 ± 1.25 |
| Com2 | 788 ± 5.96 | 1052 ± 18.51 | 853 ± 13.71 | 397 ± 0.42 |
| Com3 | 1584 ± 6.52 | 1442 ± 2.59 | 38 ± 0.08 | 203 ± 1.21 |
| Com4 | 1553 ± 12.34 | 1616 ± 14.06 | n.d. | 127 ± 0.68 |

Data (mean of three determinations ±S.D.) are expressed in mg/kg of dried extracts, obtained applying the following calibration curves: luteolin 7-O-glucoside for all the glycosides and the aglycon with luteolin. n.d.: not determined.

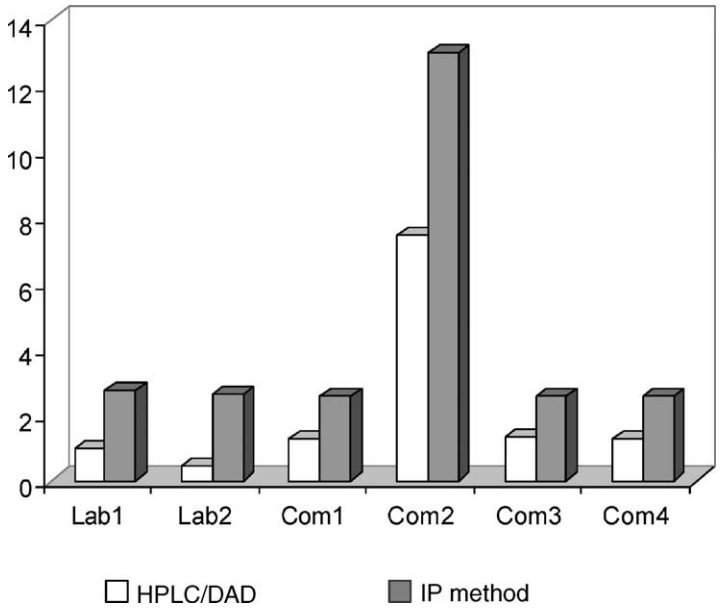



Fig. 4. Comparison between total caffeoyl derivatives, obtained by HPLC/DAD and IP determination. The data are expressed as percent of w/w (dried extracts) applying chlorogenic and caffeic acid calibration curves. Both the mono and dicaffeoylquinic acids are expressed as sum.

## 4. Conclusions

The availability of appropriate analytical procedures to investigate the quali-quantitative content of the active and/or characteristic compounds of herbal mixtures, used as medicinal herbal products, could be of relevant interest to guarantee safety, efficacy, and quality. In light of this consideration, the choice of a rapid, efficient and accurate method able to completely characterize and quantify these complex matrices is one of the main targets in this research field. This work represents a contribution from this perspective.

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