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## DIVERSITY OF ACTIVE CONSTITUENTS IN *CICHORIUM ENDIVIA* AND *CYNARA CORNIGERA* EXTRACTS

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The present study attempts to explore the phytochemical constituents of different extracts from *Cynara cornigera* and *Cichorium endivia* plant materials. The two species studied are native in Egypt. Five different solvents, *viz.*, aqueous, methylene chloride, petroleum ether, ethyl acetate, and *n*-butanol were used. Phytochemical analysis revealed the presence of phenols, flavonoids, sterols (stigmasterol and beta-sitosterol), terpenes ( $\alpha$ -amyrin, ursolic and oleanolic acid), and hydrocarbons (*n*-alkane), the latter found in low amount. The ethyl acetate and water extracts of *C. cornigera* root showed lower mass fractions of phenolic compounds ranged from 20 to 81 g/100 g, and higher amounts in ethyl acetate extract of the inflorescences and butanol extract of the root where values ranged from 195 to 399 g/100 g. The  $\beta$ -sitosterol and stigmasterol were present in all plant extracts. Oleanolic and ursolic acids were detected in roots, leaves and inflorescences of *C. cornigera* and in *C. endivia* shoot. The ethyl acetate extracts from *C. cornigera* leaf and inflorescence attained higher chemical diversity than the other extracts. Alternatively, sterols and triterpenes were the major constituents. The high chemical diversity of active constituents justifies the future potential use of the two species at commercial level.

**Keywords:** Sterols – triterpenes – flavonoids – phenolic acids – Egypt

### INTRODUCTION

The increasing use of plant extracts in food, cosmetic and pharmaceutical industries suggests a systematic study of medicinal plants around the world [49]. Biotic and abiotic stresses exert a considerable influence on the secondary metabolite pool especially the qualitative and quantitative phenolic composition [1]. Polyphenols when associated with various carbohydrates and organic acids exhibit a wide range of pharmaceutical properties including antiallergic, antiatherogenic, antiinflammatory, antimicrobial, antioxidant, antithrombotic, cardioprotective, antioxidants, and vasodilatory effects [2, 5, 36].

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The wild cichory or endive, *Cichorium endivia* L. (Asteraceae) is a Mediterranean perennial herb indigenous to Europe, Western Asia and North Africa [17]. The genus *Cichorium* comprises seven species native to the Mediterranean basin [42]. Strong antioxidant activities were found in the polyphenolic fraction of two *Cichorium* varieties; *Cichorium endivia* var. *crispum* and var. *latifolium* [41], where both varieties contain chlorogenic acid derivatives [14, 26]. Alternatively, different active constituents of other *Cichorium* spp. were reported [27, 36a], and few other studies reported different active constituents and medicinal uses of *Cichorium endivia* [29, 45, 54].

The wild artichoke, *Cynara cornigera* Lindl. (Asteraceae) is a perennial herbaceous species, originating from the Mediterranean region. The species is widely cultivated for economic purposes all over the world [15]. Cynaroside, apigenin-7-O-glucoside, and scolymoside and two sesquiterpens, viz., grosheimin and solstitialin A were identified from the leaf extracts [6, 15, 52, 57]. Extracts from *C. scolymus* are used in folk medicine against liver complaints and to improve liver regeneration after partial hepatectomy [9, 19]. Flower head of *C. cornigera* is eaten as a vegetable and prepared for different value-added products such as salad, concentrate, and canned beverages [56]. The flavonoid kaempferol 3-O-(6-O-malonyl) was reported from the root extracts of the two species [14].

Considering the reported chemical constituents in other species of Asteraceae, this study decided to screen for additional secondary metabolites that might be present in the wild cichory and artichoke native to Egypt. The aim is to explore the active constituents in the two species, particularly the phenolic compounds, terpenes and steroid contents.

## MATERIALS AND METHODS

### *Plant extracts*

Plant materials were collected from wadi Habis, about 30 km west of Matrouh city, Mediterranean coast, Egypt. Individual plants were excavated and separated into different organ components, viz., roots, stems, leaves and inflorescences (flower heads) for wild artichoke and whole plant phytomass for wild cichory. The plant materials were cut into pieces and air-dried in shade at room temperature. The dry material (792 gm) of wild cichory; and leaves (675 gm), roots (864 gm) and flower heads (630 gm) of wild artichoke were ground into powder. Extraction was performed in 90% ethyl alcohol at room temperature till exhaustion, and was then concentrated under reduced pressure to dry at 40 °C using a rotary evaporator. Materials were subjected to successive extraction with petroleum ether, methylene chloride, ethyl acetate, and *n*-butanol using separating funnel. The solvents were used in the order to increase polarity. The residue obtained from each solvent was dried and weighed. The extracts were kept at 4 °C till used for phytochemical screening.

### Phenolic compounds

Qualitative and quantitative determinations of phenolic compounds were carried out according to Dimitrios et al. [12]. The retention time (RT) of the compounds isolated from *C. endivia* and *C. cornigera* was compared with that of corresponding standards under the same conditions and further confirmed by co-injection with isolated standards. The compounds were then injected to a High Performance Liquid Chromatography (HPLC) system at the same time as the solvent extracts and the chromatogram was run with a gradient of CH<sub>3</sub>OH in acetic water (10 to 90% CH<sub>3</sub>OH in water in the presence of 5% CH<sub>3</sub>COOH). Throughout the experiment each peak detected in the range from 254 to 280 nm in 30 min at 20 °C corresponded, as a rule, to a single molecule. Most compounds exhibited absorption peaks in the low-wavelength region 245 nm.

All the HPLC samples (one gram each) were prepared by extraction with 50 ml methanol (Lab scan) and sub-coiled condensed for one hour. After evaporation, the residue was dissolved in methanol to give a final concentration of 0.0025–0.025% (w/v). Filtration over acrodisc-CR removed the insoluble particles. The samples were analysed at room temperature (20 °C) in a Bruker LC 42 (Bruker Franzen Analytic GmbH) with UV diode array detection, LC 21 pump, LC 51 automatic sample analyzer, LC 211 oven, using a spherisorb C16 ODS column, 5 µm, 250 mm × 4.6 mm (Bischoff, Leonberg). The flow rate was 1 ml/min, and the injection volume 5 µl. The operating system was the Chromstar Data system of the Bruker LC 42 connected to an Epson computer. Both the height and the area of a well-resolved peak in a chromatogram were proportional to the concentration of the compounds detected. For quantification, either peak height or peak area was used.

### Lipids and triterpenes

Plant extracts were used for the investigation of lipid and triterpene contents using Unicam Pro-GC with [(Column: 3% OV-17 (Methyl phenyl Silicone) on Chromosorb-WHP); (Mesh: 100–120) and (Dimensions: 1.5 × 4 mm)]. The percentage composition of the extracts was computed from GC peak areas without correction factors. Qualitative analysis was based on a comparison of retention times, indices and mass spectra with the corresponding data base. The conditions of GC used are shown in Table 1.

Table 1  
Separation conditions of unsaponifiable matters during GC analysis

| Temperature Programming |           |                   |            |          |          | Gases Flow Rate |                |            |
|-------------------------|-----------|-------------------|------------|----------|----------|-----------------|----------------|------------|
| Initial temperature     | Rate      | Final temperature | Final time | Injector | Detector | N <sub>2</sub>  | H <sub>2</sub> | Air        |
| 70 °C                   | 10 °C/min | 270 °C            | 50 min     | 250 °C   | 300 °C   | 30 ml/min       | 33 ml/min      | 330 ml/min |

The quantification of Oleanolic acid (OA) and Ursolic acid (UA) followed the method described by Wang et al. [53]. Standard solutions of OA and UA were prepared in final concentration of 25 µg/ml of each compound. The petroleum ether extracts were dissolved in 5 ml methanol then an aliquot of 50 µl extract was diluted in 5 ml methanol. Samples of 20 µl were injected in the HPLC apparatus. The peaks in the chromatogram were identified in comparison to their retention time with the corresponding standards under the same conditions.

## RESULTS

### *Successive extracts yield*

The yield of ethanolic extracts and the successive solvent extraction of *C. endivia* and *C. cornigera* are presented in Table 2. The highest yield of *C. endivia* was obtained from the ethanolic and water extracts with values 18.95 and 16.47 mg/100 g, respectively. The highest yield from *C. cornigera* was obtained from the ethanolic extract of roots (111.22 mg/100 g), followed by leaves (110.24 mg/100 g) and the least amounts in the flower heads (86.38 mg/100 g). Considering the order of extract dependent yield in *C. cornigera*, the order of root extract yield was ethanol > water > ethyl acetate > *n*-butanol > methylene chloride > petroleum ether, while for leaf and flower the yields were ethanol > water > *n*-butanol > ethyl acetate > methylene chloride > petroleum ether.

### *Phenolic compounds and flavonoids*

Twenty phenolic compounds were detected in the two study species, eight flavonoids, viz., Catechin, apigenin, luteolin, luteolin-3-methoxy-7-rutinoside, rutin, quercetin, chrysin and 5-7-dihydroxy-4-methisoflavone and 12 phenolic acids (Tables 3 and 4).

Table 2  
The yield (mg/100 g) of *Cichorium endivia* and *Cynara cornigera* extracts obtained by the different solvents

| Plants                           | Total extract of dry plant material |         |                 |                    |               |                   |        |
|----------------------------------|-------------------------------------|---------|-----------------|--------------------|---------------|-------------------|--------|
|                                  | Dry weight (g)                      | Ethanol | Petroleum ether | Methylene chloride | Ethyl acetate | <i>n</i> -Butanol | Water  |
| <i>C. endivia</i>                | 812                                 | 18.95   | 0.12            | 0.56               | 0.44          | 1.34              | 16.47  |
| <i>C. cornigera</i> roots        | 675                                 | 111.22  | 0.06            | 0.19               | 1.14          | 1.13              | 108.68 |
| <i>C. cornigera</i> leaves       | 864                                 | 110.24  | 0.05            | 0.59               | 1.13          | 1.96              | 106.48 |
| <i>C. cornigera</i> flower heads | 630                                 | 86.38   | 0.05            | 0.40               | 1.48          | 1.85              | 84.44  |

Values rounded to the nearest two decimals.

Table 3  
Retention time (min) and percentage (%) of phenolic compounds in *Cynara cornigera* extracts

| Compound                           | Ethyl acetate (Flower head) |       | Ethyl acetate (Leaf) |       | Ethyl acetate (Root) |       | <i>n</i> -Butanol (Root) |       | Water (Root)         |      |
|------------------------------------|-----------------------------|-------|----------------------|-------|----------------------|-------|--------------------------|-------|----------------------|------|
|                                    | Retention time (min)        | %     | Retention time (min) | %     | Retention time (min) | %     | Retention time (min)     | %     | Retention time (min) | %    |
| Galic acid                         | 14.38                       | 0.09  | ND                   | ND    | ND                   | ND    | 14.68                    | 0.14  | 14.68                | 8.52 |
| Pyrogallol                         |                             |       | ND                   | ND    | ND                   | ND    | 17.87                    | 0.11  |                      |      |
| Chlorogenic acid                   | 22.02                       | 1.75  | ND                   | ND    | ND                   | ND    | ND                       | ND    | ND                   | ND   |
| Resorcinol                         | ND                          | ND    | ND                   | ND    | ND                   | ND    | 22.80                    | 1.11  | 22.43                | 5.55 |
| Catechin                           | 23.59                       | 0.42  |                      |       | 23.87                | 2.10  | ND                       | ND    | 23.83                | 3.60 |
| <i>p</i> -Hydroxy benzoic acid     | 26.39                       | 12.41 | 26.27                | 0.69  | ND                   | ND    | ND                       | ND    | 26.41                | 1.23 |
| Luteolin-3-methoxy-7-rutinoside    | ND                          | ND    | 27.01                | 2.46  | 26.89                | 1.30  | 27.06                    | 0.17  | ND                   | ND   |
| Caffeic acid                       | 29.40                       | 4.40  | 29.34                | 2.25  | 29.49                | 8.86  | 29.6                     | 12.65 | 29.51                | 1.81 |
| Vanillin                           | 29.88                       | 0.42  | 30.00                | 26.37 | 30.04                | 23.03 | 29.83                    | 16.74 | 30.15                | 0.80 |
| Ferulic acid                       | 30.96                       | 2.87  | 30.98                | 4.82  | ND                   | ND    | 30.86                    | 16.33 | ND                   | ND   |
| Rutin                              | ND                          | ND    | 31.53                | 0.23  | 31.47                | 4.81  | ND                       | ND    | ND                   | ND   |
| Phenol                             | 32.16                       | 0.95  | 32.16                | 7.70  | 32.21                | 2.12  | 32.22                    | 0.41  | 32.25                | 0.36 |
| <i>p</i> -Coumaric acid            | 32.39                       | 4.07  | 32.72                | 1.33  | ND                   | ND    | ND                       | ND    | ND                   | ND   |
| Luteoline                          | ND                          | ND    | 34.30                | 0.15  | 34.88                | 0.30  | 34.42                    | 0.05  | ND                   | ND   |
| Quercetin                          | 36.32                       | 0.57  | 36.54                | 2.71  | 36.44                | 1.67  | ND                       | ND    | 36.63                | 0.16 |
| Salicylic acid                     | ND                          | ND    | 37.53                | 0.43  | ND                   | ND    | 37.35                    | 1.11  | ND                   | ND   |
| Apigenin                           | ND                          | ND    | ND                   | ND    | ND                   | ND    | ND                       | ND    | ND                   | ND   |
| Euganol                            | ND                          | ND    | ND                   | ND    | ND                   | ND    | 41.44                    | 0.03  | ND                   | ND   |
| 5,7-Dihydroxy 4-methoxy isoflavone | 43.68                       | 0.35  | 43.60                | 0.38  | 43.49                | 0.63  | ND                       | ND    | ND                   | ND   |
| Chrysin                            | ND                          | ND    | ND                   | ND    | ND                   | ND    | ND                       | ND    | 44.24                | 1.80 |

Values rounded to the nearest two decimals. ND = Not detected.

Table 4  
Retention time (min) and percentage (%) of phenolic compounds of *Cichorium endivia* extracts

| Compound                           | Ethanol              |        | Ethyl acetate        |      | Water                |       |
|------------------------------------|----------------------|--------|----------------------|------|----------------------|-------|
|                                    | Retention time (min) | %      | Retention time (min) | %    | Retention time (min) | %     |
| Gallic acid                        | 14.657               | 4.04   | ND                   | ND   | 14.62                | 6.26  |
| Pyrogalllic                        | 17.70                | 2.33   | ND                   | ND   | 17.72                | 0.41  |
| Chlorogenic acid                   | 22.03                | 1.90   | ND                   | ND   | 21.95                | 0.51  |
| Resorcinol                         | ND                   |        | ND                   | ND   | 22.61                | 2.24  |
| Catechin                           | 23.69                | 2.10   | ND                   | ND   | 23.94                | 16.63 |
| <i>p</i> -Hydroxy benzoic acid     | 26.71                | 18.66  | 26.83                | 7.62 | 26.65                | 0.89  |
| Luteolin-3-methoxy-7-rutinoside    | ND                   | ND     | ND                   | ND   | 26.94                | 0.58  |
| Caffeic acid                       | 29.40                | 1.50   | ND                   | ND   | 27.31                | 0.76  |
| Vanillin                           | ND                   | ND     | 30.06                | 1.65 | 29.69                | 5.35  |
| Ferulic acid                       | 30.98                | 0.65   | 30.96                | 0.60 | ND                   | ND    |
| Rutin                              | 31.23                | 1.31   | ND                   | ND   | 31.21                | 0.55  |
| Phenol                             | ND                   | ND     | 32.02                | 2.29 | ND                   | ND    |
| <i>p</i> -Coumaric acid            | 32.46                | 1.15   | 32.50                | 0.90 | 32.41                | 0.01  |
| Luteoline                          | 34.85                | 0.09   | ND                   | ND   | 34.73                | 0.06  |
| Quercetin                          | 36.28                | 3.10   | ND                   | ND   | ND                   | ND    |
| Salicylic acid                     | 36.98                | 0.56   | ND                   | ND   | 37.36                | 0.08  |
| Apigenin                           | ND                   | ND     | ND                   | ND   | 39.75                | 0.06  |
| Euganol                            | ND                   | ND     | 41.37                | 0.49 | ND                   | ND    |
| 5,7-dihydroxy 4-methoxy isoflavone | ND                   | ND     | 43.71                | 0.50 | 43.37                | 0.04  |
| Chrysin                            | 44.050               | 0.7659 | ND                   | ND   | ND                   | ND    |

Values rounded to the nearest two decimals. ND = Not detected.

All plant organs contained phenolic compounds in different concentrations, ranging between 0.01 and 135.63 mg/100 g (Table 5). Ethyl acetate extracts displayed more phenolic content (0.476–135.63 mg/100 g), while that of the water and *n*-butanol extracts, only approached 38.03 mg/100 g, and 84.89 mg/100 g, respectively.

Only in the butanol root extract of *C. cornigera* the presence of euganol and pyrogalllic acid was observed. Chlorogenic acid, ferulic acid, phenol and *p*-coumaric acid were detected in the organic solvent extracts, while were absent in the aqueous extract. Significant amounts of chlorogenic acid, *p*-hydroxyl benzoic acid, *p*-coumaric acid, caffeic acid, and ferulic acid were detected in ethyl acetate extract of the flower heads, with values 135.63, 121.17, 56.66, 28.22 and 22.76 mg/100 g, respectively. Caffeic acid, vanillin, phenol, and quercetin were present in all *C. cornigera*

Table 5  
The quantitative determination of phenolic compounds (mg/100 g) in different extracts of *Cynara cornigera* and *Cichorium indivia*

| Phenolic compound                  | <i>Cynara cornigera</i> |        |         |        |       |       | <i>Cichorium indivia</i> |       |         |  |
|------------------------------------|-------------------------|--------|---------|--------|-------|-------|--------------------------|-------|---------|--|
|                                    | Ethyl acetate           |        | Butanol |        | Water |       | Ethyl acetate            | Water | Ethanol |  |
|                                    | Flower head             | Leaf   | Root    | Root   | Root  | Root  |                          |       |         |  |
| Galic acid                         | 1.65                    | ND     | ND      | 1.64   | 5.84  | 5.84  | ND                       | 6.05  | 20.35   |  |
| Pyrogalic                          | ND                      | ND     | ND      | 9.66   | ND    | ND    | ND                       | 2.94  | 86.26   |  |
| Chlorogenic acid                   | 135.63                  | ND     | ND      | ND     | ND    | ND    | ND                       | 2.26  | 44.03   |  |
| Resorcinol                         | ND                      | ND     | ND      | 2.43   | 7.49  | 7.49  | ND                       | 4.27  | ND      |  |
| Catechin                           | 16.99                   | ND     | 15.65   | ND     | 5.84  | 5.84  | ND                       | 38.03 | 25.14   |  |
| <i>p</i> -Hydroxy benzoic acid     | 121.17                  | 3.24   | ND      | ND     | 0.49  | 0.49  | 41.01                    | 0.50  | 54.45   |  |
| Luteolin-3-methoxy-7-rutinoside    | ND                      | 6.92   | 1.42    | 0.68   | ND    | ND    | ND                       | 0.19  | ND      |  |
| Caffeic acid                       | 28.22                   | 6.93   | 10.56   | 53.14  | 0.47  | 0.47  | ND                       | 0.27  | 2.87    |  |
| Vanillin                           | 2.67                    | 79.79  | 27.06   | 69.31  | 0.20  | 0.20  | 5.76                     | 1.93  | ND      |  |
| Ferulic acid                       | 22.76                   | 18.32  | ND      | 84.89  | ND    | ND    | 2.64                     | ND    | 1.55    |  |
| Rutin                              | ND                      | 2.22   | 17.58   | ND     | ND    | ND    | ND                       | 0.62  | 7.71    |  |
| Phenol                             | 12.43                   | 48.01  | 5.14    | 3.50   | 0.19  | 0.19  | 16.41                    | ND    | ND      |  |
| <i>p</i> -Coumaric acid            | 56.66                   | 8.87   | ND      | ND     | ND    | ND    | 6.92                     | 0.01  | 4.81    |  |
| Luteoline                          | ND                      | 0.63   | 0.47    | 0.32   | ND    | ND    | ND                       | 0.03  | 0.23    |  |
| Quercetin                          | 5.09                    | 11.57  | 2.77    | ND     | 0.06  | 0.06  | ND                       | ND    | 8.27    |  |
| Salicylic acid                     | ND                      | 8.27   | ND      | ND     | ND    | ND    | ND                       | ND    | ND      |  |
| Apigenin                           | ND                      | ND     | ND      | ND     | ND    | ND    | ND                       | 0.01  | ND      |  |
| Euganol                            | ND                      | ND     | ND      | 0.45   | ND    | ND    | 5.96                     | ND    | ND      |  |
| 5,7-Dihydroxy 4-methoxy isoflavone | 1.53                    | 0.79   | 0.50    | ND     | ND    | ND    | 1.19                     | 0.01  | ND      |  |
| Chrysin                            | ND                      | ND     | ND      | ND     | 0.72  | 0.72  | ND                       | ND    | 2.25    |  |
| Sum of compounds                   | 404.84                  | 195.61 | 81.20   | 255.34 | 21.33 | 21.33 | 79.91                    | 57.38 | 264.76  |  |

ND = Not detected.

organs while gallic acid, luteoline, luteolin-3-methoxy-7-rutinoside, *p*-hydroxyl benzoic acid, 5-7-dihydroxy-4-methoxyflavone and ferulic acid detected in three out of five extracts of *C. cornigera* (Table 5).

The flavonoid composition including catechin rutin and quercetin was the most abundant in *C. endivia*, while chrycin, 5,7-dihydroxy 4-methoxy isoflavone, luteolin, luteolin-3-methoxy-7-rutinoside and apigenin detected as traces. On the other hand, the total flavonoids content found in *C. endivia* was 43.61 mg/100 g and 38.90 mg/100 g in ethanol and water extracts, respectively, followed by ethyl acetate in *C. cornigera* root (38.43 mg/100 g).

The order of yield in the most abundant constituents of different *C. cornigera* extracts was catechin > luteolin-3-methoxy-7-rutinoside > rutin > quercetin > 5,7-dihydroxy 4-methoxy isoflavone, followed by, luteolin-3-methoxy-7-rutinoside, luteoline and chrycin. Flavonoid content ranged from traces to 38.039 mg/100 g (Table 5). It was abundant in ethyl acetate root extract except for quercetin in leaf extract and 5,7-dihydroxy 4-methoxy isoflavone in flower head extract. Total flavonoids of plant organs are in the order of root (54.59 mg/100 g) > flower head (23.62 mg/100 g) > leaf (22.15 mg/100 g). Luteolin was extremely low in all organs of *C. cornigera* and also showed an almost zero level of apigenin. Phenolic compounds were abundant in ethanolic extract of *C. endivia* with about 19 out of 20 compounds. In general, *C. cornigera* extracts had higher phenol concentration than *C. endivia*. The most abundant compounds were pyrogallol in the ethanolic extract (86.26 mg/100 g) and catechin in the aqueous extract of *C. endivia* (38.03 mg/100 g).

### *Lipids and triterpenes*

The plant extracts contained 22 *n*-alkane hydrocarbons (Tables 6 and 7). The identified compounds of methylene chloride formed 75.2–84.7%, where eicosane, octadecane, tridecane and heneicosane were found to be the major constituents in *C. cornigera* (Table 6). The petroleum ether fractions of the leaf and root were present in quite similar amounts, like the methylene chloride fractions. The amounts of octadecane (C<sub>17</sub>, C<sub>18</sub>) and tridecane were significantly higher in the roots (13.97, 12.18 and 11.85%, respectively) than flower heads (4.41, 5.77% and 4.54%, respectively), followed by leaves. The amount of eicosane was higher in leaves (18.99%) than in the flower heads (10.27%). Petroleum ether extract of the flower heads contained eight compounds higher than the leaves & roots which were characterized by high content of eicosane, C<sub>18</sub> and docosane (18.02, 16.6 and 11.7%), while the roots contained high amounts of tridecane and tetradecane (16.2–11.2%). The identified compounds in petroleum ether extract constituted 83.4–95.1% of the total content. Octadecane, eicosane, docosane, tridecane and tetracosane were the major constituents in *C. endivia* (Table 7). The petroleum ether extract contains more hydrocarbons (93.43%) than the crude ethanol extract (62.60%).

Two sterol compounds were identified as stigmasterol and  $\beta$ -sitosterol. The lowest contents were measured in the methylene chloride leaf extract. The major sterol



Table 6  
Percentage composition of hydrocarbon and sterol components in *Cynara cornigera* obtained by GC

| Hydrocarbons                                | Relative %      |                    |                 |                    |                 |                    |
|---------------------------------------------|-----------------|--------------------|-----------------|--------------------|-----------------|--------------------|
|                                             | Root            |                    | Leaf            |                    | Flower head     |                    |
|                                             | Petroleum ether | Methylene chloride | Petroleum ether | Methylene chloride | Petroleum ether | Methylene chloride |
| <i>n</i> -Nonane (C <sub>9</sub> )          | ND              | ND                 | ND              | 4.99               | ND              | 3.26               |
| <i>n</i> -Decane (C <sub>10</sub> )         | ND              | 2.64               | 1.32            | 4.95               | ND              | 1.28               |
| <i>n</i> -Henedecane (C <sub>11</sub> )     | 1.15            | 2.22               | 1.26            | 2.25               | ND              | 4.67               |
| <i>n</i> -Dodecane (C <sub>12</sub> )       | 2.40            | 2.71               | 2.07            | 3.11               | ND              | 6.70               |
| <i>n</i> -Tridecane (C <sub>13</sub> )      | 16.19           | 11.85              | 4.55            | 4.23               | ND              | 4.54               |
| <i>n</i> -Tetradecane (C <sub>14</sub> )    | 11.17           | 1.92               | 7.53            | 2.06               | 2.87            | 2.56               |
| <i>n</i> -Pentadecane (C <sub>15</sub> )    | 4.25            | 1.02               | 9.34            | 7.67               | 7.71            | 6.72               |
| <i>n</i> -Hexadecane (C <sub>16</sub> )     | 1.66            | 1.32               | 3.47            | 3.75               | 10.43           | 4.45               |
| <i>n</i> -Heptadecane (C <sub>17</sub> )    | 0.73            | 13.97              | 1.51            | 2.88               | ND              | 4.41               |
| <i>n</i> -Octadecane (C <sub>18</sub> )     | 12.95           | 12.18              | 14.49           | 4.52               | 16.63           | 5.76               |
| <i>n</i> -Nonadecane (C <sub>19</sub> )     | 0.65            | 2.11               | 0.53            | 4.23               | 3.72            | 3.16               |
| <i>n</i> -Eicosane (C <sub>20</sub> )       | 12.92           | 3.72               | 12.48           | 18.99              | 18.02           | 10.26              |
| <i>n</i> -Heneicosane (C <sub>21</sub> )    | ND              | 9.50               | 0.30            | 4.98               | 1.21            | 4.07               |
| <i>n</i> -Docosane (C <sub>22</sub> )       | 8.06            | 2.54               | 10.10           | 4.33               | 11.71           | 3.17               |
| <i>n</i> -Tricosane (C <sub>23</sub> )      | 1.98            | ND                 | 0.34            | 3.37               | ND              | 4.75               |
| <i>n</i> -Tetracosane (C <sub>24</sub> )    | 6.96            | 6.07               | 7.09            | 2.91               | 9.55            | 2.66               |
| <i>n</i> -Pentacosane (C <sub>25</sub> )    | 0.48            | 1.95               | 0.52            | 2.14               | ND              | 2.44               |
| <i>n</i> -Hexacosane (C <sub>26</sub> )     | 4.30            | 3.67               | 4.30            | 1.32               | 5.91            | 1.77               |
| <i>n</i> -Heptacosane (C <sub>27</sub> )    | 0.21            | 1.24               | 0.55            | 1.51               | ND              | 1.75               |
| <i>n</i> -Octacosane (C <sub>28</sub> )     | 3.03            | 2.37               | 3.48            | 1.20               | 3.70            | 1.29               |
| <i>n</i> -Triacontane (C <sub>30</sub> )    | 0.32            | ND                 | 2.55            | ND                 | ND              | 1.90               |
| <i>n</i> -Hentriacontane (C <sub>31</sub> ) | ND              | ND                 | 1.54            | ND                 | 1.23            | ND                 |
| Total                                       | 89.50           | 83.09              | 89.42           | 85.50              | 92.75           | 81.66              |
| Sterols and triterpene                      |                 |                    |                 |                    |                 |                    |
| $\alpha$ -Amyrin                            | 0.36            | 0.90               | 0.49            | 0.71               | ND              | 1.32               |
| $\beta$ -Sitosterol                         | 1.52            | 0.68               | 0.45            | 0.96               | 1.84            | 1.35               |
| Stigmasterol                                | 2.19            | 1.20               | 3.05            | 0.19               | 2.49            | 1.30               |

Values rounded to the nearest two decimals. ND = Not detected.

components in *C. cornigera* were 4.33% in petroleum ether flower head extract, followed by root extract (4.08%) then leaf extract (3.99%). The main component of methylene chloride extract is  $\alpha$ -amyrin found in the flower head (1.36%), followed by  $\beta$ -sitosterol and stigmasterol (1.35% and 1.30%, respectively).  $\alpha$ -Amyrin was not found in petroleum ether flower head extract, while  $\beta$ -sitosterol was detected in flower head and root extracts with values 1.84% and 1.52%, respectively. Stigmasterol

Table 7  
Percentage composition of hydrocarbon and sterol components  
in *Cichorium endivia* obtained by GC

| Hydrocarbons                               | Relative % |                 |
|--------------------------------------------|------------|-----------------|
|                                            | Ethanol    | Petroleum ether |
| <i>n</i> -Octane (C <sub>8</sub> )         | ND         | 2.59            |
| <i>n</i> -Nonane (C <sub>9</sub> )         | ND         | 7.44            |
| <i>n</i> -Decane (C <sub>10</sub> )        | ND         | 9.24            |
| <i>n</i> -Henedecane (C <sub>11</sub> )    | 10.72      | 11.12           |
| <i>n</i> -Dodecane (C <sub>12</sub> )      | 5.20       | 8.71            |
| <i>n</i> -Tridecane (C <sub>13</sub> )     | 1.70       | 7.38            |
| <i>n</i> -Tetradecane (C <sub>14</sub> )   | 2.29       | 5.85            |
| <i>n</i> -Pentadecane (C <sub>15</sub> )   | 4.01       | ND              |
| <i>n</i> -Hexadecane (C <sub>16</sub> )    | 2.15       | 7.38            |
| <i>n</i> -Heptadecane (C <sub>17</sub> )   | 2.88       | 0.37            |
| <i>n</i> -Octadecane (C <sub>18</sub> )    | 3.80       | 7.51            |
| <i>n</i> -Nonadecane (C <sub>19</sub> )    | 2.11       | ND              |
| <i>n</i> -Eicosane (C <sub>20</sub> )      | 4.19       | 5.77            |
| <i>n</i> -Heneicosane (C <sub>21</sub> )   | 2.75       | 1.67            |
| <i>n</i> -Docosane (C <sub>22</sub> )      | 1.41       | 4.62            |
| <i>n</i> -Tricosane (C <sub>23</sub> )     | 1.93       | ND              |
| <i>n</i> -Tetracosane (C <sub>24</sub> )   | 5.68       | 4.20            |
| <i>n</i> -Pentacosane (C <sub>25</sub> )   | 1.45       | 3.40            |
| <i>n</i> -Hexacosane (C <sub>26</sub> )    | 6.03       | 1.30            |
| <i>n</i> -Heptacosane (C <sub>27</sub> )   | 2.51       | 2.48            |
| <i>n</i> -Octacosane (C <sub>28</sub> )    | 3.65       | 1.69            |
| <i>n</i> -Triacotane (C <sub>30</sub> )    | ND         | 0.63            |
| <i>n</i> -Hentriacotane (C <sub>31</sub> ) | ND         | ND              |
| Total                                      | 93.43      | 62.60           |
| Sterols and triterpene                     |            |                 |
| $\alpha$ -Amyrin                           | 11.33      | 1.17            |
| $\beta$ -Setosterol                        | 6.76       | 0.97            |
| Stigmasterol                               | 3.27       | 1.61            |

Values rounded to the nearest two decimals. ND = Not detected.

was high in petroleum ether shoot extract of *C. endivia* (3.05%), followed by that of the flower head extract (2.49%) and root extract (2.19%) of *C. cornigera*.

Two types of triterpenes were detected in both *C. cornigera* and *C. endivia* extracts. Petroleum ether extract of *C. cornigera* flower heads showed the highest amount (54.7%) of oleanolic acid (Table 8), while *C. endivia* had the highest amount of ursolic acid (88.5%) (Table 9).

Table 8  
Retention time (min) and percentage (%) of triterpene compounds in *Cynara cornigera* extracts of petroleum ether and ethanol

| Compound       | <i>Cynara cornigera</i> |      |                      |      |                      |       |
|----------------|-------------------------|------|----------------------|------|----------------------|-------|
|                | Root                    |      | Leaf                 |      | Flower head          |       |
|                | Retention time (min)    | %    | Retention time (min) | %    | Retention time (min) | %     |
| Oleanolic acid | 2.21                    | 1.70 | 2.60                 | 2.20 | 0.98                 | 54.70 |
| Ursolic acid   | 0.98                    | 95.5 | 0.96                 | 96.3 | 0.26                 | 32.00 |

Table 9  
Retention time (min) and percentage (%) of triterpene compounds in *Cichorium endivia* extracts of petroleum ether and ethanol

| Compound       | <i>Cichorium endivia</i> |       |                      |       |
|----------------|--------------------------|-------|----------------------|-------|
|                | Petroleum ether          |       | Ethanol              |       |
|                | Retention time (min)     | %     | Retention time (min) | %     |
| Oleanolic acid | 2.58                     | 3.40  | 2.26                 | 4.40  |
| Ursolic acid   | 0.93                     | 96.60 | 0.93                 | 88.50 |

## DISCUSSION

The results of phenolic compounds and flavonoids showed that apigenin was not detected in the extracts of *C. cornigera*, while traces of chrysin was found in water extract of the root. These two compounds are reported here for the first time in the two species studied. Apigenin and luteolin were identified in *C. cardunculus* [31]. The chlorogenic acid was identified in the aqueous and alcoholic extracts of *Aster ageratoides* flower buds [11].

The results indicate that *C. cornigera* organs, especially the flower heads could represent an important source of polyphenols with therapeutic activity [34]. According to Gupta et al. [22], flavonoids are generally found in substantial concentrations in plant leaves or flowers. Brás et al. [8] reported the presence of quercetin and rutin in the leaves of *Eupatorium littorale* (Asteraceae). Twelve biologically active flavonoids, mainly flavonol and flavone derivatives, including two glycosides were detected in the glandular trichomes of *Chromolaena* species [25], while a total of 135 different compounds were reported from the Cichorieae (Lactuceae) tribe of the Asteraceae [44]. In *C. cornigera* and *C. endivia*, the high and variable active compound concentrations are related to the arid climate conditions, such as, hot temperature, high solar exposure, drought and salinity which stimulate the biosynthesis of secondary metabolites as polyphenols [13].

The presence of biologically active secondary metabolites such as tannins (gallic acid and catechin), sterols and terpenes contributes to its medicinal value [47]. Tannins have been reported to have several pharmacological activities such as spas-

molytic activity in smooth muscle cells [50]. Gallic acid has antineoplastic and bacteriostatic activities and anticancer properties in prostate carcinoma cells [28], and anti-inflammatory and antibacterial activity [46]. The present study revealed that gallic acid is in range from 1.64 mg/100 g in *C. cornigera* parts to 20.35 mg/100 g in *C. endivia*. There were variations in total phenolic content of different aromatic and medicinal plants ranging between 6.80–32.10 mg gallic acid/g dry weight bases [4, 21]. *p*-Coumaric and ferulic acids are also present in combination with betanidin monoglucoside in fruits of *Basella rubra* [20]. Hydroxycinnamic acid derivatives were found in *C. endivia* var. *crispum* and var. *latifolium*; three derivatives (5-O-caffeoylquinic acid, 3,4-di-O-caffeoylquinic acid, and 5-O-feruloylquinic acid) were found in both chicories, while 3,5-di-O-caffeoylquinic acid was typical of var. *crispum* and cis-caftaric acid of var. *latifolium* [40].

The presence of kaempferol 3-O-(6-O-malonyl) glucoside in endive is reported here for the first time. Methanol extract of *Lactuca sativa* L. varieties (Romaine, Iceberg and Baby) and *C. endivia* L. variety “escarole” have yielded a high phenolic content, where chicory extracts being the richest with 50 mg phenolics/g of freeze-dried extract [14, 35]. Tannins, sesquiterpene lactones, cinnamic acid, caffeic and chlorogenic acids were reported in chicory [27]. Monocaffeoyl tartaric acid, chlorogenic acid, and chicoric acid were detected in different varieties of *Cichorium intybus*, while cyanidin 3-O-glucoside, delphinidin 3-O-glucoside, and cyanidin 3-O-glucoside were the main phenolic compounds in the red varieties [26]. Also, apigenin and apigenin-7-O- $\alpha$ -1-arabinoside were isolated from *Cichorium intybus* aerial parts [55].

The *p*-hydroxybenzoic acid (4-hydroxybenzoic acid) found in *C. cornigera* showed a broad range of pharmacological activities, for instance, antifungal, antimutagenic, antisickling, estrogenic and antimicrobial effects [10, 43]. Chlorogenic acid has been reported to have a number of biological properties, such as antibacterial, antioxidant, hepatocyte protective and antimutagenic activity [51]. Artichoke capitula was characterized by significant amounts of chlorogenic acid and various dicaffeoylquinic esters, especially 1,3-dicaffeoylquinic acid, known as cynarin [7].

Eight phenolic compounds were isolated from *n*-butanol leaf fraction of *Cynara scolymus* which is known for its antimicrobial activity [56, 57]. Also, Orlovskaya et al. [39] have previously reported qualitative and quantitative results on phenolic compounds on raw material and extracts of *C. scolymus* leaves. The phenolic contents in leaf and seed of *Cynara cardunculus* were similar and two times higher than those in flower heads [16]. The organic subfraction of artichoke is rich in polyphenolic compounds, with caffeoylquinic acids and flavonoids as the major chemical constituent [32]. In leaf extracts of commercially available artichoke (*C. scolymus*) a number of compounds were identified, including 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 [18]. Phenolic acids, up to 2%, caffeic acid, mono- and dicaffeoylquinic acid derivatives, e.g., cynarin, and chlorogenic acid

were reported to be present in artichoke [24]. The flavonoids, triterpenoids and saponins were reported to possess hepatoprotective activity in animals [3].

As for lipid and triterpene content, twenty-two compounds of the hydrocarbons were identified in petroleum ether of *C. cornigera* parts. The major components constitute up to 85% of the plant chemical composition, while the minor components reported in trace amounts [37]. A high content of derivatives was also characteristic in *C. endivia* extracts, including hendecane, decane, dodecane, octane and heptadecane. The presence of terpenoids in *C. cornigera* and *C. endivia* has also been reported to show several biological and pharmacological activities such as antioxidative [48], anti-inflammatory [38], hepato-protective effects [33]. The mechanism of action of terpenes is not fully understood but it is speculated to involve membrane disruption by the lipophilic compounds [23].

In conclusion, our findings indicate that *C. cornigera* and *C. endivia* are rich sources of eight important biologically active flavonoids, twelve phenolic acids, two triterpenes and three steroids, which were detected for the first time in the two species studied. Considering that chlorogenic acid and rutin are highly valuable natural polyphenol compounds used as medical and industrial materials, the identification and quantification of these main polyphenols in the present study can be important for potential use at commercial level in the pharmaceutical industry.

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