

COMPOSITION AND PROPERTIES OF CHICORY EXTRACTS RICH IN FRUCTANS AND POLYPHENOLS

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The aim of this work was to evaluate the usability of different parts of chicory for obtaining extracts rich in fructans and polyphenols. The raw material consisted of dried chicory roots, chicory peels, leaves and seeds. It was subjected to water-ethanol extraction and extracts were subsequently lyophilized. The content of fructans and polyphenols was analysed as well as antioxidant capacity using DPPH method was determined in all preparations obtained. It was shown that multistage extraction of chicory roots or leaves using 75% ethanol combined with distillation of the solvent, followed by concentration of the solution and lyophilization led to obtaining dry preparations rich in fructans and polyphenols. Lyophilized fructan-polyphenol extracts produced from roots and peels contained 61.8% and 47.7% of fructans in dry matter, respectively. The content of the most beneficial FOS with DP3÷10 in those preparations reached 47.3% and 34.5% of dry matter, respectively. The preparations contained from 0.5 to 1.7% of polyphenols, with chlorogenic and dicaffeoylquinic acids predominating. The lyophilized extract obtained from chicory seeds was the richest in polyphenol compounds and contained over 10% of total phenolics, including 71% of dicaffeoylquinic acids, and was characterised by the highest antioxidant activity (0.513 mmol TAEC/g of preparation).

INTRODUCTION

Chicory (*Cichorium intybus* L., var. *silvestre* Bisch) roots and leaves are widely used in herbal preparations which display a beneficial influence on bile excretion, diuretic action, gastric juice excretion, as well as stimulation of digestion and metabolism of food ingredients [Jaroniewski, 1994]. These properties are linked to the contents of many bioactive compounds such as fructans, polyphenolic acids (5-caffeoylquinic – chlorogenic, dicaffeoylquinic, chicoric acid), polyphenol glycosides, including derivatives of quercetin, apigenin, luteolin and sesquiterpenes [Hoste *et al.*, 2006; Mulinacci *et al.*, 2001]. The root of chicory (var. *sativum* Bisch) – a plant of commercial usage whose roots are used for the production of coffee substitutes, mixtures of coffee and invigorating drinks [Bais & Ravishankar, 2001; Gałązka, 2002], belongs to the same variety: *Cichorium intybus* L. The roots of chicory are also used for the production of fructose syrups and rarely for the production of ethanol [Bais & Ravishankar, 2001].

The main groups of compounds of the chicory root are carbohydrates, including saccharose, glucose and fructose, fructooligosaccharides and inulin, whose contents reach 21% on average. Inulin is a soluble polyfructan and belongs to a group of dietary fibre. Inulin chains consist of up to 100 D-fructofuranose units linked *via* β -(2→1) glycosidic bonds [Gałązka, 2002]. Fructooligosaccharides, short chain fructans built of 2-10 residues linked *via* β -(2→1) glycosidic

bond and with terminal unit of a glucose molecule linked with a fructose molecule by α -(1→2) glycosidic bond, are structurally similar to inulin [De Leenheer, 1996]. These fructans express prebiotic properties manifested by the stimulation of the growth of beneficial microflora and inhibition of pathogenic and putrid bacteria. Moreover, fructans enhance the absorption of minerals from diet (especially calcium, magnesium and ferrum), improve absorption of vitamins by an organism (mainly from B group) and stimulate gut peristalsis. They reduce the risk of hypercholesterolemia and colonic cancer by decreasing the content of toxic metabolites (including ammonia) and harmful enzymes in faeces (including β -glucuronidase) [Bornet *et al.*, 2002; Król & Zduńczyk, 2005]. Daily intake of 2.5-10 g of fructooligosaccharides (FOS) is recognised as a dose expressing prebiotic activity in humans, however, the average daily intake from natural sources is lower, *ca.* 0.8 g [L'homme *et al.*, 2001]. This fact indicates the necessity of food supplementation with prebiotic FOS.

Root chicory, likewise wild chicory, is a source of sesquiterpenes and polyphenols [Mares *et al.*, 2005; Kocsis *et al.*, 2003; Hoste *et al.*, 2006]. Chicory root has been demonstrated to contain lactones that occur mainly as lactucin, 8-deoxylactucin, lactupicrin and their 11 β 13-dihydro derivatives [Mares *et al.*, 2005; Peters & Amerongen, 1997, 1998], with eudesmanolides and germacranolides occurring in smaller amounts [Peters & Amerongen, 1997; De Kraker *et al.*, 2001].

The predominating group of polyphenolic compounds in chicory are hydroxycinnamic acids represented by mono- (mainly 5-caffeoylquinic acid) and (3,4-, 3,5- and 4,5-) dicaffeoylquinic acids and chicoric acid [Haffke & Engelhard, 1986; Clifford, 2000]. Polyphenolic acids of chicory root express a wide range of health-promoting activities such as antiviral, anti-carcinogenic, antibacterial, anti-inflammatory, antifungal, antimutagenic, immunostimulating and antioxidant. Moreover, they can act against HIV virus, they can protect the alimentary tract and influence the reduction of cholesterol level in blood [Wang *et al.*, 2003; Innocenti *et al.*, 2005; Mares *et al.*, 2005]. Therefore, preparations rich in prebiotic saccharides and polyphenols produced from chicory can be used as supplements promoting healthy properties of a diet.

The aim of this work was to evaluate the usability of different parts of chicory, *i.e.* roots, leaves, and seeds, for obtaining extracts rich in fructans and polyphenols. The production of fructan-polyphenol preparations was performed by multistage alcohol extraction in a laboratory extractor, followed by evaporation of ethanol, concentration and lyophilization of the extracts.

MATERIALS AND METHODS

Material

The material for the research consisted of roots, leaves and seeds of root chicory provided by CYKORIA S.A Co. in Wierchosławice from the harvest of 2006. The roots and the leaves of the chicory were collected at harvest maturity in the middle of October. The roots and the leaves were washed. The roots were divided into two parts. The first one was cut into cubes with overall dimensions 1 cm × 1 cm × 1 cm, the second was decorticated and a peel fraction was obtained. Leaves, roots cut in cubes and the peel fraction after drying in the air-drier at a temperature below 70°C as well as seeds were the material to obtain fructan-polyphenol preparations.

Preparation of freeze-dried extracts rich in fructans and polyphenols

The extractions of fructans and polyphenols were carried in the multistage process, the volume of the extractors (5 or 10 L) as well as the amount of 75% ethanol were adjusted to the amount and the properties of the sample: 2 kg of the cubes of the roots were extracted with 18 L of ethanol, 2.5 kg of dried peels were extracted with 20 L of ethanol, while 1.3 kg of dried leaves and 1 kg of seeds were extracted with ethanol in the proportion: 1 part of the sample to 3 parts of the solvent. Dried materials from the roots and from the peels were extracted once for 3 h, while dried leaves and seeds were extracted 4 times, each time for 5 days. The extracts obtained were distilled and evaporated under vacuum to the concentration of 18 Bx in the case of peels and the cubes, 8 Bx in the case of leaves and 10 Bx in the case of seeds. Concentrated extracts were freeze-dried in a Christ Delta 1-24 LSC lyophilizer (initial temperature of -30°C, the time of lyophilisation 24 h, additional drying at 40°C for 2 h). The yields of extraction ($n=2$) were $17.6 \pm 3\%$ of extract from peels, $33 \pm 2\%$ of extract from root cubes, $10 \pm 1.2\%$

extract from leaves and $3.5 \pm 1\%$ extract from seeds, respectively.

Determination of polyphenol content

The sample of lyophilized chicory extracts (0.05–0.1 g) were dissolved in 80% methanol, then polyphenol content was determined in triplicates by two methods: HPLC with DAD detection and spectrophotometrically.

In chromatographic analysis Dionex system with DAD detector was used (Germering, Germany), coupled with column 4 μ m Fussion-RP 80A 150 × 2.00 mm Phenomenex Synergi (Torrance, CA, USA). Phase A – 0.05% phosphoric acid in water, phase B – 0.05% phosphoric acid in acetonitrile, flow rate 0.25 mL/min, temperature 25°C. Gradient: stabilization for 10 min with 4% of phase B, 0–33 min, 4–50% B, 33–34 min–50% B, 34–35 min–4% B. Absorbance was measured at 325 and 360 nm. All hydroxycinnamic acids were calculated as chlorogenic acid and all phenolic glycosides as quercetin (both standards provided by SIGMA). Hyperoside, quercetin glucoside, apigenin and luteolin were purchased from EXTRASYNTHÈSE Co. Chicoric acid was isolated from leaves as described by Scarpati [1958] and its purity was confirmed by UV spectrum, optical rotation and HPLC. Dicaffeoylquinic acid was an individual substance isolated by semipreparative HPLC (Phenomenex column 250 × 10 mm Torrance, CA, USA, volume of injection 200 μ L, flow rate 5 mL/min, eluent as above) from root extracts and was confirmed by MS spectrum (FAB MS [M-H]⁻ 515,3). Quercetin glucuronide was isolated from the leaves extract by semipreparative HPLC and was confirmed by UV and MS spectrum (Phenomenex column 250 × 10 mm Torrance, CA, USA volume of injection 200 μ L, flow rate 5 mL/min, eluent as above). All aforementioned substances were used as identification standards by comparison of retention times and UV spectra.

For determination of total polyphenols Folin-Ciocalteu method was used [De Pascual-Teresa & Santos-Buelga, 2000]. To 0.5 mL of an extract 0.25 mL of Folin-Ciocalteu reagent was added, then 2.5 mL of 20% Na₂CO₃ and finally the volume was filled-up with water up to 25 mL and mixed. The incubation was carried at room temperature for 1 h. Absorbance was measured with an SP 880 Meratech spectrophotometer at $\lambda=720$ nm. Chlorogenic acid was used as a standard.

Determination of contents and composition of carbohydrates

Ca. 0.5–1 g of the lyophilized extract was dissolved in 10 mL of water and passed through a column filled with a mixture of cation and anion exchangers (1:2 ratio). First 5 mL of the filtrate were rejected and next 4 mL were collected; 2 mL of the filtrate were directly used for analysis.

Fructans, saccharose, glucose and fructose were determined with the HPLC system Knauer Smartline with RI K-2301 Knauer detector (Berlin, Germany) and Aminex HPX-87C (300 × 7.8 mm) column. The elution was carried with water at 85°C at a flow rate of 0.5 mL/min. Glucose, fructose and saccharose were used as standards (SIGMA). In dissolved samples of extracts after desalting and mix-

ing with acetonitrile at a ratio of 1:1 the contents of FOS of DP3 ÷ 10 were determined according to the procedure published elsewhere [Król & Grzelak, 2006]. All analyses were performed in triplicates.

Determination of the antioxidant activity with DPPH radical

The determination was carried according to the modified method of Brand-Williams *et al.* [1995]. A solution of DPPH radical at the concentration of 24 mg/L (60.8 µmol/L) in 80% methanol was prepared. A calibration curve was prepared with Trolox at 0.124 mmol/L (31 mg/L), 248 mmol/L (62 mg/L), 0.495 mmol/L (124 mg/L), and 0.991 mmol/L (248 mg/L) concentration.

The lyophilized extract (1 g) was dissolved in 10 mL of 80% methanol. The sample (0.05 mL) was then added to 1.95 mL of DPPH radical solution, then mixed and incubated for 30 min in the dark. The absorbance of the samples and blank were measured with an SP 880 Meretech spectrophotometer at $\lambda = 515$ nm in comparison to the blank containing 80% methanol. Antioxidant activity was calculated on the basis of a calibration curve prepared for Trolox solutions and expressed as mmol TAEC/g of sample (or mg of TAEC/g of the sample). Antioxidant activity determinations were performed in triplicates.

RESULTS AND DISCUSSION

Lyophilized fructan-polyphenol extracts containing from 95% to 98% of dry matter were obtained as a result of water-ethanol extraction, concentration and freeze-drying. The chemical composition of the extracts is shown in Figures 1, 2 and 3 and in Tables 1 and 2.

The resultant preparations significantly differed in the contents of individual monosaccharides, saccharose, short-chain FOS with DP3 ÷ 10 and total fructans (Table 1). In the composition of carbohydrates of the extracts from peels and root, FOS with DP3 ÷ 10 predominated with the contents of 34.5% and 47.3% respectively, while in the extracts from leaves and seeds, it was glucose (28.5%) and fructose (22.5%), respectively. The content of fructans in the leaf extract was 3.3%, while in the seed one only 1.9%. Data presented in Table 1 show

TABLE 1. Contents and composition of the carbohydrate fraction of lyophilized fructan-polyphenol extracts obtained from different parts of chicory (in per cent of dry matter).

	Source of extract (n=3)			
	Peels	Roots	Leaves	Seeds
Fructose (F)	6.5±0.05	5.9±0.04	22.8±0.2	22.5±0.2
Glucose(G)	3.5±0.03	2.8±0.02	28.5±0.2	12.7±0.1
Saccharose (S)	16.1±0.1	16.3±0.1	12.5±0.1	1.5±0.01
Σ sugars (F+G+S)	26.1±0.2	25.0±0.2	63.8±0.4	36.7±0.3
FOS DP3 ÷ 10	34.5±0.2	47.3±0.2	3.3±0.02	1.6±0.01
Inulin	13.2±0.1	14.5±0.09	nd	0.3±0.03
Fructans (inulin + FOS DP3÷10)	47.7±0.4	61.8±0.3	3.3±0.02	1.9±0.04

nd-not detected

that the preparations obtained using water-ethanol extraction of chicory roots are rich in FOS with DP3 ÷ 10 which have been characterised as prebiotic oligosaccharides [Roberfroid, 2005; Spiegel *et al.*, 1994]. It suggests that the extraction method used in this study can be an alternative way to an effective hydrolysis of aqueous extracts from root chicory, resulting in preparations rich in FOS [De Leenheer, 1994].

The preparations obtained differed in the composition and content of polyphenolic compounds. Figure 1 shows the chromatographic profile of a lyophilized extract from flakes and peels of chicory. Chlorogenic acid was identified on the basis of spectrum and retention time as compared to the standard. Derivatives of chlorogenic acid, most probably dicaffeoylquinic acids, were identified on the basis of UV spectra (Figures 2, 3, 4) and HPLC sequence previously described by Haffke & Engelhard [1986] and Truong *et al.* [2007] and by comparison with individual substance – dicaffeoylquinic acid prepared by semi preparative HPLC. Figure 1 shows that the main polyphenolic components of chicory root extracts are chlorogenic acid occurring at the retention time of 16 min and derivatives of chlorogenic acid appearing at the retention time of 22.8-24.8 min.

Figure 5 shows the chromatographic profile of a lyophilized extract from chicory seeds. The extract from chicory seed contained 5-caffeoylquinic acid (chlorogenic acid) and derivatives of chlorogenic acid with retention times of 23.3 min, 24 min and 24.7 min, respectively. Those compounds were identified as dicaffeoylquinic acids based on UV spectra shown in Figures 6, 7, and 8.

Figure 9 shows the chromatographic profile of a lyophilized extract from chicory leaves. It was found that the main polyphenolic components in this preparation were chicoric acid, quercetin glucuronide and chlorogenic acid (retention times of 24.03, 22.5 and 16.1 min, respectively). Chicoric acid and quercetin glucuronide were identified on the basis of literature data and UV spectra shown in Figures 10 and 11 [Chkhikvishvili & Kharebowa, 2001; Mulinacci *et al.*, 2001] and were compared with individual substances obtained as described in the Materials and Methods section. The content of polyphenols, determined both with HPLC and

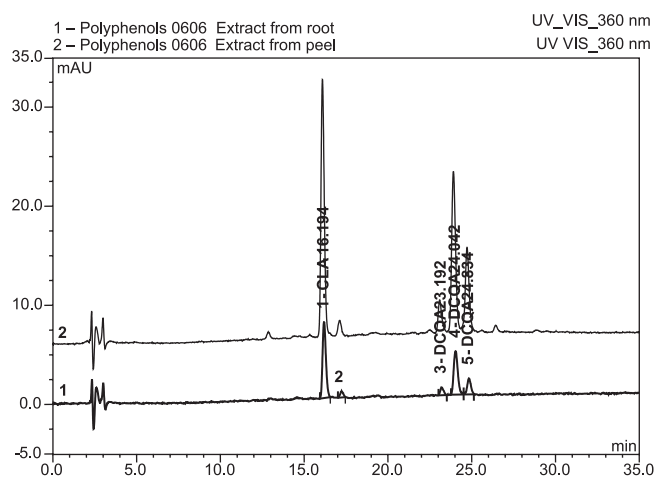


FIGURE 1. Chromatographic profile of a lyophilized extract from root (1) and peels (2) of chicory (CLA – chlorogenic acid, DCQA – dicaffeoylquinic acid).

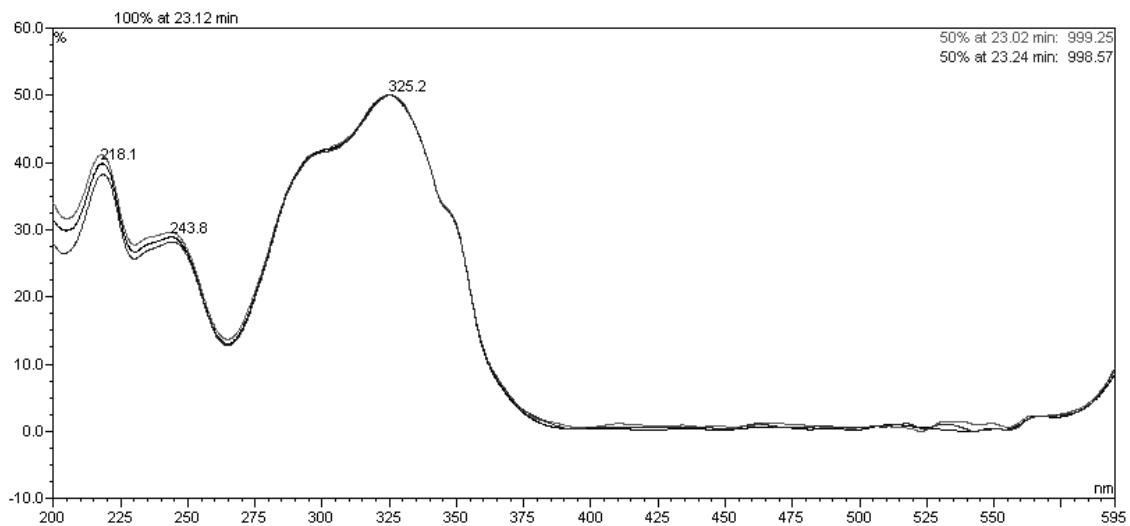


FIGURE 2. UV spectrum of peak 3 (Figure 1) at retention time of 23.1 min.

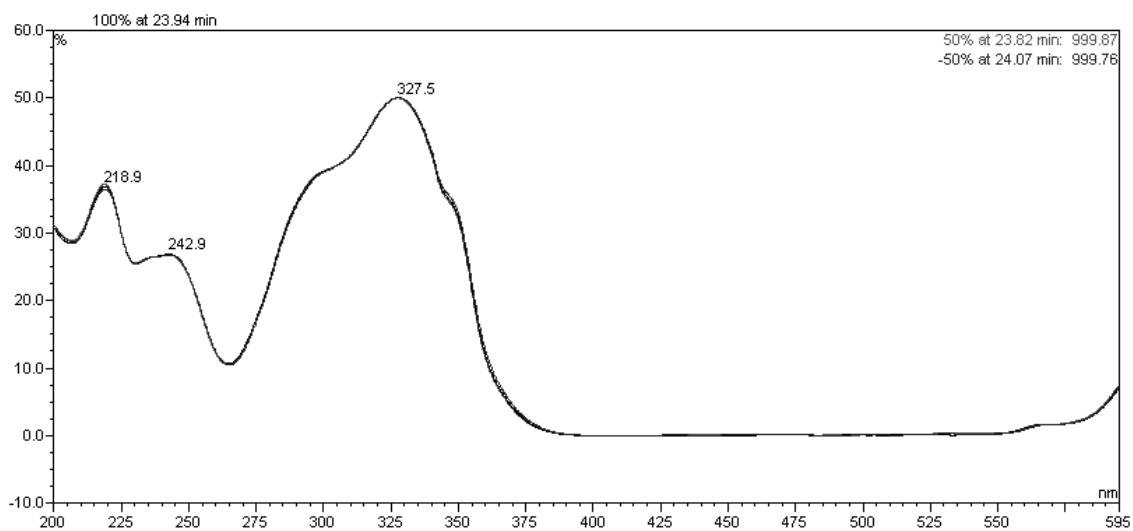


FIGURE 3. UV spectrum of peak 4 (Figure 1) at retention time of 23.9 min.

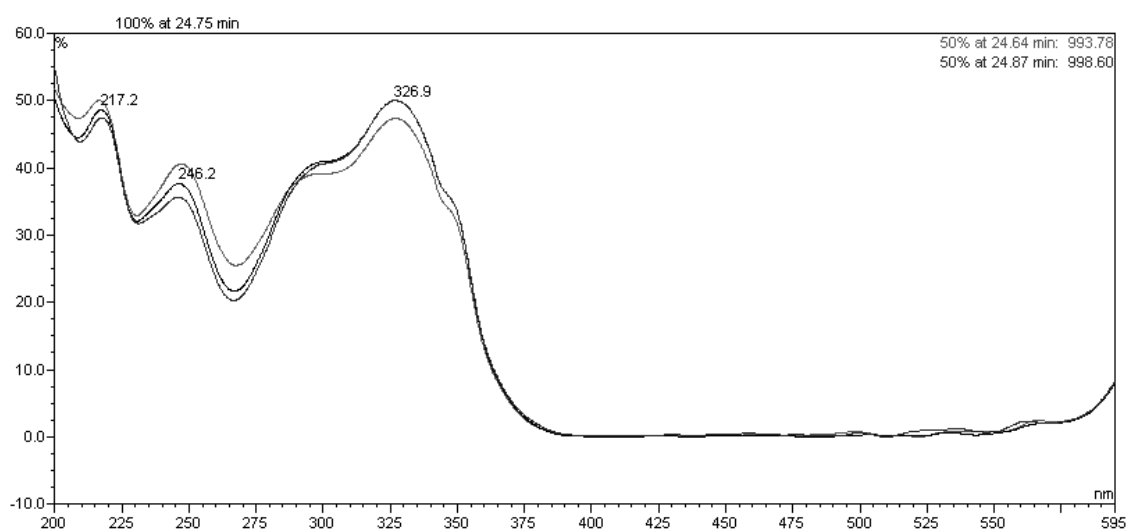


FIGURE 4. UV spectrum of peak 5 (Figure 1) at retention time of 24.8 min.

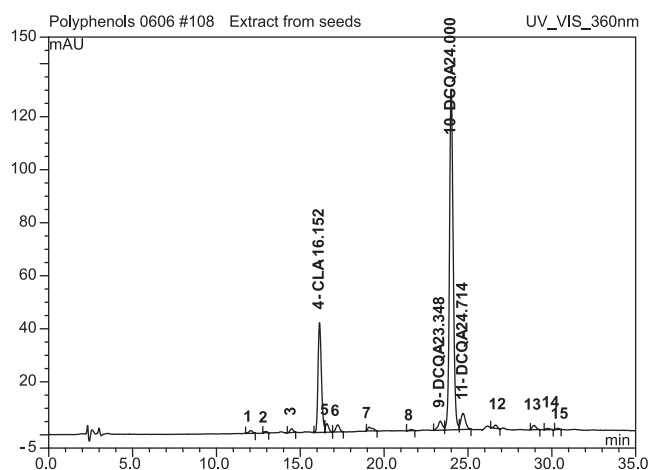


FIGURE 5. Chromatographic profile of a lyophilized extract from chicory seed (CLA – chlorogenic acid, DCQA – dicaffeoylquinic acid).

spectrophotometrically by Folin-Ciocalteu (F-C) method, is presented in Table 2.

The data presented in Table 2 indicate significant differences in the composition of mono- and dicaffeoylquinic acids, as well as chicoric acid and polyphenol glycosides in extracts obtained from different parts of chicory. The extracts obtained from roots and peels contained 5-caffeoylquinic acid (0.3-0.9%) and dicaffeoylquinic acids (0.2-0.8%), (Table 2 and Figure 1), but the extract from peels was more than 3 times richer in phenolic compounds. The content of dicaffeoylquinic acids in total polyphenols in the root extract was at a level of 40–45%. 5-Caffeoylquinic acid and dicaffeoylquinic acids (3.4-3.5- and 4.5- dicaffeoylquinic) had been determined previously in an extract from roasted chicory roots by Haffke & Engelhard [1986]. The seed extract (Table 2 and Figure 2) contained more than 10% of total polyphenols. The most dominant were dicaffeoylquinic acids (71% of total poly-

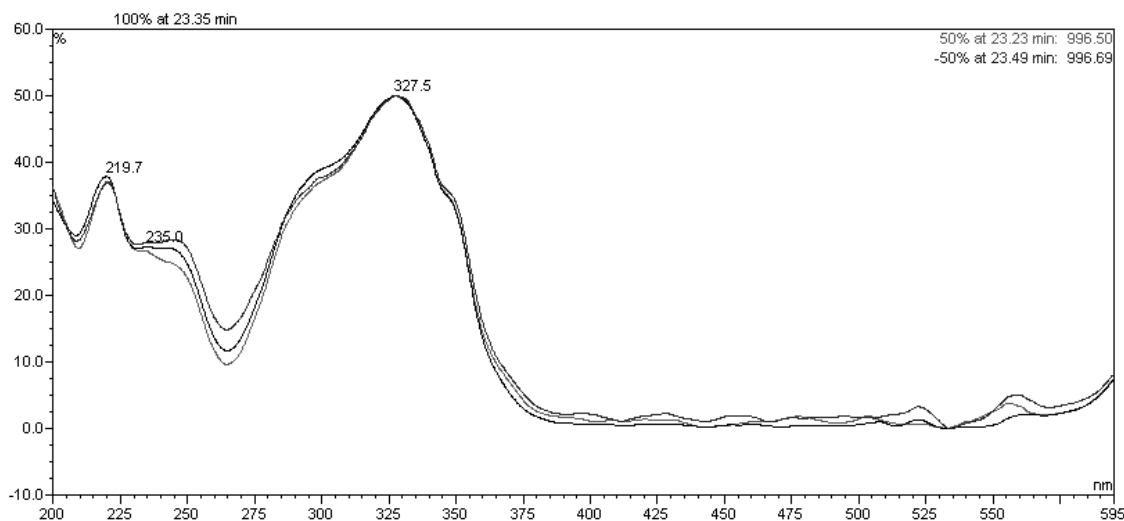


FIGURE 6. UV spectrum of peak 9 (Figure 5) at retention time of 23.3 min.

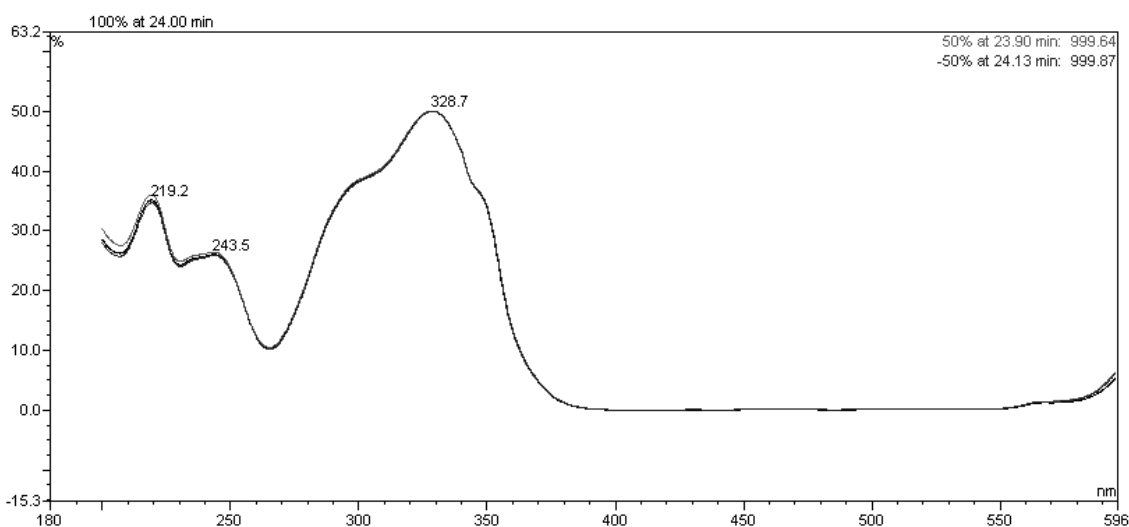


FIGURE 7. UV spectrum of peak 10 (Figure 5) at retention time of 24.0 min.

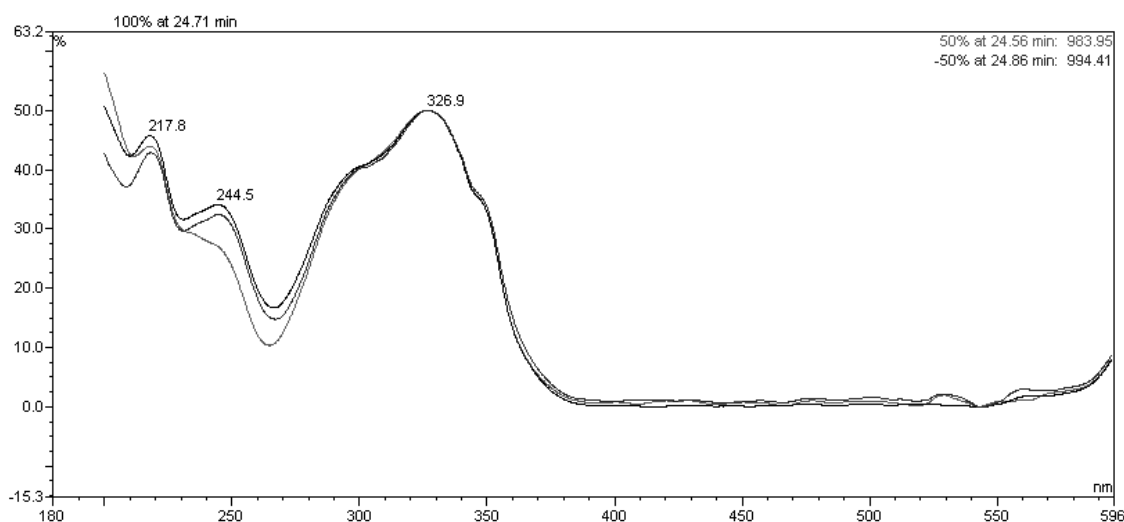


FIGURE 8. UV spectrum of peak 11 (Figure 5) at retention time of 24.7 min.

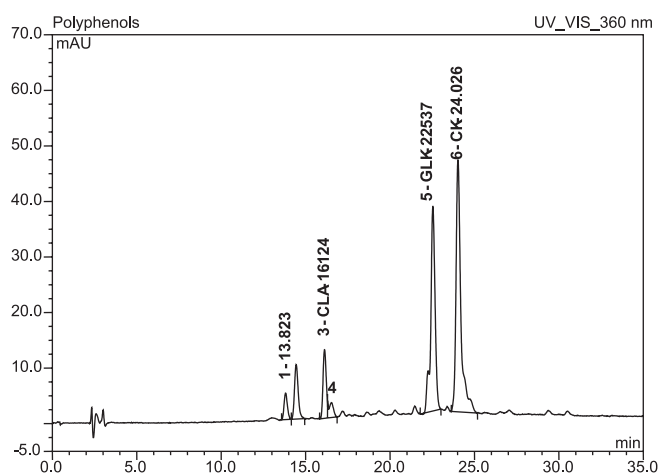


FIGURE 9. Chromatographic profile of a lyophilized extract from chicory leaves (CLA – chlorogenic acid, CK – chicoric acid, GLK – polyphenol glycosides).

phenols) and that is why the seeds of chicory were found to be a good raw material for obtaining the preparations rich in dicaffeoylquinic acids. Available literature provides some data on antihepatotoxic properties of ethanolic extracts from chicory seeds [Ahmed *et al.*, 2003]. Those authors identified the polyphenol fraction obtained from an ethanolic extract from seeds as well as methanol fraction containing polyphenols that showed higher antihepatotoxic properties than ethyl acetate and petroleum ether fractions.

According to available literature data, the leaves of wild chicory and endive are a good source of chicoric acid and quercetin glycosides [Innocenti *et al.*, 2005; Ckhikvishvili & Kharebova, 2001]. The contents of chicoric acid in endive determined by Innocenti *et al.* [2005] ranged from 0.02 to 0.15% and the contents of quercetin-3-glucuronide from 0.02 to 0.11% in fresh leaves depending on cultivar. Ckhikvishvili & Kharebova [2001] obtained chicory leaves extract containing 2.03% of chicoric acid. The contents

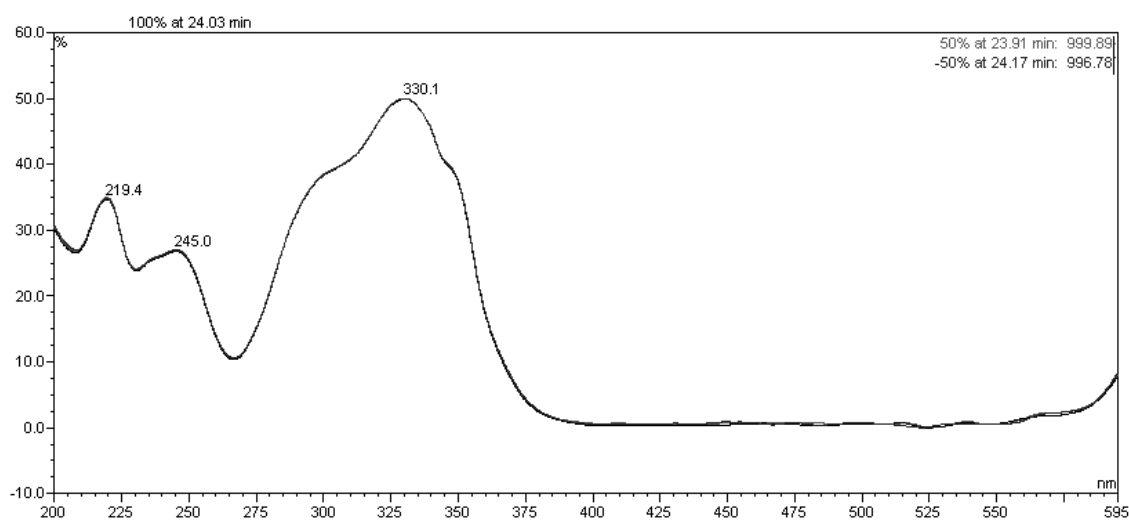


FIGURE 10. UV spectrum of peak 6 (Figure 9) at retention time of 24.03 min.

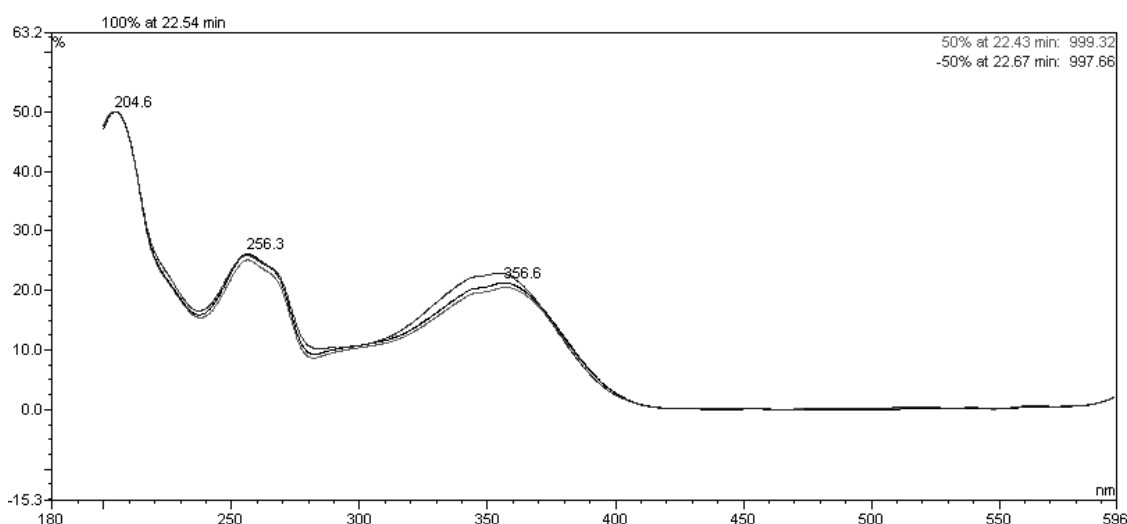


FIGURE 11. UV spectrum of peak 5 (Figure 9) at retention time of 22.54 min.

TABLE 2. Composition of lyophilized preparations of fructan-polyphenol obtained from different parts of chicory (in per cent of dry matter).

	Source of extracts (n=3)			
	Peels	Roots	Leaves	Seeds
Caffeoylquinic acids	0.9±0.01	0.3±0.01	1.2±0.02	2.9±0.01
Derivatives of chlorogenic acid (Dicafeoylquinic acids)	0.8±0.02	0.2±0.01	0.1±0.01	7.2±0.02
Total mono and dicafeoylquinic acids	1.7±0.03	0.5±0.02	1.4±0.03	10.1±0.03
Chicoric acid	-	-	2.2±0.02	-
Polyphenol glycosides	-	-	0.8±0.01	-
Total polyphenols (HPLC)	1.7±0.03	0.5±0.02	4.4±0.09	10.1±0.06
Total phenolics (F-C)	2.4±0.05	0.7±0.04	4.6±0.09	13.6±0.1

of chicoric acid and quercetin glucuronide in the preparation obtained in our study were 2.2% and 0.8%, respectively. As a result, the leaves of root chicory were found to be a valuable raw material to produce chicoric acid-rich preparations. Until present, *Echinacea purpurea* has been the main source of chicoric acid. The chicoric acid content determined by Perry *et al.* [2001] in *Echinacea purpurea* was at a level of 0.52-2.27% depending on the anatomical part of the plant as well as harvest time. Quercetin glucuronide occurs naturally in few plant materials, for example in leaves of blackberry [Fraisie *et al.*, 1996], but many data have pointed out to the formation of quercetin glucuronide during metabolism of quercetin and its glycosides [Erlund *et al.*, 2000; Hollman *et al.*, 1997].

The content of polyphenols determined with the Folin-Ciocalteu method was as follows: 2.4% for the peel preparation, 0.7% for the root extract, 4.6% for the preparation from leaves and 13.6% for the extract obtained from chicory seeds. Their contents obtained with the spectrophotometric method were higher than those obtained by means of HPLC. The former method is a quick and simple technique to characterize polyphenol content but the obtained results are only approximate. More accurate determination of polyphenols is

the HPLC method, which allows the separation of polyphenol groups and individual compounds.

The antioxidant activity of the preparations obtained expressed as TAEC (mmol Trolox/g) of freeze-dried material is illustrated in Figure 12. The extract obtained from chicory seeds was characterised by the highest antioxidant activity, *i.e.* 0.513 mmol TAEC per g of lyophilized material (128 mg TAEC/g). The lowest antioxidant activity was observed in the preparations obtained from chicory roots, *i.e.* 0.026 mmol TAEC/g (6.5 mg TAEC/g) and peels 0.077 mmol TAEC/g (19.3 mg TAEC/g). The antioxidant activity of the preparation from chicory leaves was at a level of 0.207 mmol TAEC/g (51.8 mg TAEC/g) and this was a bit higher as compared to the antioxidant activity of raw methanolic extract estimated by Llorach *et al.* [2004] at 0.143 mmol TAEC/g (36.0 mg TAEC/g), and simultaneously lower than the antioxidant activity of the extract after purification on the Amberlite XAD-2, *i.e.* 0.799 mmol TAEC/g (200 mg TAEC/g) [Llorach *et al.*, 2004]. The lyophilized seed extract possessed a similar antioxidant activity to that of the purified methanol extract from lettuce Baby cultivar described by Llorach *et al.* [2004], *i.e.* 0.513 mmol TAEC/g (128 mg TAEC/g) vs. 0.549 mmol TAEC/g (137.6 mg TAEC/g).

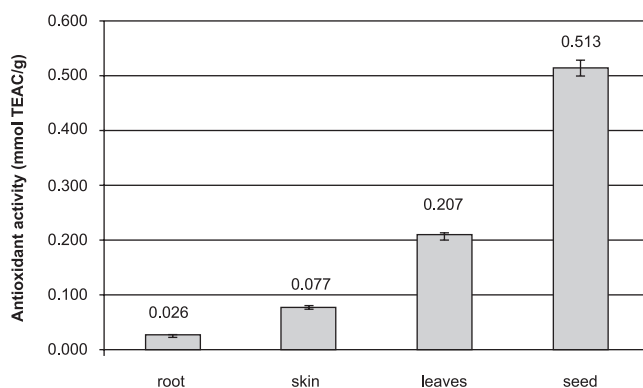


FIGURE 12. Antioxidant activity of lyophilized fructan-polyphenol preparations obtained from different parts of chicory.

CONCLUSIONS

1. Multistage extraction of chicory roots and peels with 75% ethanol followed by solvent distillation, solution concentration and lyophilization resulted in fructan-polyphenol-rich preparations. Fructan content in the preparations was 61.8% and 47.7%, and polyphenol content was 0.5% and 1.7% of dry matter, respectively. Short-chain fructooligosaccharides with DP 3–10 fraction in these preparations were present at a level of 47.3% and 34.5% respectively, and the main polyphenol compounds were chlorogenic acid and dicaffeoylquinic acids.

2. The lyophilized extract from chicory leaves was richer in polyphenols (4.4%) and contained few fructans (3.3%) when compared to extracts from roots and peels. The main phenolic compounds were chicoric acid and quercetin glucuronide. The chicoric acid content was similar as in extracts from wild chicory described by other authors.

3. The richest in polyphenols (more than 10% of dry matter) turned out to be the extract obtained from chicory seeds, in which dicaffeoylquinic acids were predominating (71% of total polyphenols) and which was characterised by the highest antioxidant activity reaching 0.513 mmol TAEC/g (128.3 mg TAEC/g of preparation).

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