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Determination of the total content of metals, polyphenols, flavonoids and the antioxidant capacity of methanolic and chloroformic extracts from *Cichorium intybus*

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Abstract

The study aimed to assess the metal content, total polyphenols, and flavonoids in the plant Cichorium intybus L *gathered from Bihor, Romania, and to emphasize the antioxidant activity of its bioactive constituents. The materials and methods involved the extraction of two types of extracts from the* Cichorium intybus L *plant using the Soxhlet extraction method. The extracts were prepared using two different solvents, namely 50% methanol and chloroform. The purpose of this extraction was to assess and compare the total phenolic and flavonoid content, as well as to determine the antioxidant capacity of the extracts using the DPPH and FRAP methods. The methanolic chicory extract had a higher total content of polyphenolic compounds (57.98 ± 0.60 mg GAE/g dry floss) and flavonoids (5.94 ± 0.08 mg QE/g dry floss) compared to the chloroform extract (55.28 ± 1.11 mg GAE/g dry floss) and (5.54 ± 0.13 mg QE/g dry floss). This indicates that methanol is a more effective solvent for extraction than chloroform. The results of the study indicate that the chloroform chicory extract exhibited a higher percentage of inhibition (74.10 ± 0.22%) compared to the methanolic extract (67.47 ± 0.65%) when tested for antioxidant capacity using the DPPH method. Conversely, the methanol extract from chicory demonstrated a higher antioxidant capacity (856.80 µmol TE/g dry chicory) than the chloroform extract (771 µmol TE/g dry chicory) according to the FRAP method. The* Cichorium intybus L *plant has the potential to yield phytopharmaceutical compounds that can help maintain or enhance human health.*

Keywords: *Cichorium intybus* L, antioxidant capacity, polyphenols, flavonoids

INTRODUCTION

Humans have been using natural plants and herbal mixtures for preventive and phytotherapeutic medical purposes since ancient times [1,2].

In recent years, there has been a growing emphasis on assessing the medicinal properties of secondary metabolites found in plants. Research on secondary metabolites has demonstrated that polyphenolic and

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flavonoid molecules provide a diverse range of favorable therapeutic qualities in humans [2]. These features include cardioprotective, antidiabetic, anti-inflammatory, anti-allergic, and antiviral effects. Additionally, their structures contain hydroxyl groups, which contribute to their antioxidant properties. This enables them to protect the body from oxidative damage produced by internally generated free radicals [3,4]. The antioxidant effects are demonstrated by the processes of free radical neutralization and inhibition of hydroperoxide breakdown into free radicals [5-8].

Assessing the antioxidant potential of extracts derived from plant products poses significant challenges due to the absence of a precise method capable of analyzing the intricate nature of such extracts. Nevertheless, the DPPH (1,1-diphenyl-2-picrylhydrazyl) and FRAP (Ferring Reducing Antioxidant Power) assays are the most commonly employed and widely acknowledged methods by researchers to assess the antioxidant capacity of plant extract samples [9-13].

Cichorium intybus L, commonly referred to as chicory, belongs to the Chicorium genus, which encompasses a total of six plant species, including this particular plant. It is primarily farmed in Europe and Asia [14-16]. The scientific classification of *Chicorium intybus* L is as follows: Kingdom Plantae, Subkingdom Tracheobionta, Division Magnoliophyta, Class Magnoliopsida, Subclass Asteridae, Order Asterales, Family Asteraceae. The genus Chicorium L. consists of the species *Chicorium intybus* [17,18].

Although it is commonly used as a coffee substitute, it has been proven to contain minerals such as potassium (K), iron (Fe), and calcium (Ca), as well as vitamins A, B1, B2, and C. Additionally, it contains bioactive compounds including inulin, sesquiterpene lactones, derivatives coumarins, cichoric acid, phenolic acids, caffeic acid derivatives, flavonoids, carotenoids, anthocyanins, tannins, coumarins, fatty acids, pectin, choline, benzo-isochromides, and alkaline acids. These compounds have significant positive effects on human health [1,15, 16,19]. It possesses choleretic, gastroprotective, antiallergic, antioxidant, antimicrobial, antihepatotoxic, antidiabetic, anti-inflammatory, prebiotic, cardioprotective, and appetite-stimulating properties. Indian medicine has historically utilized it to cure many ailments such as fever, diarrhea, jaundice, gout, and rheumatism [16]. Nevertheless, chicory is mostly employed for the treatment of gastrointestinal problems [1,15].

This study aimed to analyze the components of the plant product derived from *Cichorium intybus* L. in the Bihor region of Romania. The analysis focused on the metal content, extraction of active compounds using various solvents, and characterization of the extracts by determining the total polyphenol and flavonoid content, as well as antioxidant capacity.

MATERIALS AND METHODS

Materials

The following reagents were purchased from the specified suppliers: methanol from Silver Chemicals (Romania), chloroform from Merck Kgaa (Damstadt, Germany), ethanol, sodium carbonate, and sodium nitrite from Promochem, LGC Standards GmbH (Wesel, Germany), quercetin, gallic acid, 6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (Trolox), 2,2-diphenyl-1-picrylhydrazyl, aluminium chloride, and sodium hydroxide from Sigma Aldrich Co (Missouri, USA), Folin-Ciocalteu reagent, 2,4,6-tris(2-pyridyl)-s-triazine (TPTZ), and potassium persulfate from Merck (Darmstadt, Germany), and bidistilled water was obtained from the Milli-Q system Millipore, Bedford (MA, USA). All reagents utilised in the research possessed analytical purity.

The equipment used in this study includes a UV-VIS spectrophotometer T70+ from PG Instruments Ltd in Lutterworth, Great Britain, an analytical balance of Kern ABT 220-5DNM type from Kern and Sohn GmbH in Balingen, Germany, an IKA VORTEX stirrer with a speed range of 0-2500 rpm from Werke GmbH and Co. KG in Straufen, Germany, and rotary evaporators of Heidolph Hei-VAP Precision-Platinum 3 model from Heidolph Instruments Gmbh &Co. KG in Schwabach, Germany.

Methods

Determination of representative metal content in samples of Cichorium intybus L

Determination of zinc and strontium metal ions in the stem, leaves and flowers of the plant product of *Cichorium intybus* L was performed using X-Ray fluorescence spectrometry using an X-Ray fluorescence analyzer Niton – Thermo Fisher Scientific (Massachusetts, USA).

Identification and harvesting of the plant product of Cichorium intybus L

Dry flos of the plant *Cichorium intybus* L that was used in this study was harvested from Bihor County, from an

unpolluted area, far from the urban area. The harvest was done at the end of July, 2020 when the plant was in flowering, one morning, before the flowers open, after the dew has evaporated. Identification of the plant product of *Cichorium intybus* L was performed using literature [20].

Drying and preparation for extraction of Cichorium intybus L plant

After harvesting and washing, the plant product dried in a POL-EKO drying oven SLW 240 GmbH (Langsberger, Germany) at a temperature of 30-35° C, for 24 hours. After drying, the plant product was blended (using a SENCOR Blender SNB 6600, Czech Republic), it was packed in paper bag and stored in a dry place, away from dust and insects. Chicory plant product can thus be stored for a period of 1-2 years.

Extraction processes

Extraction of active compounds from the *Cichorium intybus* L plant product we made it using Soxhlet extraction techniques as extraction method in the following way: place 4 grams of dry, fresh and powdered vegetable product of *Cichorium intybus* L in a Soxhlet apparatus. The extraction is performed for 90 minutes using as solvents 40 mL of 50% methanol and 40 mL chloroform. The chicory extract was concentrated using a rotavapor, which operated at a speed of 80 revolutions/minute, a pressure of 200 atmospheres and a temperature of 40° C until a residue remained on the flask in the form of a film, which is then taken over with 100 mL ethanol. Extractions were carried out in triplicate and the results were expressed as an average ± SD.

Determination of total polyphenol content by Folin-Ciocalteu method

Determination of the total polyphenol content was performed by the Folin-Ciocalteu method which was previously described by Marian E et al. and Frent OD et al. [21,22], in the following way: into a test tube were added 0.1 mL chicory extract, 1.7 mL doubly distilled water and 0.2 mL diluted Folin-Ciocalteu reagent. The test tube mixture was shake with a vortex, and leave to stand for 5 minutes. Then to obtain a pH of 10 and the Folin-Ciocalteu reagent to react with phenolates present in chicory extracts 1 mL of 20 % Na2CO3 solution was added. To get the reaction to the end the test tube was left in the dark for 1.5 hours. After this time, read off the absorbance at the UV-VIS spectrophotometer, at a wavelength of 765 nm relative to the ethanol standard. All determinations were made in triplicate and the results are expressed as a mean value ± SD (standard deviation).

A gallic acid calibration curve was used to determine the concentration of polyphenols. The calibration curve equation for gallic acid is:

$y = 0.0135x + 0.0832$, $R^2 = 0.9963$ (1)

where:
x - the gallic acid equivalent (mg GAE/100 g Vegetable Product)
y - the absorbance recorded at 765 nm.

After reading the absorbance of chicory extracts at a wavelength of 765 nm, the concentration of total polyphenols, expressed in mg gallic acid equivalents (GAE)/100 g dry sample, is calculated using the calibration curve equation.

Determination of total flavonoid content

Determination of the total flavonoid content is carried out by colorimetric method with aluminum chloride, according to the method described by Frent OD et al. [21] to which small changes have been made: introduce into a test tube 1 mL sample of chicory extract over which add 4 mL of doubly distilled water, then 0.3 mL of 5% solution of $NaNO_2$ and shake using the vortex. Leave to stand for 5 minutes, then add 0.3 mL of 10 % $AlCl_3$ solution, shake again and leave to stand for 6 minutes. Then add 2 mL of 1 M NaOH solution and 2.4 mL of doubly distilled water and shake vigorously. Read off the absorbance to the UV-VIS spectrophotometer, at a wavelength of 510 nm, using quartz cells [21]. Determination is made in triplicate and the value is written as a mean value ± SD (standard deviation). Prepare a blank sample using the same way of working, only chicory extract is not added. The results were expressed in mg quercetin equivalents (QE)/g dry flos.

The calibration curve equation for quercetin is:

$y = 0.8259 x - 0.0028$ (2)

where:
y – absorbance of chicory extract sample read at 510 nm;
x – flavonoid concentration of the sample, expressed in mg quercetin equivalents/g dry flos.

Determination of antioxidant capacity of *Cichorium intybus L* ***extracts by DPPH (1,1-diphenyl-2-picrylhydrazyl) test***

To measure the antioxidant capacity of plant extracts, a colorimetric approach was applied using a DPPH reagent, with small changes to the method previously published by Marian E et al. [22].

The DPPH method consists of several steps in the following order: place 2.9 mL of 6 × 10-5 M DPPH solution in a clean, dry test tube freshly prepared, then add 0.1 mL chicory extract, shake the contents of the phial with the vortex, keep in the dark at room temperature for 15 minutes and read off the absorbance at a wavelength of 517 nm.

The blank sample is the DPPH solution. The test is performed in triplicate.

The percentage inhibition of DPPH is calculated using the equation:

$$\% \text{ inhibition} = \frac{A_{blanc} - A_{sample}}{A_{blanc}} \cdot 100 \quad (3)$$

where:
A_{blanc} – absorbance for blank read on 517 nm (t = 0 minute);
A_{sample} – absorbance for sample read on 517 nm (t = 15 minute).

Determination of antioxidant capacity by FRAP (Ferric Reducing Antioxidant Power) test

The FRAP assay was made in accordance with the procedure described by Marian E et al., with some modifications [22]: 0.1 mL chicory extract was placed in a clean, dry test tube, and then was add 0.5 mL FRAP solution and 2 mL doubly distilled water, then shake by means of a vortex. Leave to stand at room temperature for 60 minutes, then read off the absorbance at the wavelength of 595 nm using a UV-VIS spectrophotometer and use as Trolox standard. Samples are made in triplicate.

The FRAP antioxidant capacity of chicory extracts can be calculated using the regression equation (4):

$$y = 0.0017 x + 0.0872 \quad (4)$$

where:
y - absorbance of the sample read from the UV-VIS spectrophotometer at 595 nm, expressed in u. a.;
x – concentration in µmoles equivalent Trolox/sample of 0.1 mL chicory extract.

RESULTS AND DISCUSSIONS

Determination of representative metal content in samples of *Cichorium intybus L*

The experimental data on the quantification of zinc and strontium levels in the flower, leaf, and stem of *Cichorium intybus L* are depicted in Figure 1. The experimental data reveals that chicory leaves contain the highest zinc level (28 ppm), followed by the stem (16 ppm), and the blossom with the lowest concentration (15 ppm). The leaf contains the highest concentration of strontium, with 6 parts per million (ppm), followed by the flower with 5 ppm. The stem has the lowest strontium level, measuring at 3 ppm.

Furthermore, the equipment discovered two other metals (lead and silver), although their quantities are below the device's detection limit.

Determination of total polyphenol content by Folin-Ciocalteu method

In addition, the equipment detected trace amounts of lead and silver, although their concentrations were below the detection threshold of the gadget.

TABLE 1. Calculation of the average concentration of total polyphenols of chicory extracts expressed in mg GAE/100 g dry flos

Sample	Sample concentration (mg GAE/100 g dried chicory)	Mean sample concentration ± SD (mg GAE/g dry floss)
Methanolic extract	58.58 57.88 57.49	57.98 ± 0.60
Chloroform extract	55.91 54.17 55.76	55.28 ± 1.11

The analysis of the data reveals that the methanolic extract of chicory contains a significant amount of total polyphenols, specifically 57.98 ± 0.60 mg GAE/g dry floss. This amount is approximately 1.045% higher than the total polyphenol content found in the chloroform extract, which is 55.28 ± 1.11 mg GAE/g dry floss.

The obtained result is consistent with previous findings from other researchers who examined four different types of cultivated chicory. The total polyphenol content

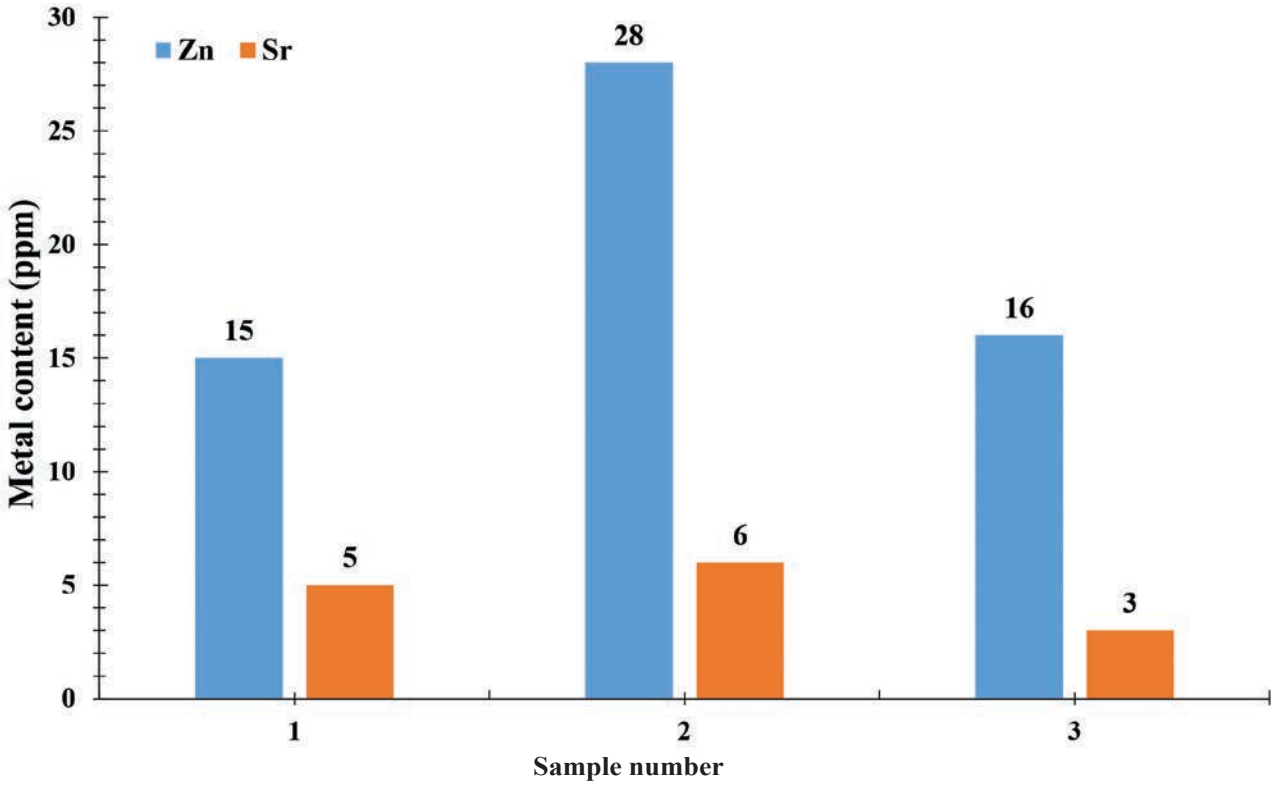

FIGURE 1. Determination of zinc and strontium content in the three parts of the plant product of *Cichorium intybus* ***L: 1 – flower, 2 – leaf, 3 – stem***

in these chicory types ranged from 39.27 - 114.93 mg GAE/100 g in the undried and unfertilized product, 40.57 - 71.23 mg GAE/100 g in the undried product grown with organic fertiliser, 37.53 - 61.20 mg GAE/100 g in the undried product grown with mineral fertiliser, and 28.27 - 120.20 mg GAE/100 g in the undried product grown with both organic and mineral fertilisers [23]. Abbas ZK et al. shown that the hydroalcoholic extract of chicory leaves contains a significant quantity of total polyphenols (85 mg GAE/g), which could be accountable for its antioxidant properties [24].

TABLE 2. Calculation of the average flavonoid concentration of chicory extracts expressed in mg QE/g dry flos

Sample	Sample concentration in flavonoids (mg QE/g dry matter)	Average sample concentration in flavonoids ± SD (mg QE/g dry flos)
Methanolic extract	5.88 6.02 5.91	5.94 ± 0.08
Chloroform extract	5.53 5.67 5.41	5.54 ± 0.13

Quantification of total flavonoid concentration

The outcomes acquired for the quantification of the overall concentration of flavonoids utilising the colorimetric technique, aided by equation (2), are presented in Table 2.

Upon analysing the data in Table 2, it is evident that the methanolic extract of chicory contains a greater quantity of flavonoids (5.94 mg QE/g dry chicory) compared to the chloroform extract (5.54 QE/g dry flos). Once again, this demonstrates that methanol is a superior solvent for extraction compared to chloroform.

The facts acquired align with the findings of current investigations conducted by other researchers. In a study conducted by Abbas ZK et al. it was shown that the dried chicory leaves had a total flavonoid concentration of 6.82 ± 0.07 mg RE/g dry weight [24]. In their study, Sinkovic L et al. examined four different types of cultivated chicory. The total flavonoid content ranged from 1.89 - 9.22 mg QE/100 g in the undried and unfertilized product, from 0.94 - 2.29 mg QE/100 g in the undried product grown with organic fertiliser, from 2.10 - 6.83 mg QE/100 g in the undried product grown with mineral fertiliser, and from 1.25 - 12.58 mg QE/100 g in the undried product grown with both organic and mineral fertilisers [23].

Determination of antioxidant capacity by the DPPH method of *Cichorium intybus L* extracts

The outcomes derived from the implementation of the DPPH approach are displayed as the average value ± SD (standard deviation) in Table 3.

TABLE 3. DPPH test results on chicory extracts

Sample	Percentage of inhibition, %	Percent inhibition mean ± SD, %
Methanolic chicory extract	67.27 68.12 67.01	67.47 ± 0.65
Chloroform extract of chicory	73.88 74.25 74.18	74.10 ± 0.22

Table 3 data indicates that the chloroform extract exhibits a greater inhibition percentage, approximately 1.098% higher than the methanolic extract, against DPPH radicals. Specifically, the chloroform extract demonstrates an average inhibition percentage of 74.10 ± 0.22%, whereas the methanolic extract shows 67.47 ± 0.65%. Based on these findings, it can be concluded that the chicory extracts exhibit a significant level of inhibition, indicating a strong ability to counteract free radicals. The findings align with the outcomes documented in other investigations. Epure A et al. found that the methanolic extract of chicory exhibited an inhibition level of 70.83 ± 2.95% [25].

Determination of the antioxidant capacity by the FRAP (Ferric Reducing Antioxidant Power) method

The antioxidant effect of chicory extracts was determined using the FRAP method, and the results are presented in Table 4.

The FRAP method results indicate that the methanol extract from chicory has a greater antioxidant capacity (856.80 µmol TE/g dry chicory) in comparison to the chloroform extract (771 µmol TE/g dry chicory). The results we obtained for the methanolic extract of chicory are lower than those reported by Epure A et al., who used a 70% methanolic extract of the aerial part of chicory (896.68 ± 27.79 µM TE/g). However, our results are higher than the result obtained for chicory tincture (594.62 ± 18.43 µM TE/g) [25].

TABLE 4. Results of the FRAP method for chicory extracts

Sample	Sample concentration (µmol TE/g dry matter)	Average sample concentration (µmol TE/g dry floss)
Methanolic extract from chicory	882.88 872.35 815.17	856.80 ± 41.63
Chloroform extract from chicory	752.64 781.01 779.62	771.09 ± 18.45

CONCLUSIONS

X-ray fluorescence spectrometry revealed that the leaves of chicory, scientifically known as *Cichorium intybus* L, contain the highest concentration of metals, specifically zinc and strontium, at 28 ppm and 6 ppm, respectively. It was observed that the methanolic extract contained a greater quantity of total polyphenols (57.98 ± 0.60 mg GAE/g dry floss) and total flavonoids (5.94 ± 0.08 mg QE/g dry floss) compared to the chloroform extract (55.28 ± 1.11 mg GAE/g dry floss, 5.54 ± 0.13 mg QE/g dry floss). This indicates that methanol is a more effective solvent for extraction than chloroform. The chicory plant extract contains both flavonoids and polyphenolic components, which possess antioxidant properties and can effectively counteract free radicals.

The chicory extract was evaluated for its in vitro antioxidant capacity using the DPPH and FRAP procedures, both of which are commonly employed spectrophotometric techniques to analyse the potential of certain chemicals found in different plant products. The DPPH method was used to determine the antioxidant capacity of chicory extracts. The results indicate that the chloroform chicory extract exhibited a higher inhibition percentage (74.10 ± 0.22%) compared to the methanolic extract (67.47 ± 0.65%). On the other hand, the FRAP method revealed that the methanol extract from chicory displayed a higher antioxidant capacity (856.80 µmol TE/g dry chicory) in comparison to the chloroform extract (771 µmol TE/g dry chicory). Based on these findings, it can be inferred that chicory, scientifically known as *Cichorium intybus,* is a noteworthy plant for acquiring phytopharmaceutical compounds, which are substances that can contribute to the preservation or enhancement of human health.

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