FOOD PRODUCTION TECHNOLOGY

PHYTOCHEMICAL CONTENTS IN SOLID–LIQUID EXTRACTION OF AQUEOUS ALCOHOLIC EXTRACT OF CHICORY (*CICHORIUM INTYBUS* L.) LEAVES

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Abstract: The object of our current study is to study the phytochemicalc contents in solid-liquid extraction of chicory (*Cichorium intybus* L.) dry leaves grown in Bulgaria. *Cichorium intybus*, commonly known as chicory, is well known as a coffee substitute but is also discretely used as the natural product in food industry and medicine throughout its long history. Solid–liquid extraction was performed by using the 50% aqueous ethanol for 120 min which results in concentration of phytochemical contents and the findings of our present results are well consistent with those obtained in other works. The chicory leaves were analysed for the content of tannin by titrimetric method; rutin was determined spectrophotometrically by using ammonium molybdate; the total phenolics was determined by the Folin-Ciocalteu assay and the total flavonoids was identified through the colorimetric reaction with aluminum (III) chloride. The content of total phenolics and total flavonoids of chicory varied between 2.71 mg GAE/mL for 10 min and 5.65 mg GAE/mL for 120 minutes and 0.84 mg CE/mL for 10 minutes and 2.45 mg CE/mL for 120 min. The content of rutin and tannins that varied within 0.71 percent for 10 minutes and 1.39 percent for 120 min of rutin and tannins was higher than that in 50% aqueous ethanol extract of *Cichorium intybus* L. for 120 min at 1.56% and 1.08% for 10 min, respectively. Extracts obtained positively correlated with their phenolic and flavonoid contents, rutin and tannins, respectively. Therefore, the complex of phytochemical active substance in dry leaves of *Cichorium intybus* L. offers lots of opportunities for future application in herbal medicine and nutrition industry to produce healthy food.

Keywords: Total phenolics, total flavonoids, rutin, tannins, 50% aqueous ethanol extract of Bulgarian dry leaves of chicory (*Cichorium intybus* L.)

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INTRODUCTION

Since ancient times natural phytochemical compounds have been used as food and beverage, as the dye and in traditional medicine sphere. Recently, the capacity of polyphenol has generated too much interest for the large content of the compound to be identified in mere plants whereas the consumption of fruits, vegetables, spices and beverages with the high content of polyphenol may reduce the risk of several diseases due to their antioxidant power, among other factors. The conventional method of polyphenol recovery from plant is based on the solid-liquid solvent extraction. When extracting by the solid-liquid method, soluble components are removed from solids using aqueous organic solvents well mixed. Solvents should be carefully selected to minimize matrix interferences [1, 2], while experimental parameters (temperature, time, pH, solid-to-liquid ratio, particle size, stirring rate, solvent polarity) should all be optimized to obtain the quantitative extraction of molecules required [1, 3].

As a method, solid-liquid extraction is widely applied in various industries. For instance, in the production of herb-based food products; currently, it is used when the plant matrix requests extraction for further processing. Non-expensive and non-toxic solvents (*i.e.*, water) are used and tend to be used in prospective researches in combination with other mild extraction techniques [1].

Cichorium intybus L., commonly known as chicory, refers to *Asteraceae* family and widely distributed in Asia and Europe [4, 5]. All parts of this plant are of great medicinal importance due to a number of compounds of medicinally significance such as alkaloids, inulin, sesquiterpene lactones, coumarins, vitamins, chlorophyll pigments, unsaturated sterols, flavonoids, saponins and tannins [4–9]. The whole plant is validated for numerous applications in food industry and medicine [10, 11]. Its dried roots are used as the substitute or adulterant in coffee powder [10, 12]. The young leaves can be added to salads and vegetable cuisine, while chicory extracts are used for production of invigorating drinks [10]. Chicory leaf is the good sources of phenols, vitamins A and C as well as potassium, calcium, and phosphorus [4, 8]. *C. intybus* has been traditionally used to treat fever, diarrhea, jaundice and gallstones [4, 13, 14]. During the past decade, the interest grew in natural plant extracts with potential antioxidant activity that contributes to the health improvement [10, 15, 16]. The expanded application is due to their properties to protect against oxidative stress disorders, as well as oxidative damage in food products [10, 17]. Polyphenols in plant extracts are well known to have strong antioxidant activity. Found in medicinal plants which are the natural source of inulin-type fructans prebiotics, polyphenols further increase the biological activity of extracts obtained [10, 18, 19]. However, the data on total phenols, total flavonoids, rutin and tannins available in leaves of medicinal plants is not complete. The common chicory (*Cichorium intybus* L.) grows in different regions of Bulgaria. Therefore, this study was intended to evaluate the phytochemical content in solid–liquid extraction of 50% aqueous alcoholic extract of chicory leaves (*Cichorium intybus* L.) grown in Bulgaria.

OBJECTS AND METHODS OF STUDY

Plant material. The chicory leaves (*Cichorium intybus* L.) were harvested from different regions of Bulgaria. All sample data is shown in the sampling report. The dried leaves were kept in a dry place for further use.

Sample preparation. A dry sample of 0.5 g was accurately weighted followed by the extraction of phenolic and flavonoid compounds with 50 mL of 50% aqueous ethanol in the ultrasonic bath for 10 to 120 min. The extract aliquot (2 mL) was ultra-centrifugated for 5 min at 14 000 rpm. The extract preparation was further used for polyphenol determination by spectrophotometric method.

Determination of total phenolics assay. The total phenolic content of *Cichorium intybus* L. was determined by the Folin-Ciocalteu assay [20].

Determination of total flavonoids assay. The total flavonoid content was measured by the aluminum chloride colorimetric assay [20].

Rutin assay. The rutin content in *Cichorium intybus* L.wasanalysed as per the The International Pharmacopoeia and AOAC method, after modified methods with 50% aqueous ethanol [21].

$$R(\%) = \frac{A_{sample} \times C \times 50 \times 100}{A_{stand} \times W \times 2},$$

where A_{sample} is the sample absorbency was determined at 360 nm, A_{stand} is the absorbancy of standard solution was determined at 360 nm, C is the concentration of the rutin standard solution (g/mL), W is the weight (g) of sample for analyses, 2 is the volume (mL) of sample for analyses, 100 is the percent, %.

Tannins assay. The content of tannins in *Cichorium intybus* L.ws analysed as per The International Pharmacopoeia and AOAC method, after modified methods [22].

Calculations. Calculations are based on the averaging analysis results of duplicate samples.

Calculation of the content (%) of tannins (T) in the sample is as follows:

$$T(\%) = \frac{V - V_0 \times 0.004157 \times 250 \times 100}{g \times 25},$$

where V is the volume of 0.1 N water solution of $KMnO_4$ for sample titration, mL; V_0 is the volume of 0.1 N water solution of $KMnO_4$ for titration of blank sample, mL; 0.004157 is the tannins equivalent in 1 mL of 0.1 N water solution of $KMnO_4$; g is the sample mass for analyses, g; 250 is the volume of volumetric flask, mL; 100 is the percent, %.

Statistical analysis. All experiments were performed in triplicates. At every time point, each experiment was carried out in duplicate or triplicate. Statistical parameters are calculated in terms of reproducibility of experimental data using the general Analysis of variance (ANOVA).

RESULTS AND DISCUSSION

The dry matter content in all experimental runs was determined and results were expressed on dry basis to provide more accurate and reliable data comparison. The 50% aqueous ethanol extract of *Cichorium intybus* L. showed the Table 1 and Table 2 in the appendixe of total phenols, total flavonoids, rutin and tannins in qualitative chemical analysis. In our works, we used the solid–liquid extraction by 50% aqueous ethanol for 10 min to 120 min, which gave concentration of polyphenols. The content for total phenolics and total flavonoids of *Cichorium intybus* L. varied within 2.71 mg GAE/mL for 10 min and 5.65 mg GAE/mL for 120 min and 0.84 mg CE/mL for 10 min and 2.45 mg CE/mL for 120 min, accordingly. This is shown in the Table 1 using the gallic acid and catechin as standards. These results indicate that 50% aqueous ethanol extract *Cichorium intybus* L. obtained for 90 min and stopped in 120 min has higher antioxidant activity than 50% aqueous ethanol extract of *Cichorium intybus* L. obtained in 10 min that may

relate to the phenolic and flavonoid content of the leaf extracts.

The flavonoids and phenolic acids are known to have antioxidant activities due to presence of structural hydroxyl groups and they significantly contribute to protect against the oxidative damage due to endogenous free radicals [4, 23]. Phenolic or polyphenols are secondary plant metabolites that ubiquitously present in plants and their products. Many of them are reported to have high levels of antioxidant activities [4, 24]. Due to their redox properties, these compounds contribute to overall antioxidant activities of plants. Usually, the antioxidant activity is to neutralize lipid free radicals and prevent decomposition of hydroperoxides into free radicals [4, 25, 26]. The chicory leaf extract used in this study was partially described with reference to total phenolic and total flavonoid compounds, rutin and tannins. *Cichorium intybus* L. is reorted to be of high medicinal importance due to phytochemical content. The results show that leaves of *Cichorium intybus* L.are a good source of phenolic compounds.

Rutin and tannins contained in 50% aqueous ethanol extract of *Cichorium intybus* L. are of significance. The level of rutin and tannins varied between 0.71 percent for 10 minutes and 1.39 percent for 120 minutes of rutin and tannins. It was found higher as compared with that in 50% aqueous ethanol extract of *Cichorium intybus* L. (120 minutes between 1.56% and 1.08% in 10 minutes, respectively). The results are shown in the Table 2 with the data obtained using rutin as the standard and the potassium permanganate as the titrant. It is important to note that is not correct to compare results for rutin and tannin contents in 50% aqueous ethanol extract of *Cichorium intybus* L. due to different methods of analysis used.

Table 1. Kinetic varieties of 50% aqueous ethanol extract of *Cichorium intybus* L. leaves in total phenols and total flavonoids

min	Total phenols, mg/mL DW	Total flavonoids, mg/mL DW
10	2.71 ± 0.03 RSD 1.3% (n = 3)	0.84 ± 0.05 RSD 6.2% (n = 3)
15	3.23 ± 0.22 RSD 6.9% (n = 3)	1.11 ± 0.01 RSD 0.9% (n = 3)
20	4.10 ± 0.17 RSD 4.2% (n = 3)	1.58 ± 0.05 RSD 3.2% (n = 3)
30	4.76 ± 0.03 RSD 0.7% (n = 3)	2.12 ± 0.03 RSD 1.6% (n = 3)
60	5.43 ± 0.05 RSD 0.9% (n = 3)	2.31 ± 0.02 RSD 3.1% (n = 3)
90	5.65 ± 0.26 RSD 4.5% (n = 3)	2.45 ± 0.08 RSD 3.5% (n = 3)
120	5.65 ± 0.26 RSD 4.5% (n = 3)	2.45 ± 0.08 RSD 3.5% (n = 3)

Table 2. Kinetic varieties of 50% aqueous ethanol extract of *Cichorium intybus* L. leaves in rutin and tannins

min	Rutin, %	Tannins, %
10	0.71 ± 0.02 RSD 2.4 (n = 3)	1.08 ± 0.02 RSD1.6 (n = 3)
15	0.82 ± 0.03 RSD 4.2 (n = 3)	1.15 ± 0.08 RSD 7.5 (n = 3)
20	0.98 ± 0.02 RSD1.7 (n = 3)	1.28 ± 0.02 RSD1.3 (n = 3)
30	1.20 ± 0.03 RSD 2.8 (n = 3)	1.37 ± 0.03 RSD 2.5 (n = 3)
60	1.28 ± 0.02 RSD 1.3 (n = 3)	1.47 ± 0.03 RSD 2.3 (n = 3)
90	1.39 ± 0.06 RSD 4.9 (n = 3)	1.56 ± 0.02 RSD 1.1 (n = 3)
120	1.39 ± 0.06 RSD 4.9 (n = 3)	1.56 ± 0.02 RSD 1.1 (n = 3)

Tannins can then generate smaller phenolic compounds (pyrogallol, catechol, and ellagic acid) with the known bactericidal activity. Tannins are polyphenolic substances with different molecular weight and a variable complexity [22, 27, 28]. Tannins, the polyphenolic compounds with high molecular weight found naturally in lots of plants proved to protect plants against micro-organisms, unfavorable climatic conditions and animal damage. On the other hand, they can form multiple hydrogen bonds with carboxylic groups of dietary proteins and proteolytic enzymes in the gastrointestinal tract which results to the reduced digestibility of proteins and finally the retardation of animal growth [4, 29]. Tannins have many biologically significant functions, such as protection against oxidative stress, and degenerative diseases [22, 27]. Rutin is the glycoside between the flavonol quercetin and the disaccharide rutinose [21, 27]. Rutin is one of bioactive flavonoid compounds found in plants in the considerable amount. The content of total phenolics, tannins, rutin and total flavonoids of the *Cichorium intybus* L. extract and its kinetics are given in Tables 1 and 2 in the annex. Phenolic compounds have such multiple biological effects as anti-atherogenic, antioxidant, anti-inflammatory, cardioprotective, antimicrobial, anticarcinogenic and neuroprotective. Secondary metabolites of plants such as phenolic compounds, terpenoids, alkaloids and lectins have an antimicrobial effect [30–32]

The kinetic study was performed by continuously measuring the absorbency of the extract by the UV–VIS spectrophotometer. The continuous measurement is faster and more accurate for kinetic studies of extraction compared to conventional discontinuous methods. In conventional methods, sampling is manual at given time intervals which is not precise, as there is always a time gap between sampling and analysis, which contributes to errors during kinetic measurements.

In all experiments, the extraction yield was significantly time-dependent and the profile of *Cichorium intybus* L. rises rapidly with time at first, getting less and less quick as the extraction progresses. This behavior can be explained by the fact that during the initial stage of extraction, when the solvent penetrates into the solid, an extremely high concentration gradients develop resulting in higher rates of mass transfer into the liquid phase. As the extraction time increases, the mass transfer of solutes from the solid to the fluid phase gets more difficult, due to the decrease in concentration driving force due to solid and liquid phases. In addition, as the extraction time proceeds, the concentration in the solid phase decreases and both the mixture solubility and the extraction rate decrease simultaneously. In all experiments, a higher extraction yield was reported, especially within 10 to 90 min, with the lower yield from 90 to 120 min.

The analysis of aqueous ethanol extract of *Cichorium intybus* L. for its phytoconstituentsshowed that dry leaves of *Cichorium intybus* L .are rich in total phenols, total flavonoids, rutin and tannins to some extent. It is well known that in general plant flavonoids and phenols act as highly effective free radical scavenging and antioxidants. The phytochemical screening and quantitative estimation of the chemical constituent percentage were evaluated in plants to prove that dry leaves of *Cichorium intybus* L. are rich in rutin and tannins. Phytochemicals, the plant-derived non-nutritive compounds, refer ещ one of different types of alimentary factors which play an important role in various functions of the human body. A great number of natural compounds found in food materials are reported to have antioxidant properties due to hydroxyl groups available in their structure. The antioxidants are the synthetic as well as natural compounds that prevent the oxidative damage to most important macromolecules such as lipids, proteins and nucleic acids present in human body as well as in food products by removal of free radicals generated through various biochemical processes [4, 33]. Free radicals generated through the reaction between oxidative stress radicals and lipids, proteins and nucleic acids cause apoptosis stimulation resulting in various neurological, cardiovascular and some other physiological disorders [4, 34].

CONCLUSION

In conclusion, the results of this research showed that total phenolic, total flavonoid, rutin and tannin contents are significant components in 50% aqueous ethanol extract of dry leaves of *Cichorium intybus* L. grown in Bulgaria. Extracts positively correlated with their phenolic contents and flavonoids contents, and rutin and tannins respectively. Therefore, this complex of phytochemical active substances in dry leaves of *Cichorium intybus* L. offers various fields of prospective applications in herbal medicine and nutrition for healthy food production.

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