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QUANTITATIVE DETERMINATION OF HYDROXYCINNAMIC ACIDS IN CHICORY ROOT

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Abstract. The possibility for applying of a direct spectrophotometry to quantify totality of hydroxycinnamic acid derivatives is investigated. As a quantitative criterion for quality assessment of chicory roots the total content of hydroxycinnamic acid derivatives of at least 0.3 %, expressed as chlorogenic acid, is suggested.

Keywords: chicory roots, quantitative assay, hydroxycinnamic acids, spectrophotometry.

1. Introduction

Chicory (*Cichorium intybus*) is one of the most popular medicinal plants and has a wide range of applications. For many centuries, it has been used in folk medicine for diabetes, diseases liver, biliary tract, kidney, and other [1]. Biologically active substances chicory roots – phenolic compounds, polysaccharides, flavonoids – have a broad spectrum of activity, therefore, the plant material is considered as a promising source for the development of new effective drugs. Despite the long positive experience of its use in traditional medicine the roots of chicory are studied insufficiently and have not been standardized, which to some extent reduces their application for the development of medical products. In turn, diversification of phytotherapeutic medicines leads to increase of quality requirements for medicinal plant material, improved approach to its analysis, as well as standardization and quality control [2] in order to provide the expected and reproducible efficacy and safety of finished products developed on its basis.

The hepatoprotective properties of chicory are due to its antioxidant effect [3]. Hepatoprotective activity of the chicory root extract is caused by phenolic compounds, among which the most important representatives are represented by phenolic acids and their derivatives: phenylacetic, chlorogenic, neochlorogenic, isochlorogenic, 3-feruloylchinnic, 3-*n*-coumaroylchinnic, cichoric or 2.3-dicaffeoyltartaric acids [4].

Therefore, the aim of our work was to study the content of hydroxycinnamic acids in chicory roots and choose quality indices of the plant material.

2. Experimental

The objects of the study were roots of chicory collected at different growth locations and samples of the biologically active additive “chicory roots” of different manufacturers.

While developing a method for quantitative determination of total hydroxycinnamic acid derivatives in chicory roots, the impact of extractant concentration on the extraction of these compounds was studied. Extracts for analysis were obtained by boiling the ground plant sample with the corresponding extractant (water and water-alcohol solutions of different concentration of alcohol, ranged from 10 to 95 % of the latter) in a water bath under the same conditions. Analysis of the obtained extracts was performed by recording the electronic absorption spectra and measuring the absorbance of the solution at the wavelength for maximum absorption of hydroxycinnamic acids (325 ± 2 nm). Recording of spectra and absorption measurements were performed on a spectrophotometer Carry-50 M.

The standard sample of chlorogenic acid (Fluka) was applied in the research.

3. Results and Discussion

In the study of absorption spectra of obtained water-alcohol extracts of chicory roots it was found that the spectra are changed depending on the used extract. In the spectra of extracts obtained using water and 10–20 % ethyl alcohol, no absorption maximum, characteristic of hydroxycinnamic acids, occurred. The spectra of other extracts, obtained applying higher concentrations of alcohol solutions, were characterized by the presence of a distinct peak (Fig. 1).

The same extractant is used for the extraction of hydroxycinnamic acids according to the requirements of the European Pharmacopoeia in the analysis of balm leaves [5] and the requirements of the State Pharmacopoeia of Ukraine (3rd edn.) for sample preparation within the analysis of the Echinacea roots [6].

By applying a selected extractant, aqueous alcohol extracts for different samples of chicory roots were obtained. Investigation of electronic absorption spectra of obtained water-alcohol extracts pointed at two subspecies of the plant material. In the absorption spectra of some samples a light absorbance curve with a maximum at the wavelength of 325 ± 2 nm (Fig. 2), characteristic of phenolic acids (Fig. 3), was distinctly observed. Spectra of other samples had slightly distinguishing curves, but they contained a distinct absorption maximum at the wavelength of 325 ± 2 nm (Fig. 4). Thus, the quantitative determination of hydroxycinnamic acids by a direct spectrophotometry at the wavelength of 325 ± 2 nm is a correct method.

The highest absorbance value at maximum absorption of hydroxycinnamic acids, and hence their content was observed for an extract obtained in 50 % (v/v) ethyl alcohol (Table 1), thus for complete extraction of phenolic acids it is necessary to use 50 % (v/v) ethanol in the process of sample preparation.

Table 1

The effect of alcohol concentration in an extractant on the content of hydroxycinnamic acids in the obtained liquid extracts

Concentration of alcohol in the extractant, % (v/v)	Content of hydroxycinnamic acids in liquid extracts in terms of chlorogenic acid x10^3 %
0 (water)	0.45 ± 0.01
10	0.78 ± 0.02
20	1.24 ± 0.03
30	1.62 ± 0.03
40	1.76 ± 0.04
50	2.42 ± 0.03
60	2.28 ± 0.03
70	2.21 ± 0.02
80	2.15 ± 0.03
90	1.33 ± 0.02
95	0.60 ± 0.01

The following method of quantitative determination of the total hydroxycinnamic acid derivatives, expressed as chlorogenic acid using specific absorbance value has been developed.

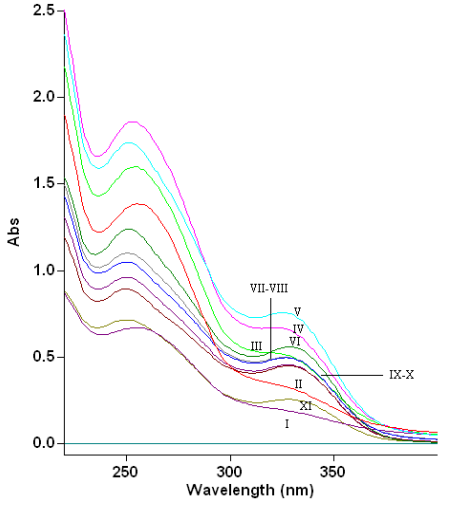

Fig. 1. Absorption spectra of aqueous-alcoholic solutions (50 % v/v) of extracts from chicory roots, depending on the concentration of the applied extractant: water (I) and 10–95 % alcoholic solutions (II-XI)

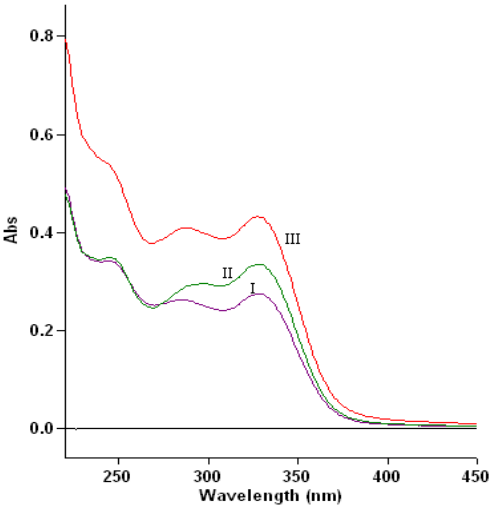

Fig. 2. Absorption spectra of alcohol solutions (50 % v/v) of chicory root extracts for samples of plant materials: 4 (I); 6 (II) and 7 (III)

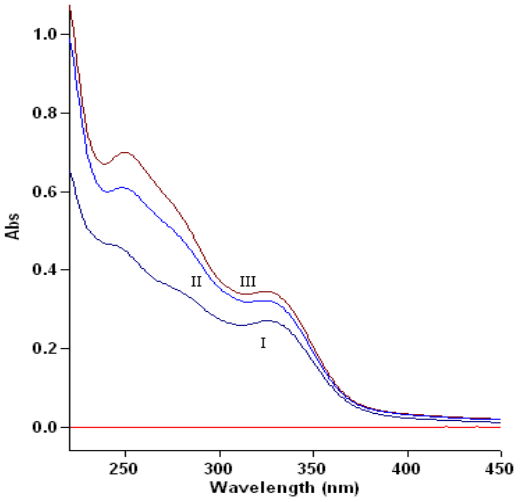

Fig. 3. Absorption spectra of aqueous-alcoholic solutions (50 % v/v) of extracts from chicory roots for samples of plant materials: 8 (I); 3 (II) and 5 (III)

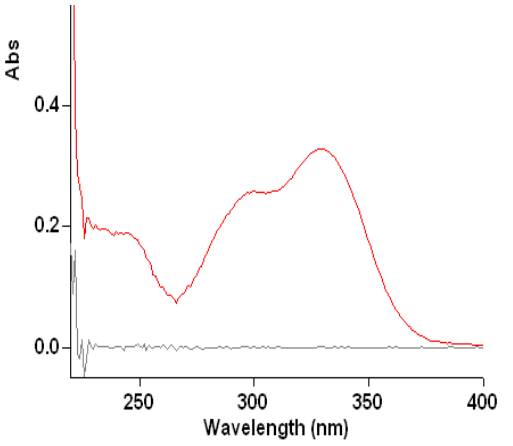

Fig. 4. Absorption spectrum of alcohol solution (50 % v/v) for a standard sample of chlorogenic acid

Table 2

Determination of totality of hydroxycinnamic acid derivatives in chicory roots

No. of the sample	Sample of plant materials	Content of hydroxycinnamic acid derivatives,%
1	The roots of chicory, LTD "LikFarma" Adonis "	0.34 ± 0.01
2	The roots of chicory, JSC "Liktravy"	0.36 ± 0.01
3	The roots of chicory, collected wild (Navariya Nova, Lviv region)	0.31 ± 0.01
4	The roots of chicory, collected wild (Myklashiv, Lviv region).	1.13 ± 0.02
5	The roots of chicory, industrial ("Halka")	0.34 ± 0.01
6	The roots of chicory, industrial (Slavuta, Khmelnytsky region).	0.33 ± 0.01
7	The roots of chicory, collected wild (Zboriv, Ternopil region).	2.09 ± 0.02
8	The roots of chicory, collected wild (Kosiv, Ivano-Frankivsk region).	1.27 ± 0.02

3.1. Method for Quantitative Determination of the Totality of Hydroxycinnamic Acid Derivatives in Chicory Roots

Stock solution. Place 1.2 g (accurately weighted sample) of ground to powder material in a 100 ml flat-bottomed flask, add 50 ml of alcohol (50 % v/v) *R* and heat it on a water-bath under a reflux condenser for 30 min. Allow to cool. Filter the obtained extract into a 100 ml volumetric flask, removing liquid. Add 25 ml of alcohol (50 % v/v) *R* and continue to boil in a water-bath under a reflux condenser for 15 min. Cool the flask, containing an extract, and filter the latter into the same flask, combining the filtrates. Add 15 ml of alcohol (50 % v/v) *R* to the residue into a flask and boil in a water-bath under a reflux condenser for 15 min. Cool and filter the extract into the same flask, combining the filtrates. Wash the residue in the flask and the filter with alcohol (50 % v/v) *R*, diluting the filtrate volume in a volumetric flask to 100 ml, and mix.

Test solution. Place 5.0 ml of stock solution in a 25 ml volumetric flask, dilute with alcohol (50 % v/v) *R* to 25 ml, and mix.

Measure the absorbance of *test solution* at the wavelength of 325 ± 2 nm in 10 mm cuvette, using alcoholic solution (50 % v/v) *R* as the compensation liquid.

Calculate the percentage content of totality of hydroxycinnamic acid derivatives (*X*), expressed as chlorogenic acid (anhydrous drug), using the following expression:

$$X = \frac{A \cdot 25 \cdot 100 \cdot 100}{5 \cdot 556 \cdot m \cdot (100 - W)}$$

where *A* – absorbance of the test solution; 556 – specific absorbance of chlorogenic acid at 325 nm; *m* – mass of

the plant sample to be examined, in grams; *W* – the loss on drying as a percentage.

We analyzed various samples of the plant material by this method and quantified totality of hydroxycinnamic acid derivatives (data are shown in Table 2).

Quantitative content of hydroxycinnamic acid derivatives in different samples of chicory roots, except three (Table 2), is almost identical. The difference ofthe three samples may be due to various growth conditions. Quantitative analysis of other samples allows us to propose the totality of hydroxycinnamic acid derivatives not less than 0.3 % in terms of chlorogenic acid (anhydrouos drug) as a quality criterion for a chicory root plant material.

4. Conclusions

As the result of the carried out investigations, the spectrophotometric method for determination of the totality of hydroxycinnamic acid derivatives, expressed as chlorogenic acid, has been developed. It has been established that in different samples of chicory roots the total content of hydroxycinnamic acid derivatives varies within 0.3–2.0 %.

Considering an antioxidant activity of hydroxycinnamic acid derivatives the content of the latter not less than 0.3 % is offered as the quality criterion for chicory roots.

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КІЛЬКІСНЕ ВИЗНАЧЕННЯ ГІДРОКСИКОРИЧНИХ КИСЛОТ У КОРЕНЯХ ЦИКОРІЮ

Анотація. *Досліджено можливість застосування прямої спектрофотометрії для кількісного визначення гідроксикоричних кислот. Як кількісний критерій якості коренів цикорію запропоновано вміст гідроксикоричних кислот не менше 0,3 % у перерахунку на кислоту хлорогенову.*

Ключові слова: *корені цикорію, кількісне визначення, гідроксикоричні кислоти, спектрофотометрія.*