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STUDY OF ALCOHOLIC-WATER EXTRACTS OF *THYMUS VULGARIS* OBTAINED BY ULTRASONIC EXTRACTION METHOD

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Alcohol-water extracts of *Thymus vulgaris* have obtained by ultrasonic extraction method for different times extraction. The obtained extracts have been tested for the quantitative content of extractive substances, phenolic compounds and flavonoids and for the presence of antioxidant activity. The best studied indicators were found in extract obtained by ultrasound extraction for 40 minutes (content of extractive substances 25 mg/ml, sum of polyphenolic compounds 30 mg/ml, flavonoids content 45 mg/ml).

Key words: polyphenolic compounds; flavonoids; antioxidant activity; DPPH; ABTS; FRAP.

Introduction

Today, one of the main issues in obtaining extractive preparations is the choice of the extraction method, which would ensure the maximum extraction of the expected biologically active substances (BAS). The effectiveness of the technology significantly depends on the level of hardware and technological support of this process. The latest developments (ultrasound extraction, extraction with liquefied gases, dynamic extraction) ensure the complete extraction of active substances from raw plant materials and reduce labor costs [1–4].

Depending on the type of BAR, which must be isolated from natural raw materials, various extraction methods are used: maceration, remaceration, percolation, repercolation, countercurrent and circulation extraction methods [3, 5]. Most of these methods involve the use of elevated temperatures, therefore, when choosing an extraction method, attention should be paid to the thermal stability of the extracted substances. Classical methods of extraction (maceration, remaceration), which consist in extracting BAR at room temperature, are often the most effective [6].

At the current stage of scientific development, special attention is being paid to the technologies of so-called "cold" extracts, which, unlike "hot" extracts, contain a full range of biologically active substances in their natural, unchanged form [5].

An alternative to temperature in modern technologies can be ultrasound to accelerate extraction. The basis of this extraction method is that the process takes place under the influence of ultrasonic waves of a specific strength, which contribute to the destruction of cell walls and the faster release of BAR from the intracellular space. Ultrasonic waves cause additional mechanical deformation of particles, which can lead to faster percolation of plant raw materials by the extractant. Under the influence of ultrasound, phenomena that are not inherent to other physical factors or exceed them in intensity are observed. Thus, under the influence of ultrasound in the liquid, its particles can move tens of thousands of times per second around the raw material particles, thereby "shaking" the surface liquid from the particles of the solid phase both under the influence of variable powerful ultrasonic pressure and hydraulic shocks at the moment of collapse of the cavitation caverns [7].

The advantage of ultrasonic extraction over other extraction methods is, first of all, the high permeability of cell walls in plant raw materials for the extractant, resulting in a more complete dissolution of the BAR content of the cell and their release into the extractant. Also, a significant advantage is an ability to accurately control all parameters of ultrasonic treatment (temperature, pressure, amplitude). It ensures that the extracted compounds do not degrade during the extraction process [7, 8].

As an object for studying the influence of ultrasound on the output of BAR, the raw material of the representative of the family Lamiaceae, *Thymus vulgaris*, was chosen. Representatives of the genus Lamiaceae have found wide application in medical practice, as well as other spheres of human activity, and are the objects of study by scientists of various scientific fields [9–18]. *Thymus vulgaris* is popular among people not only due to its spicy taste and aroma but also due to its wide spectrum of pharmacological action. Thyme herb *(Herba Thymi vulgaris)* is used in fresh or dried form for medicinal purposes. Fresh grass is used to obtain essential oil (*Oleum Thymi*), while dry grass is used to extract BAR or to use it in its native form.

The chemical composition of common thyme grass contains a large number of substances, in particular, Albanian scientists using gas chromatography-mass spectrometry (GC-MS), discovered 86 compounds, the main of which are: p-cymene (7.76–43.75 %), γ-terpinene (4.20–27.62 %), thymol (21.38–60.15 %), carvacrol (1.15–3.04 %), β-caryophyllene (1.30–3 .07 %). In general, the content of essential oil can vary in the range of 0.8–1.2 %. Also, *Thymus vulgaris* raw material is rich in flavonoids (luteolin, luteolin 7-glucoside), triterpenes (ursolic, olean, thymunic) and other acids, resins, and tannins [10]. The popularity of this plant among researchers is due to the presence of thymol and carvacrol in its composition. Scientists prove that *Thymus vulgaris* has a positive effect on the body and is one of the medicinal plants with the highest content of antioxidants, which is another confirmation of the prospect of developing new medicines based on *Thymus vulgaris* [11].

The purpose of the work. Investigate the effect of extraction time using ultrasound of *Thymus vulgaris* on the output of extractive substances, phenolic compounds, and flavonoids. Determine the antioxidant activity of the obtained extracts.

Materials and research methods

Raw material for research. Native raw material *Thymus vulgaris*, collected in the vicinity of the village of Slavske of Lviv region during the flowering period, was chosen for the research. The raw materials were air-dried and then stored in sealed containers.

Preparation of extracts

Measurements of 5 g of crushed grass of *Thymus vulgaris* were placed in three flasks, filled with 70 % ethyl alcohol in a ratio of 1:20, and left to swell for 24 hours at a room temperature of 20 ± 2–3 °C. At the end of the time, the flasks were placed in an ultrasonic bath and kept with the ultrasound on for 20 (extract-1 (E-1)), 40 (extract-2 (E-2)), and 60 (extract-3 (E-3)) minutes. The extracts were drained and made up to 100 ml with 70 % ethyl alcohol, after which they were filtered through a filter moistened with pure extractant into a dry vessel, kept in a refrigerator at 2 °C for better coagulation of the particles, and centrifuged.

Determination of the total content of extractive substances

1 ml of the studied extract was placed in a beaker, weighed, and brought to a constant mass. The solvent was evaporated in a water bath, and the blocks were dried in an oven at 105 °C to a constant mass.

Quantitative determination of the content of polyphenolic compounds

To determine the quantitative content of polyphenolic compounds, a spectrophotometric method [19] was used using the Folin-Chocalteu reagent. Determination was carried out on a Hitachi U-2810 spectrophotometer.

The studied extracts were diluted with 70 % ethyl alcohol to a content of active substances of 1 mg/ml. To carry out the determination, 0.1 ml of the extract was mixed with 2.9 ml of water and 0.5 ml of the Folin-Chocalteau reagent, kept for 3 minutes in a dark place at room temperature, then 1.5 ml of a 20 % solution of Na_2CO_3 and 5 ml of water. Leave for 2 hours in a dark place at room temperature. The optical density was measured at 760 nm in cuvettes with a layer thickness of 10 mm. Composition of the comparison solution: 70 % ethyl alcohol – 0.1 ml, distilled water – 7.9 ml, Folin-Chocalteu reagent – 0.5 ml, 20 % Na_2CO_3 solution – 1.5 ml. The quantitative content of polyphenolic compounds was calculated in mg equivalents of gallic acid (HA) per gram of extract (mgHA/g), for this, a curve of dependence of the optical density indicator on the concentration of a standard sample of gallic acid was constructed. The multiplicity of measurement repetitions is equal to three.

Quantitative determination of flavonoid content

Quantitative determination of flavonoid content was carried out by the method of spectrophotometry analysis [19] using a Hitachi U-2810 spectrophotometer. 8.4 ml of 70 % ethyl alcohol and

0.8 ml of 2 % aluminum chloride solution were added to 0.8 ml of extracts containing 1 mg/ml of extractive substances and left for 40 min at room temperature in a dark place. The optical density was measured at 420 nm in cuvettes with a layer thickness of 10 mm. The composition of the comparison solution: extract – 0.8 ml, ethyl alcohol 70 % – 9.2 ml. In parallel, a standard solution of quercetin was prepared. The quantitative content of flavonoids was calculated in mg equivalents of quercetin (K) per gram of extract (mgK/g), for this, a curve of dependence of the optical density indicator on the concentration of a standard sample of quercetin was constructed. The multiplicity of measurement repetitions is equal to three.

Determination of radical scavenging activity of extracts using the DPPH method

Interaction with 2,2-diphenyl-1-picrylhydrazyl (DPPH reagent – 2,2-diphenyl-1-picrylhydrazyl) was used to determine the radical absorbing capacity of the studied extracts [19, 20]. This method belongs to the spectrophotometry methods of analysis and is based on the determination of the optical density of colored working solutions, its advantages are the speed and accuracy of repetitions.

To conduct the study, 1 ml of the extract was mixed with 9 ml (0.04 mg/ml) of DPPH solution in ethyl alcohol. The exposure time is 30 minutes in a dark place. The control solution contained 1 ml of 70 % ethyl alcohol and 9 ml of DPPH solution. The optical density was measured at a wavelength of 517 nm in cuvettes with a layer thickness of 10 mm. The multiplicity of measurement repetitions is equal to three.

Antioxidant activity was calculated according to the formula:

$$AOA\ (\%) = (A_0\text{-}A)/A_0*100,$$

where A_0 – optical density of DPPH solution; A – optical density of the solution of the studied extract.

Trolox solution was used to compare the antioxidant activity of the studied extracts.

Determination of the antiradical activity of extracts using the ABTS reagent

To determine the antiradical activity of extracts of medicinal plants, a method using the reagent 2,2-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) ($ABTS^{\cdot}+$ – 2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid)) [20]. This method makes it possible to determine the antioxidant properties of hydrophobic and hydrophilic compounds.

For the experiment, 5 ml of ABTS solution and 0.5 ml of the extract under study were mixed in volumetric flasks with a capacity of 10 ml and kept for 30 seconds. The control solution contained: 70 % ethyl alcohol – 0.5 ml, ABTS solution – 5 ml. The optical density was measured at a wavelength of 734 nm in cuvettes with a layer thickness of 10 mm. The multiplicity of measurement repetitions is equal to three.

Antioxidant activity was calculated according to the formula:

$$AOA\ (\%) = (A_0\text{-}A)/A_0*100,$$

where A_0 – optical density of ABTS solution; A – optical density of the solution of the studied extract.

Determination of antioxidant activity of extracts by the FRAP method

To determine the antioxidant activity, a method using the FRAP (ferric reducing antioxidant power) reagent is used as a reducing substrate [20].

For the experiment, 7.5 ml of FRAP solution, 0.75 ml of water and 0.25 ml of the extract under study were mixed in measuring flasks with a capacity of 10 ml and kept for 4 minutes. The control solution contained: 70 % ethyl alcohol – 0.25 mland FRAP solution – 8.25 ml. The optical density was measured at a wavelength of 593 nm in cuvettes with a layer thickness of 10 mm. The multiplicity of measurement repetitions is equal to three.

Results and discussion

As a result of the conducted experiments, it was established that the highest content of extractive substances is observed in the E-40 extract obtained during ultrasound treatment for 40 minutes. With longer exposure in these conditions, the content of extractive substances decreased. This fact is probably caused by the destruction of biologically active components and the formation of volatile compounds. Similar data were obtained when determining the amount of polyphenolic compounds shown in Fig. 1.

According to experimental data, the content of extractive substances in the respective extracts was: E-20 – 7.2 mg/ml, E-40 – 8.8 mg/mland E-60 – 7.9 mg/ml. Accordingly, the content of the sum of polyphenolic substances in terms of gallic acid (equation of the curve of dependence of the value of optical density on the concentration of solutions of a standard sample of gallic acid y = 1.662x-0.000, R2 = 0.999): E-20–0.211 mg/ml, E-40 – 0.300 mg/ml, E-60 – 0.223 mg/ml.

In contrast to polyphenolic compounds, the content of flavonoids increased with longer exposure under the influence of ultrasound (Fig. 2).

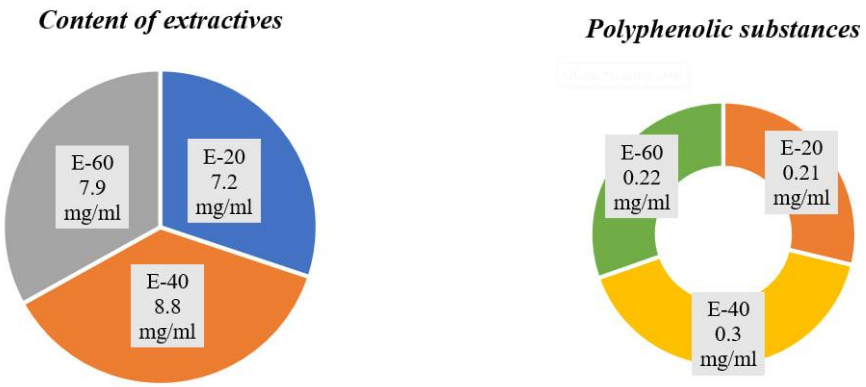

Fig. 1. Content of extractives (mg/ml) and polyphenolic substances (mg/ml) in extracts of Thymus vulgaris obtained at different times of ultrasound treatment

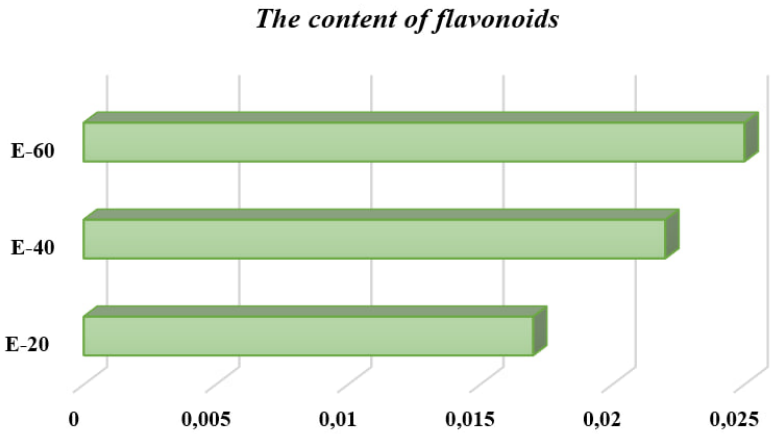

Fig. 2. The content of flavonoids mg/ml in extracts of Thymus vulgaris obtained at different times of ultrasound treatment

The content of flavonoids in terms of quercetin (the equation of the dependence curve of the value of optical density on the concentration of solutions of the standard sample of quercetin y = 10.01x-0.008, R2 = 0.999) in the extracts was: E-20 – 0.017 mg/ml, E-40 – 0.022 mg /ml, E-60 – 0.025 mg/ml.

The results of the study of the antioxidant properties of E-20, E-40 and E-60 extracts with a content of extractive substances of 1 mg/ml using the DPPH, ABTS and FRAP methods confirmed their effectiveness as radical scavengers and reducing agents.

The highest AOA values were obtained for E-60 by the three methods used. Thus, by the DPPH method, the percentage of radical scavenging activity was 72.3 %, ABTS – 86.3 %, and by the FRAP method – 61.6 %, which indicates a sufficiently high indicator of the antioxidant activity of *Thymus vulgaris* extract obtained during extraction in this time regime.

Conclusions

The conducted experimental study shows the expediency of obtaining the extract of *Thymus vulgaris* extract for the purpose of extracting a complex of biologically active substances (flavonoids and polyphenols) by the ultrasonic extraction method. The obtained results of the study of antioxidant activity by various methods confirm the prospect of using the herb Thymus vulgaris for the production of extracts and the creation of pharmaceutical and cosmetology products based on them.

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ДОСЛІДЖЕННЯ СПИРТОВО-ВОДНИХ ЕКСТРАКТІВ *THYMUS VULGARIS*, ОДЕРЖАНИХ МЕТОДОМ УЛЬТРАЗВУКОВОЇ ЕКСТРАКЦІЇ

Наведено результати аналізу спиртово-водних екстрактів *Thymus vulgaris*, одержаних методом ультразвукової екстракції. Визначено залежність кількісного вмісту екстрактивних речовин, фенольних сполук та флавоноїдів від часу екстрагування. Одержані екстракти перевірено на наявність антиоксидантної активності. Найвищий вміст екстрактивних речовин та поліфенолів одержано при 40 хв екстракції ультразвуком, тоді як найвищий вміст флавоноїдів та найкращі показники антиоксидантної активності продемонстрували екстракти, одержані при витримці 60 хв.

Ключові слова: поліфенольні сполуки; флавоноїди; антиоксидантна активність; DPPH; ABTS; FRAP.