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PRELIMINARY PHYTOCHEMICAL SCREENING AND ANTIOXIDANT ACTIVITIES OF THE EXTRACT OF TEUCRIUM CHAMAEDRYS L.

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Abstract

In modern conditions of development of the pharmaceutical industry, there is a general trend to increase consumer demand for herbal medicines on the overall pharmaceutical market as an alternative treatment for alleviating several health problems including heart diseases, diabetes, high blood pressure, and even certain types of cancer.

Pharmacologists, microbiologists, biochemists, botanists, and natural-products chemists all over the world are currently investigating medicinal plants for phytochemicals and looking for compounds that could be developed for the treatment of various diseases.

The aim of our research was preliminary phytochemical screening and antioxidant activity of plant extracts of Teucrium chamaedrys L.

Materials and methods. Raw materials were harvested during vegetative growth (May) and flowering phase (June) in 2018-2020 in Kyiv. Teucrium chamaedrys L. was used as objects for the study. The raw materials were air-dried and grounded into a powder.

The antioxidant activity of ethanol and aqueous herbal extracts of Teucrium chamaedrys L. was studied by the spectrophotometric method, according to the classical method, which should be determined according to the reactivity of the substrate of the stable radical 1,1- diphenyl-2-picrylhydrazyl (DPPH).

Results and discussion. The herb of Teucrium chamaedrys L. contains compounds like glycosides, flavonoids, triterpenoids, aminoacids, tannins, and phenols.

The greatest ability to exhibit antioxidant properties is shown by the aqueous herbal extract of the Teucrium chamaedrys L. which is almost not inferior to the ethanol herbal extract on 70% methyl alcohol, showing a decrease in the concentration of the radical form of DPPH in 10 minutes by 89.6% and 87.1% respectively.

The values of the correlation coefficients antioxidant activity of oxycinnamic acids and antioxidant activity of polyphenols are close to one, we can tell about the relationship between the antioxidation activities of herbal extracts of the Teucrium chamaedrys L. and the content of oxycinnamic acids and polyphenols.

Keywords: *herbal medicines, phytochemicals, extraction, antioxidant activity, DPPH.*

Introduction

In modern conditions of development of the pharmaceutical industry, there is a general trend to increase consumer demand for herbal medicines on the overall pharmaceutical market as an alternative treatment for alleviating several health problems including heart diseases, diabetes, high blood pressure, and even certain types of cancer. This phenomenon is due to some advantages that distinguish them from synthetic or semi-synthetic analogs. A wide range of therapeutic effects, low toxicity, and the associated possibility of longer-term use of herbal medicines make them the undisputed market leaders [1].

Pharmacologists, microbiologists, biochemists, botanists, and natural-products chemists all over the world are currently investigating medicinal plants for phytochemicals and looking for compounds that could be developed for the treatment of various diseases [2].

Phytotherapy is mainly used to treat intensive and permanent diseases. There are thousands of herbs with thousands of different applications, which are used in both traditional medicine and natural remedies.

According to the World Health Organization, about 80% of people prefer herbal remedies in the treatment, and this number is growing, to decreasing the effects of drug intolerance, the frequent occurrence of allergic reactions to drugs that have synthetic substances, the resistance of the microflora to broad-spectrum antibacterial agents [3,4].

There are a number of advantages associated with using herbal medicines as opposed to pharmaceutical products, such as the reduced risk of side effects. Most phytotherapy remedies are tolerated for the patient, with lower consequences than traditional medicine. They usually have fewer side effects and can be safer to use for an extended period [5].

One of the main advantages of herbal remedies is cost. Even generic pharmaceutical drugs will have a high price than phytotherapy medicines. Researching, testing, and marketing add to the price of prescription medicines. You can also buy most herbal drugs over the counter, which means you don't need to have expensive health insurance [2,6].

Another virtue of phytotherapy is its availability. You can even grow some simple herbs, such as chamomile and calendula, at home. Sometimes, plants can be the only accessible treatment to the people.

Considering critic's opinions and supporters of traditional medicine in studying many alternative therapies such as acupuncture, homeopathy, and meditation, phytotherapy lends itself well to standard evaluation methods [1,7].

The aim of our research was preliminary phytochemical screening and antioxidant activity of plant extracts of Teucrium chamaedrys L.

Methods

Text Raw materials were harvested during vegetative growth (May) and flowering phase (June) in 2018-2020 in Kyiv. Teucrium chamaedrys L. was used as objects for the study. The raw materials were air-dried and grounded into a powder.

Sample Preparation

Preparation of the liquid extract was carried out by maceration: to the crushed raw material (2-3 mm) was added ethanol in the ratio (1:10) and extracted in a water bath at a temperature of 80-90°C for 30 minutes. The resulting solution was filtered using filter paper (red tape - 125 mm). The extraction was repeated twice more using new portions of ethyl alcohol. The extracts were defended, mixed, and brought to the required volume.

Preparation of lipophilic extracts was performed by exhaustive extraction of raw materials in a Soxhlet apparatus. The obtained extracts were concentrated by rotary evaporator.

Results

Preliminary phytochemical screening:

A. Tests for carbohydrates:

Procedure: 10 drops of the sample were poured into a test tube. Solution of sodium hydroxide (30%) was added until the alkaline reaction, then 5 drops of β-naphthol. Slowly added concentrated sulfuric acid along the walls of the tube to form a red-colored ring at the fluid boundary. After shaking a dark purple solution will form [8].

B. Test for glycosides.

Procedure: in two round-bottomed flasks were added 5 ml of extract of the object of study. To the first sample was added 0.5 ml of concentrated hydrochloric acid and to the second one 0.5 ml of water was. Solutions were heated for 20 minutes. For the first sample with hydrochloric acid acidity the medium was adjusted to pH 7 according to a universal indicator using a solution of potassium hydroxide 20%, and in another was added an equivalent amount of water. To both samples were added 2 ml of Fehling's reagent, boiled for 2 minutes, and left for 10 minutes. Observed the formation of a brick-red precipitate of copper oxide, which indicates the presence of free sugars [9].

C. Test for flavonoids:

Procedure: 5 ml of 2.0% NaOH mixture was added to the equal volume of plant extract. The formation of orange-yellow, yellow, red, or purple-red coloration is indicated the presence of xanthones, flavones, flavonols, chalcones, and anthocyanins, respectively. The presence of flavonoids will be confirmed after adding 2 drops of diluted acid by changing the yellow to a colorless solution [10].

D. Test for triterpenoids

Procedure: 5 mL of chloroform was added to 2 ml of extract. 2 mL of concentrated sulfuric acid were carefully added by drops till double phase formation. The formation of a reddish-brown coloration in the middle layer is indicative of the terpenoid's positive results [11].

E. Test for aminoacids.

Procedure: Few drops of 1% Ninhydrin solution were added to 1.5 ml of extract and heated on a spirit lamp. The blue-violet coloration indicated positive results, the presence of aminoacids [9,12].

F. Test for tannins.

Procedure: 2 ml of distilled water was added to 2 ml of extract. The solution was heated and filtered. Few drops of FeCl3 were taken. The presence of tannins will be confirmed after the formation of a green-colored solution [13].

G. Test for phenols.

Procedure: 2 ml of extract was added to a dry test tube, heated with an equal amount of phthalic anhydride (or phthalic acid), and slowly taken 3-5 drops of concentrate sulfuric acid, left for 2 minutes. Cooled and poured the mixture into a beaker containing the solution of dilute sodium hydroxide. The formation of pink, blue, green, red coloration indicates the presence of phenol with a free para position [10,14].

Determination of antioxidant activities

The antioxidant activity of ethanol and aqueous herbal extracts of Teucrium chamaedrys L. was studied by the spectrophotometric method, according to the classical method, which should be determined according to the reactivity of the substrate of the stable radical 1,1- diphenyl-2-picrylhydrazyl (DPPH). The optical density of ethanol solutions of DFPG before (Do516) and after incubation with ethanol solutions of the studied extracts (Dt516), as well as the value of the ratio (Dt516 / Do516)×100% characterize the antiradical properties, the ability of extracts to trap free radicals. Measurements were performed on a spectrophotometer HP-845-2A (USA) in quartz cuvettes with an optical path length of 1 cm, at a controlled incubation temperature of 25° C [15,16].

As comparison drugs were used: synthetic inhibitor of free radical reactions Dibunol (Ionol, 2,6-di-tert-butyl-4-methylphenol) [10] and tincture of walnut leaves ("Kyiv PF"), which were purchased at the pharmacy, and also the standard samples of rutin ("Fluka") and chicory acid ("Fluka").

Statistical data analysis was performed using t - Student's test.

The table. 2 shows the optical density of solutions of the stable radical DPPH (control) and solutions of DPPH, which contained the studied extracts for a period of 2, 5, 10 minutes.

For a more convenient analysis of the data obtained the table. 2 also shows the percentage of antioxidant activity (AOA), which characterizes the ability of the studied extracts to reduce the content of the radical form of DPPH in a solution due to a period of 10 minutes [17,18].

The percentage of hydroxycinnamic acids and flavonoids in the studied extracts was determined in terms of chicory acid and rutin, solutions of these compounds were used as reference substances in the determination of antioxidant activity [19].

We also considered it necessary to compare the antioxidant activity of the studied extracts of the herb of Teucrium chamaedrys L. with the antioxidant activity of synthetic antioxidant Dibunol

(Ionol) and tincture of walnut leaves, because this drug, which has antioxidant properties, is made from medicinal plant raw materials.

The obtained data show that all the studied extracts can reduce the content of the radical form of DPPH, that is, to some extent detect antioxidant activity.

The greatest ability to exhibit antioxidant properties is shown by the aqueous herbal extract of the Teucrium chamaedrys L. which is almost not inferior to the ethanol herbal extract on 70% methyl alcohol, showing a decrease in the concentration of the radical form of DPPH in 10 minutes by 89.6% and 87.1% respectively.

Herbal extracts of the Teucrium chamaedrys L. made from 96% ethyl alcohol, exhibits very little antioxidant activity. Herbal extract on 96% alcohol for 10 minutes of reaction reduces the concentration of the radical form of DPPH only by 15%.

Slightly inferior to the antiradical properties of chicory acid aqueous herbal extracts of the Teucrium chamaedrys L. and extracts on 70% ethanol.

Analyzing the data of the kinetics of the interaction of the radical form of DPPH with aqueous and spirituous extracts on 70% ethanol, it should be noted a rapid course of the reaction, which in 2 minutes almost reaches the end.

Therefore, when aqueous herbal extract and 70% ethanol extract are added to the solution of DPPH, the concentration of its radical form decreases (in 2 minutes) by 85% and 67%, respectively.

The interaction of chicory acid with DPPH for 10 minutes revealed a high level of antioxidation properties (91.3%), which is consistent with the data available to us in the literature [20].

In addition, the reaction between DPPH and this compound is quite fast - in 2 minutes, the content of the radical form of DPPH in the ethanol solution is reduced by 84%. Compared to it, the flavonoid rutin and especially the classic antioxidant Dibunol interact slowly with a stable radical. After 2 minutes of reaction, rutin inactivates 55% of the radical form of DPPH, and in 10 minutes only 45%. For 10 min of reaction, Dibunol reduces the content of the radical form of DPPH only by 12%.

In vitro studies have shown that the aqueous extract and 70% ethanol herbal extract of the Teucrium chamaedrys L. have antioxidation properties, in which they are inferior to chicory acid and are not inferior to the tincture of walnut leaves.

A correlation analysis was also performed between antioxidation activity and the percentage of oxycinnamic acids, flavonoids, and polyphenols in the studied extracts by the method of least squares.

After calculations, the following values of correlation coefficients (K) were obtained:

AOA (%) - oxycinnamic acids (%) K≈0.90;

AOA (%) - flavonoids (%) K≈0,42;

APA (%) - polyphenols (%) K≈0.68.

Since the values of the correlation coefficients AOA - oxycinnamic acids and AOA - polyphenols are close to one, we can tell about the relationship between the antioxidation activities of herbal extracts of the Teucrium chamaedrys L. and the content of oxycinnamic acids and polyphenols.

The linear dependence was determined between the content of oxycinnamic acids and AOA and the content of polyphenolic compounds and AOA in the herbal extracts of the Teucrium chamaedrys L.

Discussion

The herb of Teucrium chamaedrys L. contains compounds like glycosides, flavonoids, triterpenoids, aminoacids, tannins, and phenols. All of them have their inherent pharmaceutical values.

Based on the data obtained in the process of studying the antioxidant properties, we consider it necessary to further study the properties of extracts of Teucrium chamaedrys L..

Acknowledgments

The authors declare that there are no conflicts of Interest.

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Table 01. Phytochemical composition of ethanol and aqueous extracts

#	Test	Ethanol extract	Aqueous extract
A	Carbohydrates	++	+
B	Glycosides	+	+++
C	Flavonoids	+++	++
D	Triterpenoids	+	+
E	Aminoacids	+	+++
F	Tannins	+	+++
G	Phelols	+++	+++

Key: ++ = strongly present, + = present, - =absent of tested phytochemical.

Table 02. The value of the optical density of solutions of a stable radical DPPH (D0520) and DPPH + extract (Dt520) in the interaction of DPPH solution with studied extracts and compared drugs (t = 25° C).

Solutions	Number of measurements	The optical density (D0520), t = 25° C			AOA, in 10 min
		2 min	5 min	10 min	
DPPH	10	0,967±0,009	0,957±0,008	0,951±0,005	0
DPPH + Ionol (Dibunol)	5	0,823±0,005*	0,723±0,007*	0,695±0,008*	27
DPPH + tincture of walnut leaves	10	0,493±0,009*	0,337±0,008*	0,128±0,009*	86,9
DPPH + chicory acid	5	0,141±0,006*	0,118±0,005*	0,086±0,005*	91,3
DPPH + rutin	5	0,514±0,008*	0.418±0.009*	0,344±0,008*	62,3
DPPH + herbal extract (96 % ethanol)	10	0,921±0,007*	0,918±0,012*	0,917±0,010*	15
DPPH + herbal extract (70 % ethanol)	5	0,282±0,008*	0,214±0,009*	0,113±0,011*	87,1
DPPH + herbal extract (50 % ethanol)	5	0,364±0,007*	0,326±0,008*	0,242±0,010*	74,5
DPPH + herbal extract (aqueous)	5	0,133±0,010*	0,127±0,006*	0,104±0,008*	89,6

Keys: P <0,05 - in relation to the ethanol solution of DPPH (control).