The study of the qualitative and quantitative content of the amount of flavonoids and hydroxycinnamic acids in a dense extract of common tansy flowers

In recent years, herbal medicines have become increasingly popular in the pharmacotherapy of many diseases. One of the medicinal plants widely distributed in the wild on the territory of Ukraine is common tansy (*Tanacetum vulgare* L.) of the *Asteraceae* family; it is a promising raw material for the development of new medicines. In this regard, it is relevant to study and standardize a dense extract of common tansy flowers (DECTF) to predict the possible pharmacological action and the feasibility of further use in the production of solid dosage forms.

**Aim.** To study the qualitative and quantitative content of the amount of flavonoids and the amount of hydroxycinnamic acids in a dense extract of common tansy flowers.

**Materials and methods.** The study object was DECTF. The qualitative composition of phenolic compounds in the extract was determined by TLC. The quantitative determination of the amount of flavonoids calculated with reference to luteolin was performed by the spectrophotometric method.

**Results and discussion.** The identification of DECTF was performed by TLC. It allowed us to identify 3 dominant compounds of phenolic nature – luteolin, luteolin-7-glycoside and chlorogenic acid. The quantitative content of phenolic substances in DECTF was studied by spectrophotometry; it was found that the amount of flavonoids (calculated with reference to luteolin) was 3.69 ± 0.01 %; the amount of hydroxycinnamic acids (calculated with reference to chlorogenic acid) was 16.88 ± 0.02 %.

**Conclusions.** A significant content of flavonoids and hydroxycinnamic acids in DECTF indicates the prospects for further research and development of new drugs with the anti-inflammatory, antimicrobial and choleretic action based on it.

**Key words:** TLC; spectrophotometry; dense extract; common tansy; luteolin; hydroxycinnamic acids
В последние годы лекарственные средства растительного происхождения приобретают все большую популярность в фармакотерапии многих заболеваний. Одним из лекарственных растений, широко распространённым в диком виде на территории Украины, является пижма обыкновенная (Tanacetum vulgare L.) семейства Астровые (Asteraceae), которая представляет перспективным сырьем для разработки новых лекарственных средств. В связи с этим актуальным является исследование и стандартизация густого экстракта цветков пижмы обыкновенной (ГЭЦПО) для прогнозирования возможного фармакологического действия и целесообразности дальнейшего использования в производстве твердых дозированных лекарственных форм.

Цель работы – исследовать качественное и количественное содержание суммы флавоноидов и суммы гидроксикоричных кислот в густом экстракте цветков пижмы обыкновенной.

Материалы и методы. Объектом исследования был ГЭЦПО. Определение качественного состава фенольных соединений в экстракте проводили методом ТСХ, а количественное определение суммы флавоноидов в пересчете на лютеолин – спектрофотометрическим методом.

Результаты и их обсуждение. Проведена идентификация ГЭЦПО методом ТСХ, которая позволила определить 3 доминирующие соединения фенольной природы – лютеолин, лютеолин-7-гликозид и хлорогеновую кислоту. Исследовано количественное содержание фенольных веществ в ГЭЦПО методом спектрофотометрии и установлено, что сумма флавоноидов (в пересчете на лютеолин) составляет 3,69 ± 0,01%; содержание суммы гидроксикоричных кислот (в пересчете на хлорогеновую кислоту) – 16,88 ± 0,02%.

Выводы. Значительное содержание флавоноидов и гидроксикоричных кислот в ГЭЦПО указывает на перспективность проведения дальнейших исследований и разработки новых лекарственных препаратов противовоспалительного, антимикробного и жёлчегонного действия на его основе.

Ключевые слова: ТСХ; спектрофотометрия; густой экстракт; пижма обыкновенная; лютеолин; оксикоричные кислоты

Introduction. In recent years, herbal medicines have become increasingly popular in the pharmacotherapy of many diseases. According to the WHO statistics, up to 80% of the world’s population prefers drugs of natural origin. Interest in the use of medicinal plants and medicines obtained on their basis is due to their low toxicity in most cases, and therefore, the possibility of the long-term use (months, years), as well as a complex effect on the human body. The role of herbal medicines in chronic diseases where they can be used as the maintenance therapy between courses of the main treatment is increasing. They are used in the prevention of diseases since the complex of biologically active substances (BAS) of plants has a greater affinity with the human body than isolated chemically pure active substances that determine the polyvalence of pharmacological properties, and simultaneously it safely affects various body systems involved in the pathological process [1].

In addition, an important prerequisite for creating herbal medicines is the availability of the raw material and the possibility of cultivating species, control of BAS accumulation by plant vegetation phases during the raw material harvesting [2].

One of the medicinal plants widely distributed in the wild on the territory of Ukraine is common tansy (Tanacetum vulgare L.) of the Asteraceae family. The main groups of BAS of this type are flavonoids, hydroxycinnamic acids, volatile components of essential oil – α- and β-thujone, and others. Common tansy has the choleric, antimicrobial, anthelmintic, phytocidal, anti-inflammatory, antispasmodic effects and is mainly used to treat diseases of the liver, gallbladder, gastrointestinal tract, and as an anthelmintic agent [3-6].

At the pharmaceutical market of Ukraine there are only two medicines based on common tansy: the medicinal plant raw material “Tansy flowers” in packs and the herbal tea “Fitohepatol” in sachets produced by PJSC Lectravy”. Therefore, the development of new medicinal herbal medicines based on it is a promising direction [7-8].

In this regard, it is relevant to study and standardize a dense extract of common tansy flowers (DECTF) in order to predict the possible pharmacological action and the feasibility of further use in the production of solid dosage forms.

The aim of this work was to study the qualitative and quantitative content of the amount of flavonoids and the amount of hydroxycinnamic acids in a dense extract of common tansy flowers.

Materials and methods. The study object was DECTF obtained at the premises of the Department of Botany, National University of Pharmacy. Tansy flowers were crushed to a particle size of 2-3 mm and extracted three times with 70% water-ethanol solution. The ratio of the mass of the raw material to the total volume of the extractant was 1 : 5. The resulting extracts were combined and allowed to stand for 24 hours at a temperature of 2-4 °C, after that they were filtered and evaporated on a rotary vacuum evaporator until a thick mass with humidity of not more than 25% was obtained.

The dense extract obtained was a viscous mass of a dark brown color with a specific odor; it stretched into threads and again mixed into a solid mass.

The qualitative composition of phenolic compounds in the extract was determined by TLC, taking as a basis the unified TLC methodology presented in monograph
of the State Pharmacopoeia of Ukraine (SPhU) Ed. 2 Suppl. 2 “Tansy flowers” [9, 10] Luteolin, luteolin-7-glycoside, chlorogenic and caffeic acids were selected as standard marker substances.

**Test solution.** For its preparation, dissolve the extract in the amount of 0.5 g in 10 ml of methanol when heated on a water bath at the temperature of 60 °C under reflux for 10 min, then cool and filter.

**Reference solutions.** Use the official reference standards (RS) of the SPhU – 2.5 mg of luteolin, 2.5 mg of luteolin-7-glycoside, 2.5 mg of chlorogenic acid, 2.5 mg of caffeic acid in 10 ml of methanol.

**Plate.** TLC plates with a silica gel 60 layer.


**Injection volume.** Apply 10 µL of each solution in strips.

**Distance that the mobile phase must pass.** 10 cm from the start line.

**Detection.** Detect after drying at a temperature of 100-105 °C for 5 min. Treat a warm plate with the solution of 10 g/L aminoethyl ether of diphenylboric acid RS in methanol RS and the solution of 50 g/L macrogol RS 400 in methanol RS. Evaluate the results after drying in the air for 30 min and examine in the UV light at a wave-length of 365 nm (Fig. 1).

The quantitative determination of the amount of flavonoids calculated with reference to luteolin was performed by the unified spectrophotometric method described in the monograph of the SPhU Ed. 2 Suppl. 2.

“Tansy flowers” using a HP-8453 UV-VIS spectrophotometer [9, 11].

**Stock solution 1.** Collect 0.16 g of a dense extract in a 100 ml flask and dilute to the volume with the same solvent (70 % ethanol). Then the study is carried out according to the method.

**Test solution 1.** Place 5.0 ml of Stock solution 1 in a round-bottomed flask and evaporate to dryness under reduced pressure. Transfer the resulting residue to a 25 ml volumetric flask using 8 ml of the mixture of methanol RS – anhydrous acetic acid RS (10 : 100). Rinse a round-bottomed flask with 3 ml of the mixture of methanol RS – anhydrous acetic acid RS (10:100), and place the washing liquid in the same 25 ml volumetric flask. Add 10.0 ml of the solution containing 25.0 g/L of boric acid RS, 20.0 g/L of oxalic acid RS in anhydrous formic acid RS to the solution obtained, and dilute the solution to 25.0 ml with anhydrous acetic acid RS.

**Compensation solution 1.** Place 5.0 ml of Stock solution 1 in a round-bottomed flask and evaporate to dryness under reduced pressure. Transfer the residue to a 25 ml volumetric flask using 8 ml of the mixture of methanol RS – anhydrous acetic acid RS (10 : 100). Rinse a round-bottomed flask with 3 ml of the mixture of methanol RS – anhydrous acetic acid RS (10 : 100), and place the washing liquid in the same 25 ml volumetric flask. Add 10.0 ml of anhydrous formic acid RS to the solution obtained, and dilute the solution to 25.0 ml with anhydrous acetic acid RS.

**Stock solution 2.** Place approximately 0.010 g (accurate weight) of luteolin RS (SPhU) in a 100 ml volumetric flask, dissolve in 70 ml of methanol RS, dilute the solution to the volume with the same solvent and mix.

**Reference solution.** Transfer 1.0 ml of Stock solution 2 to a 25 ml volumetric flask. Add 10.0 ml of the solution containing 25.0 g/L of boric acid RS, 20.0 g/L of oxalic acid RS in anhydrous formic acid RS and dilute the solution to 25.0 ml with anhydrous acetic acid RS.

**Compensation solution 2.** Transfer 1.0 ml of Stock solution 2 to a 25 ml volumetric flask, add 10.0 ml of anhydrous formic acid RS, and dilute the solution to 25.0 ml with anhydrous acetic acid RS.

The optical density of Test solution was measured 30 min after the preparation at a wavelength of 410 nm in relation to Compensation solution 1. In parallel, the optical density of Reference solution in relation Compensation solution 2 was measured.

The content of the amount of flavonoids calculated with reference to luteolin in a dense extract was calculated by the formula:

\[ X, \% = \frac{A_l \times m \times (100-w) \times 100}{A_w \times (100-P) \times 100} \]

where: \( A_l \) – is the optical density of Test solution at a wavelength of 410 nm; \( A_w \) – is the optical density of Reference solution at a wavelength of 410 nm; \( m \) – is the sample weight of luteolin RS (SPhU), g; \( w \) – is the sample weight of the raw material tested, g; \( P \) – is the luteolin content in luteolin RS (SPhU), %; \( w \) – is the loss of the raw material on drying, %.
The quantitative content of the amount of hydroxycinnamic acids in DECTF was determined by the spectrophotometric method calculated with reference to chlorogenic acid described in the monograph of the SPhU 2.3 “Nettle leaves”.

**Stock solution.** Place 0.16 g of a dense extract in a 100 ml volumetric flask and dilute to the volume with 70 % ethanol.

**Test solution.** Place 1 ml of Stock solution to a 10 ml volumetric flask, successively add 2 ml of 0.5 M hydrochloric acid solution, 2 ml of a freshly prepared solution of 10 g of sodium nitrite RS and 10 g of sodium molybdate in 100 ml of water RS and 2 ml of diluted sodium hydroxide solution RS stirring after each addition, dilute to the volume with water RS and mix.

**Compensation solution.** Place 1 ml of Stock solution to a 10 ml volumetric flask, successively add 2 ml of 0.5 M hydrochloric acid solution and 2 ml of diluted sodium hydroxide solution RS stirring after each addition, dilute to the volume with water RS and mix.

The optical density of Test solution was measured immediately at a wavelength of 525 nm in a cuvette with the layer thickness of 10 mm, using Compensation solution as a reference solution.

The content of the amount of hydroxycinnamic acids calculated with reference to chlorogenic acid (%) in a dense extract was calculated by the formula:

\[ X = \frac{A \times 1000 \times 100}{188 \times m \times (100 - W)} \]

where: \( A \) – is the optical density of Test solution at a wavelength of 525 nm; \( W \) – is the loss of the raw material on drying, %; \( m \) – is the sample weight of the raw material tested, g.

For the calculation, the specific absorption index of chlorogenic acid equal to 188 was used.

**Results and discussion.** The identification of the DECTF sample studied was performed compared to the selected RS (SPhU) on a single chromatographic plate under the conditions of the method. For this purpose, the selected RS (SPhU) of luteolin, luteolin-7-glycoside, chlorogenic and caffeic acids were applied to the chromatographic plate in parallel with the samples of the DECTF studied. The chromatography results obtained were described in relation to the selected RS: color, color intensity, location on the plate. The results are shown in Fig. 1.

On the chromatogram of the DECTF solution, 6 zones were observed; of them, the yellow and yellowish-brown fluorescence zones corresponded to the zones of reference solutions of luteolin and luteolin-7-glycoside. Below these zones there was a blue fluorescence zone, which corresponded to the zone of the reference solution of chlorogenic acid. The results of the study indicate the presence of the substances studied in DECTF.

Therefore, the next step was to determine the quantitative content of luteolin and chlorogenic acid in DECTF. It was found that the content of the amount of flavonoids mainly represented by flavones calculated with reference to luteolin reached 3.69 ± 0.01 % (Fig. 2, Tab.1).

Metrological characteristics are given in Tab. 1.

The unified spectrophotometric method selected for determination of the amount of hydroxycinnamic acids is acceptable for the raw material studied; it is confirmed by the UV spectrum of the amount of hydroxycinnamic acids obtained in DECTF (Fig. 3).

The content of the amount of hydroxycinnamic acids was at the level of 16.88 ± 0.02 % calculated with reference to chlorogenic acid.

Table 1

| m | n | 3.69 | 3.6867 | 0.000033 | 0.0033 | 0.95 | 2.78 | 3.69 ± 0.01 | 0.25 |
Metrological characteristics for determining the quantitative content of hydroxycinnamic acids in DECTF are given in Tab. 2.

### Conclusions and prospects of further research

1. The identification of DECTF has been performed by TLC. It has allowed us to identify 3 dominant compounds of phenolic nature – luteolin, luteolin-7-glycoside and chlorogenic acid.

2. The quantitative content of phenolic substances in DECTF has been studied by spectrophotometry; it has been found that the amount of flavonoids (calculated with reference to luteolin) is 3.69 ± 0.01 %; the amount of hydroxycinnamic acids (calculated with reference to chlorogenic acid) is 16.88 ± 0.02 %.

3. A significant content of flavonoids and hydroxycinnamic acids in DECTF indicates the prospects for further research and development of new drugs with the anti-inflammatory, antimicrobial and choleretic action based on it.

**Conflict of interest:** authors have no conflict of interests to declare.

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