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The study of the chemical composition of the components of the motherwort herb

Aim. To study phytochemically the components of the motherwort (*Leonurus cardiaca*) herb and determine which overground organs contain the greatest number of active substances and affect the quality of medicines based on this raw material.

Materials and methods. The object of the study was the motherwort herb and its components, such as leaves, flowers and stems. The alcoholic tincture from the motherwort herb and its components was obtained by maceration in the ratio of 1 : 5. Substances of phenolic nature and iridoids were identified by TLC. The phenolic composition of the objects was studied by HPLC on a Shimadzu LC20 Prominence liquid chromatograph under standard conditions. The content of the amount of phenolic compounds, hydroxycinnamic acids and flavonoids was determined by spectrophotometric methods.

Results and discussion. The qualitative composition and the quantitative content of phenolic compounds of the components of the motherwort herb were studied. The component composition of the motherwort herb was determined; in it leaves were 36 %, stems – 34 % and flowers – 30 %. The quantitative content of 6 substances of phenolic nature was identified in the objects studied: 3 flavonoids (rutin, apigenin, catechine) and 3 hydroxycinnamic acids (chlorogenic, caffeic and rosmarinic acids). Iridoid compounds (harpagide, harpagide acetate and ajugoside) were identified in the raw material by TLC. The content of main groups of BAS was determined in the tinctures obtained from herb, leaves, flowers and stems of motherwort by the spectrophotometric method. It has been found that flowers largely accumulate hydroxycinnamic acids, while leaves have a considerable amount of flavonoids. The content of all substances identified is several times lower in stems than in other components of the herb, therefore, it is advisable to normalize their content.

Conclusions. When collecting the motherwort herb it is necessary to reject coarse, woody stems, which significantly reduce the content of active substances in the medicinal plant raw material and affect the quality of the raw material in general and galenic agents based on it.

***Key words*:** motherwort; herb; leaves; flowers; stems; biologically active substances

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Дослідження хімічного складу компонентів трави собачої кропиви

Мета роботи. Провести фітохімічне вивчення компонентів трави собачої кропиви та встановити, які надземні органи мають найбільший вміст діючих речовин та впливають на якість лікарських засобів на основі цієї сировини.

Матеріали та методи. Об'єктом дослідження була трава собачої кропиви та її компоненти: листя, квітки та стебла. Спиртові настойки з трави собачої кропиви та її компонентів одержували методом мацерації у співвідношенні 1 : 5. Речовини фенольної природи та іридоїди ідентифікували методом ТШХ. Дослідження фенольного складу об'єктів проводили методом ВЕРХ на рідинному хроматографі Shimadzu LC20 Prominence за стандартних умов. Вміст суми фенольних сполук, гідроксикоричних кислот та флавоноїдів визначали спектрофотометричними методами.

Результати та їх обговорення. Вивчено якісний склад та кількісний вміст фенольних сполук компонентів трави собачої кропиви. Встановлено компонентний склад трави собачої кропиви, в якій листя складає 36 %, стебла – 34 % та квітки – 30 %. У досліджуваних об'єктах було ідентифіковано та встановлено кількісний вміст 6 речовин фенольної природи: 3 флавоноїдів (рутину, апігеніну, катехіну) та 3 гідроксикоричних кислот (хлорогенової, кавової та розмаринової кислот). Методом ТШХ у сировині ідентифіковані іридоїдні сполуки (гарпагід, гарпагіду ацетат та аюгозид). У настойках з трави, листя, квіток та стебел собачої кропиви спектрофотометричним методом визначено вміст основних груп БАР. Встановлено, що квітки в більшій мірі накопичують гідроксикоричні кислоти, а листя в значній кількості – флавоноїди. В стеблах вміст всіх ідентифікованих речовин у декілька разів нижчий, ніж в інших компонентах трави, тому їх вміст доцільно нормувати.

Висновки. При заготівлі сировини необхідно вилучати грубі, здерев`янілі стебла, які значно знижують вміст діючих речовин у ЛРС та впливають на якість сировини в цілому та галенових засобів на її основі.

Ключові слова: собача кропива; трава; листя; квітки; стебла; біологічно активні речовини

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Исследование химического состава компонентов травы пустырника

Цель работы. Провести фитохимическое изучение компонентов травы пустырника и установить, какие надземные органы содержат наибольшее количество действующих веществ и влияют на качество лекарственных средств на ее основе.

Материалы и методы. Объектом исследования была трава пустырника и ее компоненты: листья, цветки и стебли. Спиртовые настойки из травы пустырника и ее компонентов получали методом мацерации в соотношении 1 : 5. Вещества фенольной природы и иридоиды идентифицировали методом ТСХ. Исследование фенольного состава объектов проводили методом ВЭЖХ на жидкостном хроматографе Shimadzu LC20 Prominence при стандартных условиях. Содержание суммы фенольных соединений, гидроксикоричных кислот и флавоноидов определяли спектрофотометрическими методами.

Результаты и их обсуждение. Изучен качественный состав и количественное содержание фенольных соединений компонентов травы пустырника. Установлен компонентный состав травы пустырника, в котором листья составляют 36 %, стебли – 34 % и цветки – 30 %. В исследуемых объектах было идентифицировано и установлено количественное содержание 6 веществ фенольной природы: 3 флавоноидов (рутина, апигенина, катехина) и 3 гидроксикоричных кислот (хлорогеновой, кофейной и розмариновой кислот). Методом ТСХ в сырье идентифицированы иридоидные соединения (гарпагид, гарпагида ацетат и аюгозид). В настойках из травы, листьев, цветков и стеблей пустырника спектрофотометрическим методом определено содержание основных групп БАВ. Установлено, что цветки в большей мере накапливают гидроксикоричные кислоты, а листья в значительном количестве флавоноиды. В стеблях содержание всех идентифицированных веществ в несколько раз ниже, чем в других компонентах травы, поэтому их содержание целесообразно нормировать.

Выводы. При заготовке сырья необходимо удалять грубые, одревеснившие стебли, которые значительно снижают содержание действующих веществ в ЛРС и влияют на качество сырья в целом и галеновых средств на его основе.

***Ключевые слова*:** пустырник; трава; листья; цветки; стебли; биологически активные вещества

In the modern world there is a tendency to the increase of the level of psychopathological disorders. In our country, the situation is potentiated by various factors: aggravation of political events, socio-economic and environmental problems, deterioration of the quality of life. This leads to stress, increased fatigue of the body, reduced efficiency, insomnia, irritability, anxiety, tension, constant change of mood, etc. Sedatives based on the medicinal plant raw material (MPRM), which are preferred by more than 80% of the population, are considered to be the most optimal for the treatment and prevention of these symptoms.

The motherwort herb (*Leonurus cardiaca*) belongs to medicinal plants with the sedative action, which are most often used in medical practice. The main biologically active substances (BAS) of motherwort are derivatives of flavone (apigenin), flavonol (quercetin, kaempferol), quinqueloside, quercetin-7-glucoside, hyperoside, rutin, genkwanin, quercitrin, 5,4'-dihydroxy-7-methoxyflavone, cosmosiin, 4-rutinoside of caffeic acid. The plant contains tannins (approximately 2-5 %), triterpenoid saponins (ursolic acid), steroid glycosides, traces of essential oil (approximately 0.03 %): caryophyllene, α-humulene, α-pinene, β-pinene, limonene, linalool; there is also resin, iridoids of aucubin type (ajugol, ajugoside, galiridoside) and diterpenes (leocardin and marubin) [1-6].

The tincture of motherwort is the most common drug obtained from this MPRM. However, herbal medicines from this raw material are characterized by the volatility of the chemical composition; it may be due to the amount and the chemical composition of the components of the herb, namely leaves, flowers and stems, which have an impact on the overall quality of medicines. Therefore, **the aim** of the work was to study phytochemically the components of the motherwort herb and determine which overground organs contain the greatest number of active substances and affect the quality of medicines based on this raw material.

Materials and methods

The object of the study was the motherwort herb gathered in 2017 on the pharmacopoeial area of the National University of Pharmacy. The raw material collected was divided into separate components (leaves, flowers and stems) and analyzed according to the requirements of the State Pharmacopoeia of Ukraine (SPhU) 2.0 [7].

The alcoholic tincture from the motherwort herb and its components was obtained by maceration in the ratio of 1 : 5 taking into account the absorption coefficient of the extractant by the raw material (K = 2.0). To do this, 10.0 g of the crushed raw material (leaves, flowers and stems) was placed in a flask; 50 ml of 70 % ethyl alcohol was added in it and infused for 7 days. Then the extract was poured, the residue was pressed and purified (settling and filtration).

The qualitative composition and the quantitative content of BAS in the extracts from the motherwort herb and its components were determined by the following Pharmacopeial methods: thin-layer chromatography, spectrophotometric methods and HPLC [2, 6, 7, 8].

Substances of phenolic nature were identified by TLC with authentic samples of flavonoids and hydroxycinnamic acids using the solvent system of *glacial acetic acid R – water R – ethyl acetate R* (20 : 20 : 60). Chromatograms were developed by spraying with *dimethylaminobenzaldehyde solution*, then the plate was heated at a temperature from 100 °C to 105 °C for 10 min up to development of spots and examined in the daylight [6, 7].

Table 1

The composition of phenolic compounds in the tinctures of the motherwort herb and its components

Substance	The content in a dry residue, %			
	Herb	Leaves	Flowers	Stems
Chlorogenic acid	2.20	1.83	5.53	0.60
Caffeic acid	0.16	0.16	0.09	0.03
Rutin	1.19	1.69	1.27	0.79
Rosmarinic acid	0.05	0.02	0.07	0.01
Apigenin	0.27	0.52	–	–
Catechine	0.18	0.23	0.10	0.15
A dry residue				
Tincture	3.50	3.55	2.9	1.27

Iridoids were identified by TLC with authentic samples in the solvent system of *chloroform – methanol – water* (80 : 2 : 0.1) using the ascending method. The chromatogram was developed with Stahl reagent (1.0 g of *n*-dimethylaminobenzaldehyde in the mixture of 50.0 ml of acetic acid, 5.0 ml of phosphoric acid and 100.0 ml of water) [2].

The objects was studied by HPLC on a Shimadzu LC20 Prominence liquid chromatograph in the modular-design system equipped with a four-channel pump LC20AD, a column thermostat CTO20A, automatic sampler SIL20A, a diode array detector SPDM20A and Chem Station LC20 under the following conditions: a Phenomenex Luna C18(2) column with the size of 250 × 4.6 mm, the particle size – 5 μm; the column temperature – 35 °C; detection wavelength – 330 nm (for hydroxycinnamic acids, glycosides of flavonoids), 370 nm (for aglycones of flavonoids), 280 nm (for tannins), 340 nm (for coumarins); the flow rate of the mobile phase – 1 ml/min; the injected volume – 5 μl; the mobile phase: eluent A: 0.1 % solution of trifluoroacetic acid in water and eluent B: 0.1 % solution of trifluoroacetic acid in acetonitrile in various ratios. Identification of the components was performed by the retention time and compliance of UV spectra with the reference substances [9, 10].

The content of the amount of phenolic compounds calculated with reference to gallic acid was determined by spectrophotometry at the wavelength of λ = 270 nm. The content of derivatives of hydroxycinnamic acids calculated with reference to chlorogenic acid was determined by direct spectrophotometry at the wavelength of λ = 327 nm. The content of the amount of flavonoids calculated with reference to rutin was determined at the wavelength of λ = 417 nm using the method of the SPhU 2.0 [6, 7, 8].

Results and discussion

During the studies the percentage of various overground organs in the motherwort herb was determined. The highest percentage of the components of the motherwort herb was in leaves (36 %) and stems (34 %), while in flowers it was the lowest (30 %).

During the TLC analysis in the tinctures obtained from herb, leaves, flowers and stems of motherwort the following groups of BAS were identified: flavonoids (rutin, apigenin), derivatives of hydroxycinnamic acids (caffeic and chlorogenic acids) and iridoid compounds (harpagide, harpagide acetate and ajugoside).

As a result of analysis of tinctures from herb, leaves, flowers and stems of motherwort obtained by HPLC six substances were identified: 3 flavonoids and 3 hydroxycinnamic acids, and the quantitative content was determined (Tab. 1).

It was found that flowers largely accumulated hydroxycinnamic acids, which main component was chlorogenic acid, while leaves had a considerable amount of flavonoids. Apigenin, which was absent in other overground organs of the plant, was also identified in leaves. The content of all substances identified was several times lower in stems than in other components of the herb. Therefore, it is advisable to normalize their content.

The content of main groups of BAS was also determined in the tinctures obtained from overground organs of motherwort by the spectrophotometric method (Tab. 2).

Table 2

The content of main groups of BAS was also determined in the tinctures from overground organs of motherwort

The BAS group and the method used	The quantitative content in a dry residue, %			
	Herb	Leaves	Flowers	Stems
Phenolic compounds				
Spectrophotometry (λ = 270 nm) calculated with reference to gallic acid	13.56 ± 0.03	14.28 ± 0.04	12.63 ± 0.05	5.41 ± 0.01
Derivatives of hydroxycinnamic acids				
Spectrophotometry (λ = 327 nm) calculated with reference to chlorogenic acid	12.04 ± 0.05	13.82 ± 0.03	9.59 ± 0.04	7.13 ± 0.02
Flavonoids				
Spectrophotometry (λ = 417 nm) calculated with reference to rutin	1.63 ± 0.03	3.06 ± 0.03	1.91 ± 0.04	0.80 ± 0.02

The studies have shown that leaves are in the greatest amounts in the content of flavonoids of the motherwort herb tincture, flowers are one third less, while stems have an insignificant amount of flavonoids; therefore, their amount in the raw material should be normalized. Taking this into account when collecting the motherwort herb it is necessary to reject coarse, woody stems, which significantly reduce the content of active substances in the MPRM and affect the quality of the raw material in general and galenic agents based on it. In our opinion, in the monograph "Motherwort herb" of the SPhU 2.0 the limitation norms on the content of stems (not more than 46 %) are overstated and require revision.

CONCLUSIONS

The qualitative composition and the quantitative content of phenolic compounds in the herb, leaves, flowers and stems of motherwort have been determined, namely 6 substances of phenolic nature has been identified: 3 flavonoids (rutin, apigenin, catechine) and 3 hydroxycinnamic acids (chlorogenic, caffeic and rosmarinic acids). Flowers largely accumulate hydroxycinnamic acid, while leaves have a considerable amount of flavonoids. The content of all substances identified is several times lower in stems than in other components of the herb, therefore, it is advisable to normalize their content.

Thus, when collecting the motherwort herb it is necessary to reject coarse, woody stems, which significantly reduce the content of active substances in the MPRM and affect the quality of the raw material in general and galenic agents based on it.

Conflict of Interests: authors have no conflict of interests to declare.

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