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Study of biologically active substances content in herbal preparation for the treatment and prevention of alopecia

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A medicinal herbal extract containing *Arctii Radices, Sophora Japonica* fruits, *Acorus Clamaus rhizome, Folia Urticae Dioicae* and *Humulus Lupulus* has been assayed for polyphenols, flavonoids, hydrocinnamic acids, polysaccharides, essential oil, and the total content of extractable substances. The selected endpoints have been shown to be suitable for standardization of the herbal product. Theoretical calculations of the content of the studied groups of bioactive ingredients in the herbal medicine have been shown to comply, within the satisfactory accuracy, with the experimental data.

Key words: herbal medicine, *Arctii Radices, Acorus Clamaus rhizome, Folia Urticae Dioicae, Humulus Lupulus, Sophora Japonica* fruits, phytochemical development, alopecia.

INTRODUCTION

The herbal medicine is supplied in a form of a complex extract of a tea containing (per 100 g): *Arctii Radices* — 28 g, *Sophora Japonica* fruits — 28 g, *Acorus Clamaus rhizome* — 16 g, *Folia Urticae Dioicae* — 14 g and *Humulus Lupulus* — 14 g. Phytochemical and pharmacological development of the product has been completed in the previous phase of the study [1, 2]. The results of such study and data available in the literature suggest that the herbal product should contain the following bioactive ingredients: flavonoids, terpenes, tannins, organic acids, carbohydrates, essential oils and fatty acids, proteins and amino acids, quinones, carotenoids, bitter glycosides, mineral acids and their salts etc. [3-5].

Accounting for a need in further standardisation of the product under development and a need in the development of the methods of quality control, and accounting for our own experience [6, 7] and data available in the literature, we have decided to assay the studied herbal medicine for polyphenols, flavonoids, hydrocinnamic acids, polysaccharides and essential oils and the total content of extractable substances.

Thus, the study was aimed purpose at the determination of the content of extractable substances (dry residue) in the herbal medicine and quantitative determination of polyphenols, flavonoids, hydrocinnamic acids, polysaccharides and essential oils in such product.

MATERIALS AND METHODS OF INVESTIGATION

Batches of the herbal medicine manufactured under laboratory conditions from the raw materials harvested in 2006 through 2008 were used in the study.

The *content of extractable substances (dry residue)* was determined in accordance with State Pharmacopoeia of Ukraine 1, 2.8.16, p. 514.

The *content of polyphenols* was determined in accordance with European Pharmacopoeia 5, monography: *Determination of tannins in herbal drugs*, by using the colour reaction of polyphenols contained in the herbal product with a mixture of phosphomolybdate and phosphotungstate with the further measurement of the absorbance of the obtained solution at the wavelength of 760 nm. The content of total polyphenols was determined on pyrogallol basis.

The *content of flavonoids* was determined by absorbance spectrophotometry (State Pharmacopoeia of Ukraine 1, 2.2.25) by using a method

TABLE 1

Results of the quantitative determination of bioactive ingredients in the studied herbal medicine

Characteristic	Assay,%		
	Series 10706	Series 20807	Series 30308
Extractable substances:			
In the product	4,75±0,17	4,41±0,16	5,01±0,20
On the raw material basis	23,52±0,88	22,05±0,72	25,00±0,79
Polyphenols:			
In the product	**0,18±0,01**	**0,17±0,03**	**0,18±0,02**
On the raw material basis	0,90±0,05	0,88±0,15	0,89±0,10
On the extractable substance basis	3,78±0,12	3,97±0,14	3,55±0,11
Flavonoids:			
In the product	0,31±0,01	0,27±0,01	0,33±0,02
On the raw material basis	1,55±0,05	1,35±0,05	1,65±0,10
On the extractable substance basis	6,53±0,17	6,12±0,19	6,58±0,11
Hydrocinnamic acids:			
In the product	0,11±0,001	0,12±0,002	0,10±0,001
On the raw material basis	0,55±0,005	0,60±0,010	0,49±0,005
On the extractable substance basis	2,31±0,09	2,72±0,07	1,95±0,09
Polysaccharides:			
In the product	0,39±0,007	0,43±0,004	0,49±0,006
On the raw material basis	1,95±0,035	2,15±0,020	2,60±0,030
On the extractable substance basis	8,21±0,17	9,75±0,19	10,37±0,22
Essential oil:			
In the product	0,032±0,001	0,031±0,001	0,043±0,002
On the raw material basis	0,16±0,005	0,15±0,005	0,21±0,010
On the extractable substance basis	0,67±0,08	0,69±0,07	0,85±0,09

on the basis of formation of a complex with aluminium chloride in acid medium (method of flavonoid determination described in Analitical Normative Documentation «Sophora fruits»). Prior to hydrolysis, an aliquot of the herbal product was evaporated to dryness, and the quantitative determination of total flavonoids was carried out at the wavelength of 425 nm on rutin basis.

The *content of hydrocinnamic acids* was determined by absorbance spectrophotometry (State Pharmacopoeia of Ukraine 1, 2.2.25) by using a method on the basis of formation of a complex with sodium nitrite and sodium molibdate in an alkaline medium solutions. The absorbance of the test solution was measured at the wavelength of 525 nm on chlorogenic acid basis.

The *content of polysaccharides* was determined by using gravimetry: polysaccharides were precipitated with 96% alcohol *R* and centrifuged, and the supernatant and precipitate were filtered through a glass filter previously dried at 100°C to 105°C to constant weight; filter with a precipitate on it was dried at 100°C to 105°C until the constant weight.

The *content of essential oil* was determined by using a gravimetric method. Essential oil was distilled with water into a separating funnel and further extracted with chloroform *R*. Distillation of essential oil as an azeotrope mixture with water was performed at 115°C to 120°C. The weight of distilled essential oil was determined after drying of the essential extract by filtration through anhydrous sodium sulphate and evaporation of the obtained extract in a water bath at 65°C to 70°C.

RESULTS OF INVESTIGATION AND ITS DISCUSSION

Results of the quantitative determination of bioactive ingredients in three batches of the studied herbal medicine are summarized in table 1. For the purposes of convenience, we have calculated the content of the bioactive ingredients on the basis of extractable substances in the extract.

The obtained results show only minor differences in the content of the studied bioactive ingredients in three batches of the herbal medicine, which is very important for the further work on the standardization of the product. It is noteworthy that the results of this study suggest a satisfactory reproducibility of the selected methods of determination of the mentioned bioactive ingredients.

TABLE 2

Phytochemical development of the herbal product (theoretical calculation [1, 3, 4, 8, 9] and experimental data)

Groups of bioactive substances	Quantitative content,%			
	in 100 g of collection (calculated)	In medicinal herbal extract 1:5 (calculated)	In the herbal medicine (calculated accounting for the extraction ratio of 40%)	In the herbal medicine (averaged experimental data)
Polyphenols	2,440	0,488	0,195	0,178
Flavonoids	2,900	0,580	0,232	0,303
Hydrocinnamic acids	2,054	0,411	0,164	0,113
Polysaccharides	3,940	0,788	0,315	0,440
Essential oil	0,619	0,124	0,050	0,035

Among the studied substances, polysaccharides have been found to be the most abundant (0,40% to 0,49% on a dry substance basis). The content of flavonoids ranges from 0,27% to 0,31%, on a dry substance basis. The concentration of polyphenols and hydrocinnamic acids is lower: 0,17% to 0,18% and 0,10% to 0,13%, respectively (on a dry substance basis).

It is worth to note that the obtained data are consistent with the phytochemical development of the herbal product (table 2): differences between the theoretically calculated and experimental content of the studied bioactive ingredients do not exceed 1.45-fold change. The results of the study suggest the basis for the further development of the specification of the studied herbal medicine and methods of quality control on the basis of the selected methods mentioned above.

CONCLUSIONS

The quantitative determination of extractable substances (dry residue) and content of polyphenols, flavonoids, hydrocinnamic acids, polysaccharides and essential oil in three batches of the studied herbal medicine demonstrated acceptable minor fluctuations of the content of the aforementioned ingredients in different batches of the studied herbal medicine suggesting the suitability of such characteristics for further standardization of the product. The theoretical calculation of the content of the studied group of bioactive ingredients in the herbal medicine satisfactorily comply with the experimental data.

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О.Ю.Галкін, А.Г.Котов. Дослідження вмісту біологічно активних речовин у галеновому препараті для лікування та профілактики алопеції. Київ, Харків, Україна.

Ключові слова: *галеновий препарат, корені лопуха, кореневища лепехи, листя кропиви, шишки хмелю, плоди софори, фітохімічний дизайн, алопеція.*

Проведено дослідження фітопрепарату у вигляді витягання, до складу якого входять корені лопуху справжнього, плоди софори японської, кореневища аїру, листя кропиви, хмелю супліддя (шишки), щодо кількісного вмісту поліфенолів, флавоноїдів, гідроксикоричних кислот, полісахаридів, ефірної олії, а також загальної кількості екстрактивних

речовин. Встановлено, що обрані показники можливо використовувати для стандартизації препарату. Теоретичні розрахунки щодо вмісту досліджуваних груп біологічно активних речовин у фітопрепараті із задовільною точністю співпали з експериментальними даними.

А.Ю.Галкин, А.Г.Котов. Исследование содержания биологически активных веществ в галеновом препарате для лечения и профилактики алопеции. Киев, Харьков, Украина.

Ключевые слова: *галеновый препарат, корни лопуха, корневища аира, листья крапивы, шишки хмеля, плоды софоры, фитохимический дизайн, алопеция.*

Проведено исследование фитопрепарата в виде извлечения, в состав которого входят корни лопуха настоящего, плоды софоры японской, корневища аира, листья крапивы, хмеля соплодия (шишки), по количественному содержанию полифенолов, флавоноидов, гидроксикоричных кислот, полисахаридов, эфирного масла, а также общего количества экстрактивных веществ. Установлено, что выбранные показатели можно использовать для стандартизации препарата. Теоретические расчеты по содержанию исследуемых групп биологически активных веществ в фитопрепарате с удовлетворительной точностью совпали с экспериментальными данными.

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