New Approaches to the Standardization of Raw Materials of the Sandy Immortelle - Helichrysum arenarium (L.) Moench.

A.V. Kurkina
(GOU VPO "Samara State Medical University of Roszdrav", g. Samara)

SUMMARY

The aim of the research is to improve the methods of standardization of flowers of the sand immortelle - Helichrysum arenarium (L.) Moench. The flavonoid composition of the flowers of the sand immortelle was investigated. It was determined that isosalipurposide is the dominant flavonoid of raw immortelle sand. Objective methods of qualitative and quantitative analysis of the content of flavonoids using TLC and differential spectrophotometry in the presence of the State standard sample (SSS) of isosalipurposide are proposed. The metrological characteristics of the developed analysis method indicate that the error of a single determination in the raw material of the sand immortelle is ± 3.95%.

Key words: Sandy immortelle, Helichrysum arenarium (L.) Moench., Flowers, flavonoids, isosalipurposide, thin layer chromatography, spectrophotometry.

Introduction

It is known that flavonoids as biologically active compounds have a wide spectrum of pharmacological action and are a unique source of choleretic, hepatoprotective, and antioxidant drugs [1–8]. At present, an unfavorable situation has developed in the assortment of drugs presented on the pharmaceutical market of the Russian Federation, which consists in the dominance of foreign drugs. In this regard, it seems appropriate to expand the range of domestic drugs of the above spectrum of action. One of the promising sources of choleretic and hepatoprotective action are the flowers of the sand immortelle -Helichrysum arenarium (L.) Moench. [1-5].

Sandy immortelle is a perennial herb, widespread in the steppe regions of the European part of Russia and the CIS countries [1–5]. Flowers and preparations of sandy immortelle (infusion, liquid extract, Flamin, choleretic preparations) are used in medical practice as choleretic drugs [1–5, 9], however, with regard to the chemical composition, the literature data are contradictory. According to the literature, the predominant flavonoids in the flowers of the Helichrysum areosalipurposide are chalcone isosalipurposide, the flavanones salipurposide, prunin, and naringenin [1–5], but there is no single point of view which of these components is dominant. The sandy immortelle also contains a number of related substances, including coumarins, cinnamic acids, phthalides, polysaccharides, etc. [1–5]. In our opinion,

It is the complexity of the chemical composition of the flowers of the sandy immortelle that is the reason for the fact that the analysis methods for the medicinal plant raw materials (MPR) of the Sandy immortelle do not meet the modern requirements of pharmacognosy and pharmacopoeial analysis. So, in the monograph on this raw material (Art. 9 of the USSR State Pharmacopoeia of the XI edition), the section "Qualitative reactions" is presented only by a qualitative reaction to flavonoids [10], which does not allow the determination of individual components characteristic of a given plant. In addition, in the section “Quantitative determination”, the pharmacopoeial method, according to the results of our preliminary studies, overestimates the results for the content of flavonoids by approximately 2–3 times.

The aim of this study is to improve the methods of standardization of flowers of the sand immortelle.

Methods

We studied samples of sandy immortelle flowers collected in various regions of the Russian Federation, as well as industrial samples of raw materials produced by OJSC Krasnogorskleksredstva (Moscow Region) and LLC PKF Fitofarm (Anapa, Krasnodar Territory). Methods of chemical, spectral and physicochemical analysis, including thin-layer chromatography, direct and differential spectrophotometry, were used for the phytochemical study of the medicinal plant raw material of the sandy immortelle. In the course of developing a method for the quantitative determination of the amount of flavonoids in flowers of the sand immortelle, the UV spectra of aqueous-alcoholic extracts from this raw material were studied. The spectra were recorded using a Specord 40 spectrophotometer (Analytik Jena).

Method for the quantitative determination of the amount of flavonoids in flowers sandy immortelle

An analytical sample of raw materials is crushed to a particle size passing through a sieve with holes 1 mm in diameter. About 1 g of crushed raw materials (accurately weighed) is placed in a flask with a thin section with a capacity of 100 ml, add 50 ml of 70% ethyl alcohol. The flask is closed with a stopper and weighed on a tare balance with an accuracy of ± 0.01 g. The flask is connected to a reflux condenser and heated in a boiling water bath (moderate boiling) for 60 minutes. Then the flask is closed with the same stopper, weighed again and the missing extractant is replenished to the initial mass. The extract is filtered through a filter paper and cooled for 30 minutes. The test solution is prepared as follows: 1 ml of the obtained extract is placed in a volumetric flask with a capacity of 50 ml, add 1 ml of 3% alcoholic solution of aluminum chloride and bring the volume of the solution to the mark with 95% ethyl alcohol (test solution A). As a reference solution, a solution prepared under the same conditions is used, but

without the addition of aluminum chloride (reference solution A). Measurement of optical density is carried out on a spectrophotometer at a wavelength of 418 nm. In parallel, measure the optical density of the GSO solution of isosalipurposide at a wavelength of 418 nm, prepared by analogy with the test solution (see note).

Note: Preparation of isosalipurposide standard solutionsample. About0.02 g (accurately weighed) of isosalipurposide is placed in a volumetric flask with a capacity of 50 ml, dissolved in 20-30 ml of 95% ethanol and the volume of the solution is brought to the mark with 95% ethanol (solution A). 1 ml of isosalipurposide solution A is placed in a 50 ml volumetric flask, 1 ml of a 3% alcohol solution of aluminum chloride is added and the volume of the solution is adjusted to the mark with 95% ethyl alcohol (test solution B). A solution is used as a reference solution, which is prepared as follows: 1 ml of isosalipurposide solution A is placed in a 50 ml volumetric flask and the volume of the solution is brought to the mark with 95% ethyl alcohol (reference solution B). The content of the sum of flavonoids in the flowers of the immortelle sandy in terms of isosalipurposide and absolutely dry raw materials in percent (X) is calculated by the formula:

$$X = \frac{D \times m_o \times V \times V_1 \times 100 \times 100}{m \times D_o \times V_o \times V_2 \times (100 - W)}$$

where D is the optical density of the test solution; D_O Is the optical density of the GSO solution of isosalipurposide; V — extraction volume, ml;

V_1 - the volume of the test solution A, ml;

V_O - volume of isosalipurposide GSO solution A, ml;

V_2 - the volume of the tested GSO solution of isosalipurposide (solution B), ml; m is the mass of raw materials in grams, g;

m_O Is the mass of the GSO of isosalipurposide, g;

W is the loss in mass on drying, in percent.

results

The TLC method revealed that isosalipurposide is the dominant flavonoid in the flowers of the sand immortelle. This substance is found in the flowers of the test plant in the form of a yellow spot with an Rf value of about 0.6. At the same time, other flavonoids characteristic of this plant are found - salipurposide (5-O-glucoside of naringenin) and prunin (7-O-glucoside of naringenin) in the form of one spot with violet fluorescence in UV light at a wavelength of 254 nm. The chromatogram also shows spots of coumarins and cinnamic acids, which have blue fluorescence at a wavelength of 366 nm. Subsequent development of the chromatogram with a solution of diazobenzenesulfonic acid makes it possible to more clearly detect the dominant isosalipurposide spot at the level of the isosalipurposide GSO spot. Taking into account the absence of GSO isosalipurposide in the Russian Federation, we have developed a scheme for the isolation of isosalipurposide, which is currently being studied in terms of preparation for state registration of regulatory documents (ND) for this standard. Consequently, there is a prospect of using the TLC method to determine

the authenticity of the flowers of the immortelle sandy by detecting the dominant and diagnostic flavonoid - isosalipurpusid in the presence of an appropriate standard sample.

In order to improve the method of quantitative determination, we studied the conditions for the extraction of flavonoids from flowers of the sand immortelle. It has been shown that the optimal extractant is 70% ethyl alcohol, while 50% ethyl alcohol is used in SP XI (Table 1). For the extraction of raw materials, 40%, 50%, 60%, 70%, 80% ethyl alcohol was used (Table 1). The method of quantitative determination developed by us is based on the following optimal parameters: extraction of raw materials with 70% ethyl alcohol for 1 hour under boiling conditions in a ratio of 1:50, measurement of optical density at a wavelength of 418 nm under conditions of differential spectrophotometry.

Table 1

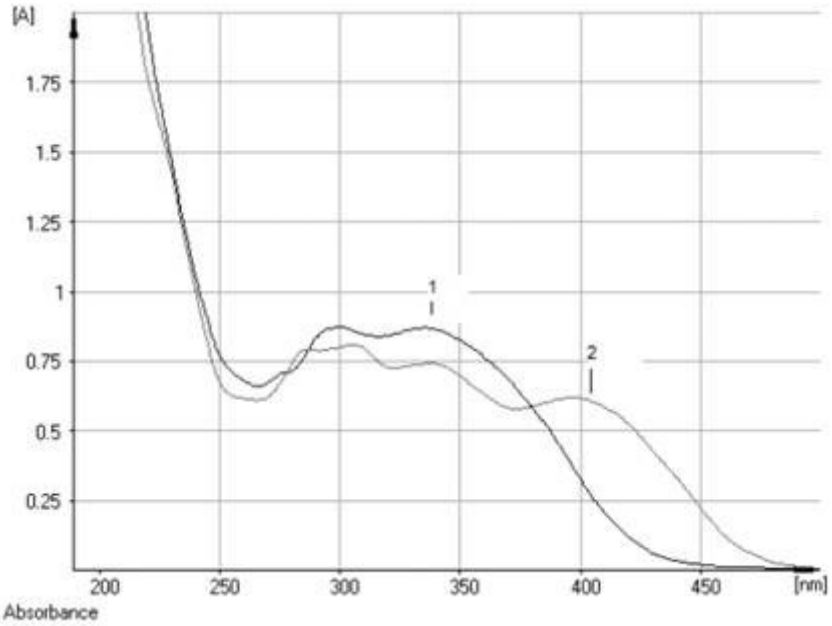
Effect of extraction conditions on the extraction of flavonoids from flowers sandy immortelle

№ п/п	Экстрагент	Соотношение «сырье-экстрагент»	Время экстракции	Содержание суммы флавоноидов в пересчете на изосалипурпозид, %	
				при длине волны 315 нм*	при длине волны 418 нм
1.	40 % этиловый спирт	1:50	1 ч	8,90 ± 0,08	2,21 ± 0,02
2.	50 % этиловый спирт	1:50	1 ч	8,30 ± 0,07	2,50 ± 0,03
3.	60 % этиловый спирт	1:50	1 ч	7,29 ± 0,03	2,37 ± 0,02
4.	70 % этиловый спирт	1:50	1 ч	10,10 ± 0,12	3,10 ± 0,03
5.	80 % этиловый спирт	1:50	1 ч	8,53 ± 0,09	2,82 ± 0,02

Примечание: * – фармакопейная методика [1].

Discussion and conclusions

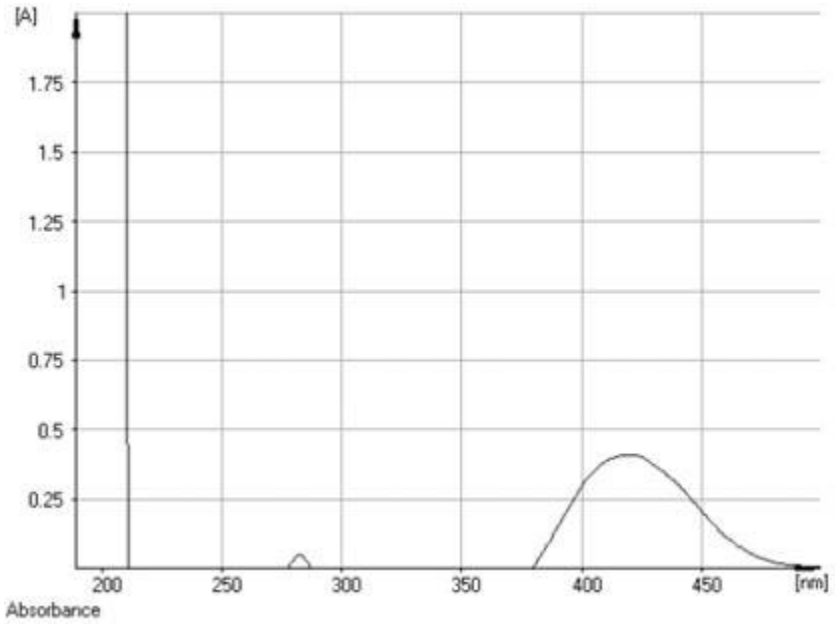
In the UV spectrum of the aqueous-alcoholic extract, two most characteristic absorption maxima were found in the region of 295 nm and 335 nm (Fig. 1). It should be noted that in the corresponding Pharmacopoeia Monograph, the analytical wavelength is 315 nm, i.e. the region of one of the minima of the absorption curve (Fig. 1). In our opinion, the overestimation of the results occurs due to the presence of accompanying substances - hydroxycinnamic acids (absorption maximum at 290, 327 nm), coumarins (absorption maximum at about 340 nm) and phthalides (absorption maximum at 270, 290 nm). In addition, the method does not use the GSO of isosalipurposide, but uses a calibration graph. In our opinion, in the analysis method, it is advisable to use the variant of differential UV spectroscopy,



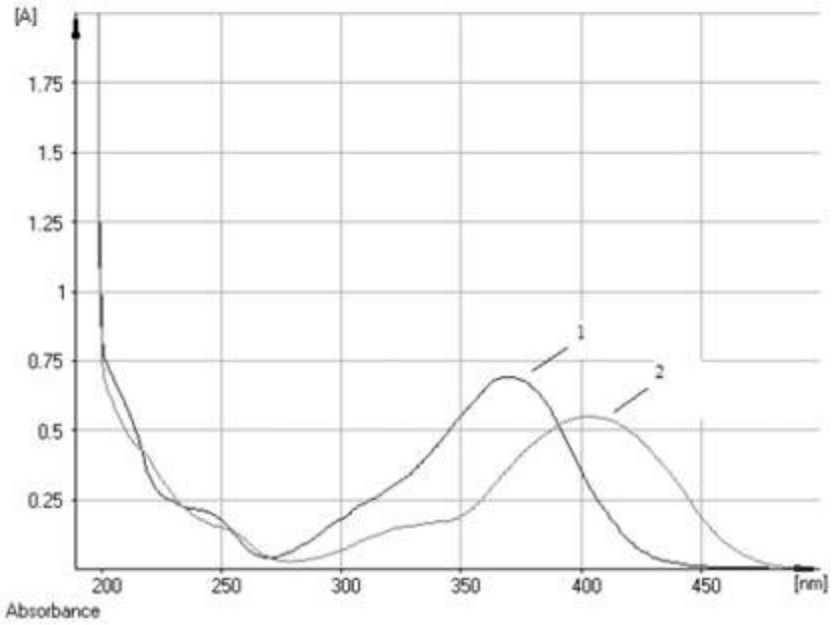
Rice. 1.UV spectra of solutions of aqueous-alcoholic extract from flowers sandy immortelle

Designations: 1 - initial solution; 2 - solution in the presence of $AlCl_3$...

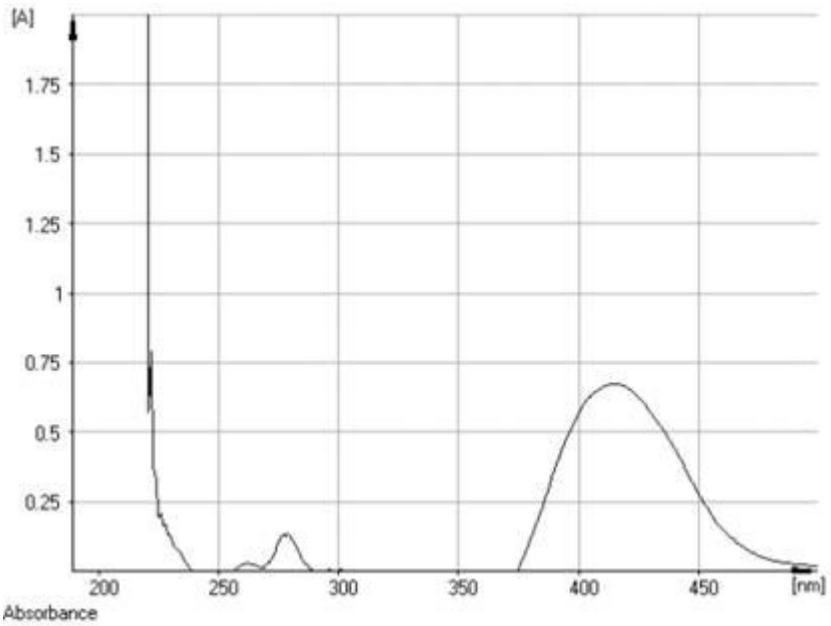
The study of the UV spectrum of the aqueous-alcoholic extraction in the presence of AlCl3 showed that the main absorption maximum is observed at a wavelength of 418 nm (Fig. 2). Moreover, the nature of the absorption of this spectrum correlates with the UV spectrum of isosalipurposide: in the presence of AlCl3, a bathochromic shift of the maximum at 370 nm to the region of 418 nm is observed (Figs. 3 and 4). This allows us to conclude that, in the presence of AlCl3, the nature of the UV absorption curve of the aqueous-alcoholic extraction of immortelle flowers is mainly due to isosalipurposide. On this basis, a method was developed for determining the sum of flavonoids using an analytical wavelength of 418 nm in the variant of differential UV spectroscopy.



Rice. 2.UV spectrum of a solution of aqueous-alcoholic extract from immortelle flowers sandy with the addition of $AlCl_3$ (differential spectrophotometry).



Rice. 3. UV spectra of isosalipurposide solutions.
Designations: 1 - initial solution; 2 - solution in the presence of $AlCl_3$...



Rice. 4. UV spectrum of a solution of isosalipurposide with the addition ofAlCl3 (differential spectrophotometry).

Using the developed technique, a number of samples of sandy immortelle flowers were analyzed (Table 2). The content of the sum of flavonoids in various samples of immortelle flowers is in the range of 2.90-3.50% (in terms of isosalipurposide). The metrological characteristics of the developed analysis methods indicate that the error of a single determination of the amount of flavonoids in the raw material of the sand immortelle is ± 3.95% (Table 3).

table 2

The content of the sum of flavonoids in various samples of immortelle flowers sandy

№ п/п	Характеристика образца сырья	Содержание суммы флавоноидов в пересчете на изосалипурпозид и абсолютно сухое сырье (в %)
1.	ОАО «Красногорсклексредства»	3,10 ± 0,05
2.	ООО ПКФ «Фитофарм» (г. Анапа)	2,95 ± 0,04
3.	Самарская обл. (с. Переволоки, 2008 г.)	3,04 ± 0,06
4.	Ульяновская обл. (г. Барыш, 2008 г.)	2,90 ± 0,04
5.	Пензенская обл. (с. Новая Елюзань, 2005 г.)	3,50 ± 0,07
6.	Саратовская обл. (с. Усовка, 2008 г.)	3,20 ± 0,05

Table 3

Metrological characteristics of the quantitative determination procedure the amount of flavonoids in the flowers of the immortelle sandy

f	X	S	P, %	t (P, f)	ΔX	E, %
10	3,10	0,0549	95	2,23	0,122	±3,95

Thus, as a result of the studies carried out, the data on the chemical composition of the flowers of sandy immortelle were revised, the dominant substance of the raw material of the studied medicinal plant (isosalipurposide) was determined, objective methods for the qualitative and quantitative analysis of the characteristics of the flavonoid content using TLC and differential spectrophotometry in the presence of GSO of isosalipurposide were proposed. The developed methods are included in the draft of the pharmacopoeial monograph "Sandy immortelle flowers".

Literature

1. Wild useful plants of Russia / Otv. ed. A.L. Budantsev, E.E. Lesiovskaya. - SPb .: Publishing house SPKhFA, 2001. - P. 74–81.

2. Kurkin V.A. Pharmacognosy: A Textbook for Pharmaceutical Students universities (faculties.). 2nd ed., Rev. and add. - Samara: OOO "Etching", GOU VPO "SamGMU Roszdrav", 2007. - 1239 p.

3. Kurkin V.A. Fundamentals of Herbal Medicine: A Study Guide for Students pharmaceutical universities. - Samara: OOO "Etching", GOU VPO "SamGMU Roszdrav", 2009. - 963 p.

4. Plant resources of the USSR: Flowering plants, their chemical composition, usage; Family Asteraceae (Compositae). - SPb .: science, 1993. - pp. 120–123.

5. Tyukavkina N.A. Act speech. Bioflavonoids. Chemistry, food, medicine, health. -M., 2002. - 56 p.

6. Phytotherapy: guidelines No. 2000/63: [approved. M-vom Healthcare of the Russian Federation] / Karpeev AA, Kiseleva T.L., Korshikova Yu.I., Lesiovskaya E.E., Sakanyan E.I .; Scientific-practical. center of trad. honey. and homeopathy. - M., 2000 .-- 28 p.

7. Phytotherapy: normative documents // Teaching aid / Under total. ed. AA Karpeeva, T.L. Kiseleva. - Publishing house of FNCEC TMDL of Roszdrav, 2006. - 362 p.

8. Wagner H. Pharmazeutische Biologie. Drogen und ihre Inhaltsstoffe. - Stuttgart-New York: Gustav Fischer Verlag, 1993.522 s.

9. State Register of Medicines. - T. 1, 2. Official edition. - M., 2008.

10. State Pharmacopoeia of the USSR: Vol. 2. General methods of analysis. Medicinal plant raw materials / Ministry of Health of the USSR. - 11th ed., Add. - M .: Medicine, 1989 .-- 400 p.

Author's address
Ph.D., Kurkina A.V.
Department of Pharmacognosy with Botany and Fundamentals of Phytotherapy, Samara State Medical University of Roszdrav
Annushkae@yandex.ru

Kurkina, A.V. New approaches to the standardization of raw materials of the Sand Immortelle - Helichrysum arenarium (L.) Moench. / A.V. Kurkina // Traditional Medicine. - 2010. - No. 1 (20). - S. 45-49.