

Chromatographic study of the flavonoid composition of fresh bulbs of *Allium cepa* L.,
used in homeopathy G.A. Zinchenko,
T.L. Kiseleva, A.V. Nefedova
(Institute of Homeopathy and Naturotherapy of the Federal Scientific Clinical Experimental
Center for Traditional Methods of Diagnostics and Treatment of the Ministry of Health of Russia, Moscow)

The method of homeopathy is approved for medical use on the territory of the Russian Federation by order of the Ministry of Health of the Russian Federation No. 335 dated November 29, 1995 [2]. However, there is still no domestic homeopathic pharmacopoeia. In this regard, the development of criteria and methods for determining the authenticity and good quality of homeopathic raw materials of plant origin is relevant.

The aim of the study was a chromatographic study of the flavonoid composition of fresh bulbs of five varieties of onion *Allium cepa* L. grown on the territory of the Russian Federation, used in homeopathy.

Pharmacopoeia monographs on raw onion are present in foreign homeopathic pharmacopoeias (HomP) in India [3], France [5] and Germany [4]. A detailed analysis of these monographs is given in the previous publication [1]. In the structure of each PS there is a section "Thin-layer chromatography", and GomF of India offers thin-layer chromatography for amino acids, GomP of Germany - for terpenoids, and GomP of France - for flavonoids.

The study was carried out on the basis of the laboratory of pharmacognosy and botany of the Institute of Homeopathy and Natural Therapy of the Federal Scientific Clinical and Experimental Center for Traditional Methods of Diagnostics and Treatment of the Ministry of Health of the Russian Federation.

The objects of the study were fresh bulbs of five varieties of onions: Myachkovsky, Strigunovsky, Gollandsky Bely, Carmen, and Stuttgarten Riesen.

When performing the work, the method of thin layer chromatography was used: Kieselgel 60 F plates²⁵⁴ (firm "Merck", Germany), as well as methods of carrying out qualitative reactions, traditional for pharmacognostic studies.

At the first stage of the study, methods were developed for carrying out qualitative reactions and chromatographic study (TLC) of flavonoids in raw materials of one variety of onion "Myachkovsky".

Extraction from fresh onion bulbs was obtained by three-fold extraction, 60% ethanol was used as an extractant, the ratio of raw material and extractant was 1:25. To 1 ml of the obtained extract were added 0.1 g of zinc powder and 5-7 drops of concentrated hydrochloric acid and heated in a water bath for 10 minutes. A pale pink color (flavonoids) gradually appeared. A few drops of 5% NaOH solution were added to 1 ml of the obtained extract. As a result of the reaction, the yellow color of the solution (flavonoids) increases.

To the start line of the Kieselgel 60 F plate²⁵⁴ (Merck, Germany) with a size of 9x7 cm using a microsyringe was applied 0.04 ml of extract from fresh onion bulbs in a strip of 1 cm at a distance of 2 cm from the edge. The plate with the applied sample was dried in air, placed in a chamber with an ethyl acetate - acetic acid - water (5: 1: 1) solvent system, and chromatographed ascending. The saturation time of the chamber is not less than 40 minutes. After passing the front of solvents about 7.5 cm, the plate was removed from the chamber, dried in air for 15 minutes and treated with 2% alcoholic solution of $AlCl_3$... After drying for 2-3 minutes in an oven at a temperature of 100-105 ° C, the plate was viewed in UV light at a wavelength of 365 nm. The chromatogram contains 3 absorption zones: Rf 0.13 (yellow color), Rf 0.24 (blue color), Rf 0.49 (yellow color).

Using the qualitative reactions to flavonoid compounds and chromatographic detection of flavonoids developed for the variety "Myachkovsky"

raw materials of various varieties of onions grown on the territory of the Russian Federation, in a comparative aspect (Tables 1 and 2, respectively).

Table 1

**RESULTS OF DETECTION OF FLAVONOIDS AND SULFUR-CONTAINING COMPOUNDS
USING QUALITATIVE REACTIONS IN VARIOUS RAW MATERIALS
VARIETIES OF ALLIUM SULFUR**

No. p / p	Onion variety	Reaction results		
		Cyanidinic try	Diluted with NaOH.	With Ag (NH ₃) ₂ NO ₃
1	"Myachkovsky"	Positive: pink staining	Positive: strengthening of yellow coloration	Positive: yellow coloration, when heated black sediment
2	"Strigunovsky"	Positive: pink staining	Positive: strengthening of yellow coloration	Positive: yellow coloration, when heated black sediment
3	"Stuttgarten Riesen"	Positive: pink staining	Positive: strengthening of yellow coloration	Positive: yellow coloration, when heated black sediment
4	"Carmen"	Positive: bright crimson staining at adding HCl	Positive: yellow coloration	Positive: yellow coloration, when heated black sediment
5	"Dutch white"	Negative: staining not appeared	Negative: staining not appeared	Positive: yellow coloration, when heated black sediment

Raw materials of all the studied varieties of onions give positive reactions to flavonoids, except for the variety "Gollandskiy belyi", and in all cases, except for onions of the Gollandskiy belyi variety, on chromatograms we found 2 yellow absorption zones (flavonoids) and 1 absorption zone blue (phenol carboxylic acids). Rf equals 0.13 (yellow), 0.23 (blue), 0.48 (yellow) (average of 5 determinations for raw materials of each grade). The luminescence intensity of the absorption zones after processing the chromatogram with chromogenic reagents was also approximately the same.

When carrying out a chromatographic analysis of the extraction of red onion varieties "Carmen" from the raw material, in addition to the absorption zones described above in visible light, one more absorption zone of violet color was identified, which disappears after about 8 hours. This allowed us to assume the presence of anthocyanins in this onion variety. Therefore, in the future, we made an attempt to chromatographically separate anthocyanin compounds.

As a result of the study, it was found that for the chromatographic separation of anthocyanins, analysis on silica gel - Kieselgel 60 F plates²⁵⁴ (Merck, Germany) in the solvent system chloroform - methanol - water (60: 37: 7). In this case, 1 absorption zone with an Rf of 0.09 is found, which has a blue color in visible light after processing the chromatogram with a 4% solution of ammonium molybdate.

CONCLUSIONS

1. Developed a method for the chromatographic determination of flavonoids in fresh raw materials onions, which consists in the chromatographic separation of extracts from raw materials in a thin layer on Kieselgel 60 F plates²⁵⁴ (Merck, Germany) in the solvent system ethyl acetate - acetic acid - water (5: 1: 1) and detection by observing the fluorescence zones in UV light (365 nm) before and after processing the chromatogram with 2% alcoholic solution of aluminum chloride (AlCl₃) (the plates were kept at a temperature of 100-105 ° C for 2-3 minutes in an oven).

2. Using the developed technique, qualitative reactions were carried out and chromatographic analysis of extracts from raw onion varieties "Strigunovsky", "Stuttgarten rizen", "Carmen", "Gollandsky white" in a comparative aspect. 2 zones of absorption with Rf 0.13 and 0.48, referred by us to flavonoids, and 1 absorption zone with Rf 0.23, referred by us to phenol carboxylic acids.

3. Chromatographic analysis of the "Gollandskiy Bely" grade raw material reveals the only absorption zone with an Rf of 0.23, corresponding to phenol carboxylic acids.

4. In the chromatographic analysis of raw materials of the "Carmen" variety, in addition to the above three zones absorption (pin 1), an absorption zone with Rf 0.09 is detected in the chloroform solvent system - methanol - water (60: 37: 7) on Kieselgel 60 F plates²⁵⁴ firm Merck, Germany, which we classified as anthocyanins.

table 2

CHARACTERISTIC OF ABSORPTION ZONES DURING TLC EXTRACTIONS FROM BULBS
DIFFERENT VARIETIES OF ONION

No. p / p	Onion variety onion	Absorption zone				
		Rf	Colour v light	visible	Color in UV light up to processing chromatograms	Color in UV light after processing chromatograms
1	"Myachkovsky"	0.14	Not identified		Dark brown	Yellow
		0.49	Yellow		Yellow	Yellow
		0.25	Not identified		Not identified	Blue
2	"Strigunovsky"	0.14	Not identified		Dark brown	Yellow
		0.48	Yellow		Yellow	Yellow
		0.25	Not identified		Not identified	Blue
3	Stuttgarten rizen "	0.13	Not identified		Dark brown	Yellow
		0.48	Yellow		Yellow	Yellow
		0.23	Not identified		Not identified	Blue
4	"Carmen"	0.13	Not identified		Dark brown	Yellow
		0.47	Yellow		Yellow	Yellow
		0.21	Not identified		Not identified	Blue
		0.09 *	Purple		Not identified	Not identified
5	"Dutch White"	0.23	Not identified		Not identified	Blue

LITERATURE

1. Nefedova A.V., Kiseleva T.L., Zinchenko GA, Kosmodemyanskiy L.V. Onions and garlic in herbal medicine and homeopathy. Publication 3: the use of raw materials and medicines in homeopathy // Traditional medicine. - 2004. - No. 3. - in print.

2. Order of the Ministry of Health and the Ministry of Health of the Russian Federation "On the use of the method of homeopathy in practical

health care "from 29.11.95, No. 335

3. Homoeopathic Pharmacopoeia of India (HPI). New Delhi: Government of India Ministry of Health and Family welfare. - Vol. 6. - 1990. - 243 p.

4. Homeopathisches Arzneibuch. - 1. Ausgabe 1978. -Gesatausgabe. - Diese Gesatausgabe nach der Neufassung 1985 enthalt die Teibande HAB 4. Nachtrag 1985. - Amtliche Ausbage. - Deuscher Apotheker Verlag Sttugart Govi - Verlag GmbH. Franctfurt. - 928 s.

5. Pharmacopee Francaise. - 10 edition, 6 supplement: Monographues de souches pour preparations homeopathiques. - Paris.

Zinchenko, G.A. Chromatographic study of the flavonoid composition of fresh *Allium cepa* L. bulbs used in homeopathy / G.A. Zinchenko, T.L. Kiseleva, A.V. Nefedova // Traditional Medicine. - 2004. - No. 2 (3). - S.49-51.

[To favorites](#)