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HPLC ANALYSIS OF *Artemisia dracunculus* EXTRACTS

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Flavonoids in extracts of Artemisia dracunculus *L. are studied. The principal component is identified as pinocembrine. Pinocembrine is analyzed quantitatively using an internal standard. The uncertainties in the chromatographic measurements are estimated.*

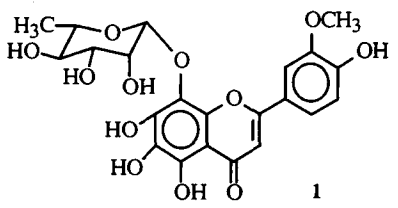
Key words: *Artemisia dracunculus*, flavonoids, HPLC.

Preparations from plant material are widely used as prophylactics and to treat various diseases. They have managed to position themselves more favorably than the synthetic compounds. Therefore, it seems timely to expand the variety of domestic natural medicinal compounds [1-3].

The above-ground part of tarragon or tarkhun (*Artemisia dracunculus* L., Asteraceae) is widely used as an aromatic herb and is included in the recipe of the nonalcoholic drink "Tarkhun." Many reports have appeared about the use of this plant as a general tonic, anti-inflammatory, wound healer, antitumor, diuretic, anti-ulcer, and digestion improving agent [4].

The timeliness of these studies is also due to the fact that industrial tarkhun plantations have now been established in Samara district at the Middle Volga regional experimental station of the NPO All-Russia Institute of Medicinal and Aromatic Plants (Antonovka). In the future, this will enable the plant to be used not only for culinary purposes (RST RSFSR 667-82, "Fresh tarragon") but also as a medicinal plant.

Flavonoids such as estragonoside (**1**), pinocembrine (**2**), naringenin (**3**), and annagenin (**4**) have previously been isolated from tarkhun extract [5, 6]. The molecular structure and certain physicochemical properties [mp, wavelength of absorption maximum (λ_{max})] of tarkhun and pinostrobine (**5**, internal standard) components are given below.



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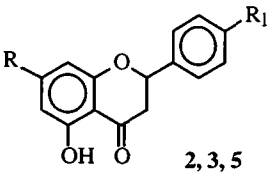
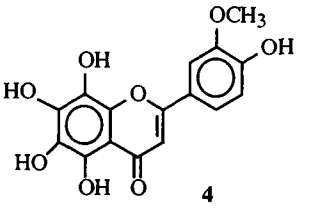


2, 3, 5

2: R = OH, R_1 = H
3: R = R_1 = OH
5: R= OCH_3, R_1 = H



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We developed a chromatographic method for analyzing tarkhun components by HPLC.

The experiment was performed using reverse-phase HPLC owing to the high polarity of this class of compounds. The peaks for tarkhun components had to be fully resolved so various eluents were studied: CH_3OH—H_2O, C_2H_5OH—H_2O, and CH_3CN—H_2O. Despite the fact that the separation of this class of compounds had been reported [7], we were unable to obtain good separation using CH_3OH—H_2O and C_2H_5OH—H_2O. The optimal mobile phase was CH_3CN—H_2O (50:50 vol. %). Qualitative and quantitative analysis of pinocembrine in tarkhun extract was carried out using it. Figure 1 shows chromatograms of tarkhun extracts that were obtained in a Separon C18 column using it.

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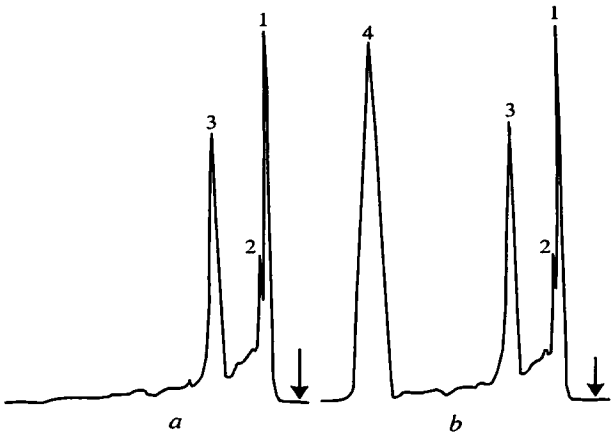

Fig. 1. Chromatograms of tarkhun extract without (*a*) and with standard (pinocembrine) (*b*): unidentified components (1, 2), pinocembrine (3), pinostrobine (4).

The pinocembrine peak was identified by comparing the retention times t_R and capacities k with a pinocembrine government standard.

The k value was calculated using

$$k = (t_R - t_M)/t_M, \quad (1)$$

where t_R and t_M are the retention times of the analyzed and nonsorbed compounds, respectively.

Sodium nitrate was used as the nonsorbed compound. Under the analysis conditions, t_R = 5 min 45 sec; k = 4.48.

Pinocembrine in the tarkhun extract was analyzed quantitatively using an internal standard. This method consists of chromatography of a known quantity of the analyte to which a known quantity of standard compound has been added. A government standard (GS) of pinostrobine [2], which like pinocembrine is a flavanone, was used.

The pinocembrine concentration in the extract was calculated using

$$C_i = (k_i - Q_i) \cdot 100 \cdot r/Q_{st} \quad (2)$$

where k_i is the detector sensitivity coefficient for pinocembrine relative to the standard (pinostrobine); Q_i and Q_{st} are the peak areas of the analyte (pinocembrine) and standard (pinostrobine) in mm^2, respectively; r is the ratio of the standard mass to the mass of the analyte without standard.

The peak area was calculated by triangulation, i.e., the peak height was multiplied by its width at half height.

If it is assumed that the detector sensitivity coefficient for pinocembrine and the standard is equal to unity owing to the similarity of the physicochemical properties of pinocembrine and pinostrobine, Eq. (2) becomes

$$C_i = (Q_i) \cdot 100 \cdot r/Q_{st}. \quad (3)$$

The advantage of the internal-standard method is that data are obtained from a single analysis cycle, which increases the accuracy. The results of the quantitative analysis are listed below:

Analysis number	Peak area, mm^2		Pinocembrine content in
	pinocembrine Q_i	pinostrobine Q_{st}	extract, C_i, mass%
1	209.91	807.80	0.0381
2	247.51	1024.53	0.0354
3	253.26	974.47	0.0381
4	226.85	914.41	0.0363
5	220.45	914.41	0.0353
6	210.87	852.75	0.0362
7	217.90	863.61	0.0370

The overall analytical uncertainty was calculated considering random and systematic contributions [8, 9]. The calculated uncertainties in the determination of pinocembrine concentration in the tarkhun extract are given below:

$S_{\bar{C}}$	Δ_{sys}	Δ_{tot}, %
0.0012	0.0895	8.9500

Here $S_{\bar{C}}$ is the standard deviation of the mean

$$S_{\bar{C}} = \sqrt{\frac{\sum_{i=1}^{n}(C_i - \bar{C})^2}{n(n-1)}}, \quad (4)$$

where $\bar{C}$ is the arithmetic mean of the pinocembrine concentration; n is the number of analyses; Δ_{sys} is the systematic component of the uncertainty that was calculated using

$$\Delta_{sys} = k\sqrt{\theta_w^2 + \theta_{TY}^2 + 0.005^2 + 0.01^2 + 0.03^2 + \left(\frac{\Delta\bar{Q}_i / Q_{st}}{\bar{Q}_i / Q_{st}}\right)^2}, \quad (5)$$

where k is a coefficient set equal to 1.1 for a confidence interval $P = 95\%$;

$$\theta_w = 1.1\sqrt{(\Delta m_0/m_0)^2 + (\Delta m_1/m_1)^2 + (\Delta m_2/m_2)^2}$$

is the uncertainty in weighing; $\Delta m_0 = \Delta m_1 = \Delta m_2 = 0.0002$ g is the uncertainty in the weights; m_0, m_1, and m_2 are the numerical values of all weighings during sample preparation; $\Theta_{TY} = (1\text{-}N)/2$ is the uncertainty in the content of principal compound; N is the percent content of the principal compound; 0.005 is the uncertainty in the linearity; 0.01 is the uncertainty in the measuring loop; 0.03 is the uncertainty in setting and regulating the chromatographic separation parameters; $(\Delta Q_i/Q_m)/(Q_i/Q_{st})$ is the uncertainty in the limits of a reliable measurement of the peak-area ratio of the i-th component and the standard. Because $\Delta_{sys}/S_{\bar{C}} = 74.58 > 8$, the overall uncertainty of the analysis will be equal to the systematic component, which was 8.95%.

EXPERIMENTAL

Preparation of Tarkhun Extract. An analytical sample was ground to a particle size capable of passing through a 1-mm sieve. An accurately weighed (~1.0 g) sample of ground material was placed in a stoppered 100-ml flask and treated with ethanol (40 ml, 70%). The flask and its contents were attached to a reflux condenser and heated on a boiling-water bath for 1 h. The contents of the flask were cooled and filtered through a paper filter (red band). The extract (1 ml) was placed in a 25-ml volumetric flask and brought to the mark with 95% ethanol.

Eluent Preparation. The eluent was CH_3CN—H_2O (50:50 vol. %). Eluent was prepared by mixing pure solvents. The mixture was irradiated with ultrasound in order to degas it. Mechanical impurities were removed by filtration through a paper filter (blue band).

Chromatographic Analysis of Tarkhun. The investigations were carried out in a Milikhrom-1 liquid-chromatography microcolumn. The conditions were: UV detection at 290 nm; steel column (120 × 2 mm), Separon C_{18} sorbent; CH_3CN—H_2O (50:50 vol. %) eluent; eluent flow rate F_c 200 μl; sample volume 15 μl; optical density scale A 1.6 and 12.8 arb. units.

The retention times of the sorbates t_R and of an unsorbed compound t_M were determined during the analysis.

Estragonoside (1). Yellow crystals, $C_{22}H_{22}O_{12}$, mp 192-195°C (ethanol), λ_{max} (ethanol) 258, 267, and 348 nm.

Pinocembrine (2). Light yellow crystals, $C_{15}H_{12}O_4$, mp 185-188°C ($CHCl_3$—C_6H_{14}), λ_{max} (ethanol) 290 and 330 nm.

Naringenin (3). Yellow crystals, $C_{15}H_{12}O_5$, mp 264-268°C (aqueous alcohol), λ_{max} (ethanol) 291 and 332 nm.

Annagenin (4). Bright yellow crystals, $C_{16}H_{12}O_8$, mp 256-258°C (aqueous alcohol), λ_{max} (ethanol) 258, 271, and 376 nm.

Pinostrobine (5). White platelike crystals, $C_{16}H_{12}O_4$, mp 112-113°C, λ_{max} (ethanol) 289 nm.

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REFERENCES

1. G. P. Kotel'nikov, O. G. Yakovlev, and N. O. Zakharova, *Gerontology and Geriatrics* [in Russian], Samarskii Dom Pechati, Moscow and Samara (1997), p. 118.
2. V. A. Kurkin, *Phenylpropanoids, Promising Natural Biologically Active Compounds* [in Russian], Samara (1996).
3. G. P. Yakovlev and K. F. Blinova, eds., *Plants for Us* [in Russian], Uchebnaya Kniga, St. Petersburg (1996).
4. *Plant Resources of the USSR. Chemical Composition and Useful Properties...* [in Russian], Nauka, St. Petersburg (1996).
5. V. A. Kurkin, G. G. Zapesochnaya, A. V. Mytsyk, A. V. Kurkina, K. S. Pimenov, and M. S. Mar'ina, *Rast. Resur.*, **32**, Nos. 1-2, 88 (1996).
6. V. A. Kurkin, G. G. Zapesochnaya, and E. V. Avdeeva, *Khim. Prir. Soedin.*, 61 (1997).
7. L. W. Wulf and C. W. Nagel, *J. Chromatogr.*, **116**, 271 (1976).
8. Yu. I. Arutyunov, I. A. Platonov, L. A. Onuchak, S. Yu. Kudryashov, S. V. Avdeev, and E. V. Revinskaya, *Quantitative Gas-Chromatographic Analysis. Laboratory Practicum* [in Russian], Samarskii Universitet, Samara (1997).
9. L. A. Onuchak, Yu. I. Arutyunov, I. A. Platonov, S. Yu. Kudryashov, S. V. Avdeev, and E. V. Revinskaya, *Chromatographic Measurements. Laboratory Special Practicum* [in Russian], Samarskii Universitet, Samara (1996).