ISSN 0027-1314, Moscow University Chemistry Bulletin, 2020, Vol. 75, No. 1, pp. 52–55. © Allerton Press, Inc., 2020.
Russian Text © The Author(s), 2020, published in Vestnik Moskovskogo Universiteta, Seriya 2: Khimiya, 2020, No. 1, pp. 65–71.

A Comparative Study of Acetonitrile Fraction Marker Substances from Leaves and Fruits of *Malus sylvestris*

N. V. Nesterova[a], *, I. A. Samylina[a], and V. N. Matveenko[b]

[a]*Sechenov University, Moscow, 119991 Russia*
[b]*Moscow State University, Moscow, 119991 Russia*
**e-mail: nestero-nadezhda@yandex.ru*

Received January 10, 2019; revised February 12, 2019; accepted February 14, 2019

Abstract—The new plant raw material from leaves and fruits of *Malus sylvestris* is analyzed. It is shown that the content of substances extracted with acetonitrile amounted to 4.7–5.4% for leaves and 2.3–2.8% for fruits. The obtained extracts were used to identify substances, the presence of which is specific for the plant's raw material and can be used in the future to identify raw material in crushed and powdered form. It is found that acetonitrile extract of *Malus sylvestris* is characterized by the presence of 10 peaks for fruits and 22 peaks for leaves, which can be considered as markers for the identification of medicinal plant material.

Keywords: fruits, leaves, *Malus sylvestris*, extractive substances, gas chromatography, marker substances
DOI: 10.3103/S0027131420010083

INTRODUCTION

According to the World Health Organization, falsification of drugs is considered one of the important and serious threats to the global pharmaceutical market [1, 2]. At the same time, the possibility of counterfeiting drugs based on herbal raw materials (HRMs) and biologically active food additives has not attracted the close attention of the medical and pharmaceutical scientific community, despite the fact that consumer demand for this group of goods is growing steadily, and the range of HRM is expanding. The identification of crushed HRM by the microscopic analysis and qualitative reactions provided by the 14th edition of the State Pharmacopoeia (SP), requires a high level of qualification and experience of the analyst for conducting the examination. At the same time, an analysis of the scientific literature shows the promise of using gas chromatography for identifying marker substances [3], the combination of which allows reliable identification of medicinal plant materials even in crushed and powdered forms, as well as in collections based on HRM and extraction drugs, which allows us to implement a unified methodological approach of "through" standardization at the stages:

HRM—substance—a herbal medicine of plant origin.

The leaves and fruits of the forest apple tree contain a complex of biologically active substances (BAS), represented by flavonoids, tannins, phenolcarboxylic acids, and polysaccharides, providing a wide range of biological activity. For reliable instrumental identification of the authenticity of raw materials in order to avoid regrading or falsification by unscrupulous manufacturers and taking into account the similarity of the chemical composition of the studied raw materials to others, including the pharmacopeia representatives of the Rosaceae family [4–7], we considered that it appropriate to identify rare substances that can act as specific marker substances typical for this HRM. It is well known that this area of research is currently considered to be one of the most important in the standardization system of HRM, which has been repeatedly underlined in the directives of the Council of the Eurasian Economic Commission "Requirements for studying the stability of drugs from medicinal plant materials." In view of the foregoing, the aim of this work was to assess the quantitative content of extractives from raw materials with acetonitrile, as well as a qualitative assessment of marker substances that allow the identification of leaves and fruits of forest apple trees.

MATERIAL AND METHODS

To identify marker substances and quantitatively determine the extractives extracted with acetonitrile from dry and fresh raw materials, the leaves and fruits of wild plants of *Malus sylvestris* collected in 2018 in the Istra and Chekhov districts of the Moscow Region were used.

Based on the experience of earlier studies, we used forest acetonitrile (*TU* (Technical Specifications) *6-09-3534-87*, analytical grade) (1 : 1) to extract and identify specific substances in the leaves and fruits of the apple tree.

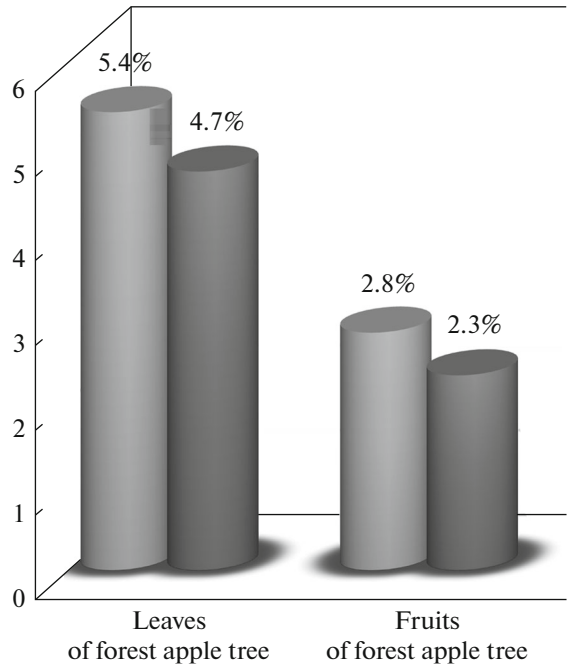

Fig. 1. Assessment of the content of substances extracted from the raw material of forest apple trees with acetonitrile.

The samples were prepared as follows. Sealed vials with the obtained filtered extracts from the test materials were placed for 10–15 min in a Sapphire mixer bath operating on ultrasound without preliminary heating, after which 10 mL of extraction was taken into a teflon cone and centrifugated in the laboratory centrifuge Ohaus Split 16000 at 16000 rpm for 120 s. After centrifugation, 1 mL of extraction from the surface layer was taken using a microdoser (to avoid the ingress of microparticles of raw materials) and placed in the injector drum of a chromatograph-mass spectrometer.

The study was performed on an Agilent Technologies 6850 Series II gas chromatograph, Agilent Technologies Network Mass Selective Detector, and HP-5MS (30 m × 0.25 mm) chromatographic column. The chromatography conditions were as follows: initial isothermal section 35°C, 5 min; 35–100°C, at a rate of temperature rise of 2°C/min; 100–200°C, at a rate of temperature rise of 5°C/min; 200–250°C, at a rate of temperature rise of 10°C/min; final isothermal section, 15 min; the temperature of the evaporator was 200°C; the injector temperature was 30°C; and the feed rate of the carrier gas (helium) was 1 mL/min.

RESULTS AND DISCUSSION

In the course of assessing the content of extractives extracted with acetonitrile from samples of fresh and dried raw material (leaves and fruits of forest apple trees), the data obtained are presented in Table 1 and Fig. 1. The obtained acetonitrile extracts were used by us to identify marker substances that allow the identification of raw materials.

The general view of the mass spectra of acetonitrile fractions of leaves and fruits of forest and domestic apple trees is shown in Figs. 2 and 3.

As can be seen from the data in Tables 1–3, there are 10 peaks for the acetonitrile extract from the fruits of the forest apple tree, among which tributyl acetyl citrate, 1,2-cyclohexanedicarboxylic acid dinonyl ester, and 1,2-cyclohexane dicarboxylic acid cyclohexyl-

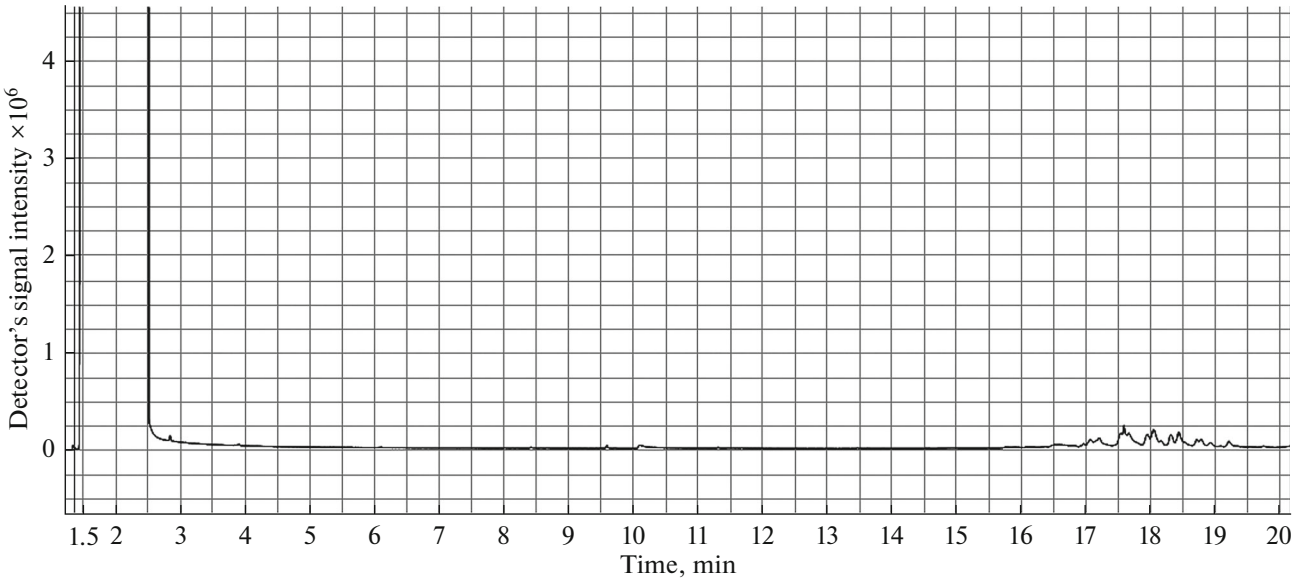

Fig. 2. General view of the chromatography-mass spectra of the fruits of forest apple tree.

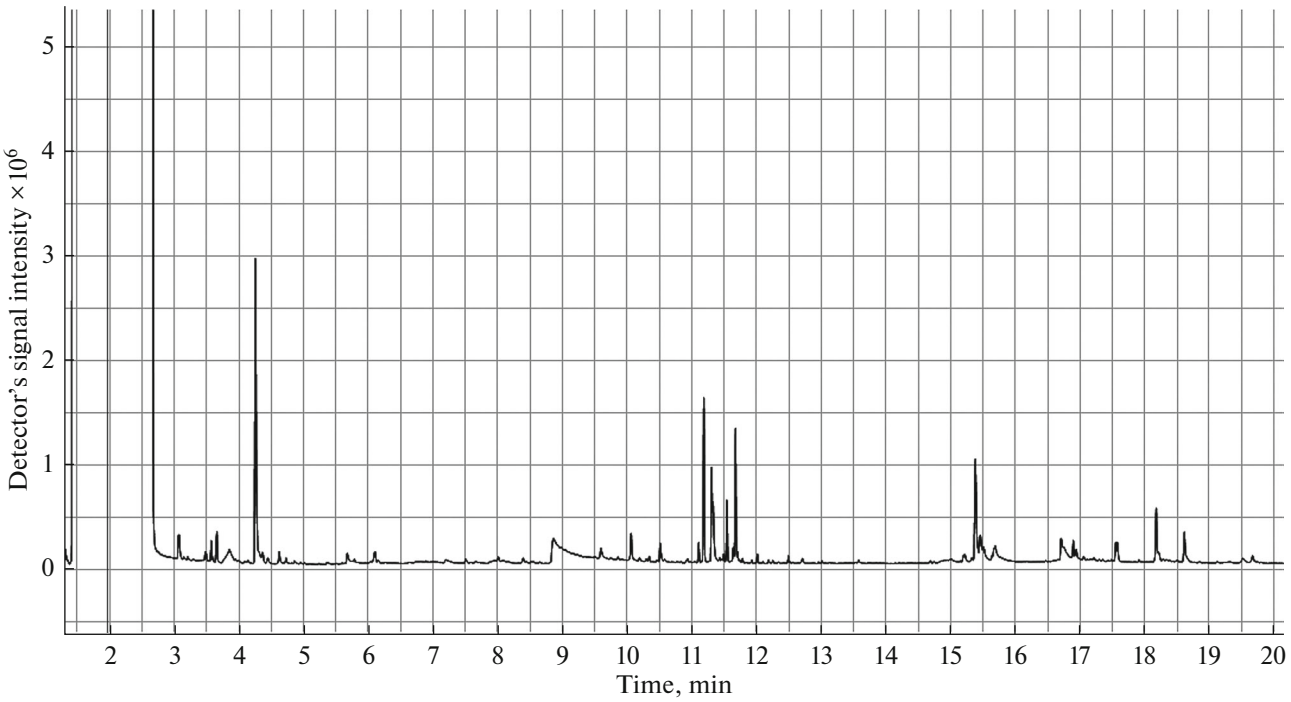

Fig. 3. General view of the chromatography-mass spectra of the leaves of forest apple tree.

methylnonyl ester can be considered as marker substances. There are also 22 peaks for the leaves, among which furfural, 5-hydroxy methylfurfural, *cis*-β-farnesene, α-farnesen, 8,11-octodecanoic acid methyl ester, and tributylacetyl citrate can be used as marker substances. It should be noted that all the listed substances were isolated from the studied raw material for the first time.

Table 1. Assessment of the content of extractives extracted from the raw materials of apple trees with acetonitrile forest

Extractant used	The content (%) of extractive substances in the studied raw material from forest apple trees			
	fruits		leaves	
	fresh	dried	fresh	dried
Acetonitrile (*TU 6-09-3534-87*, analytical grade)	2.8	2.3	5.4	4.7

Table 2. The composition of the acetonitrile fraction from the fruits of the forest apple tree

Retention time, min	Name of substance	Gross formula
2.10	Cyclopropane	C_3H_6
2.838	1-Butanol	$C_4H_{10}O$
3.904	Furfural	$C_5H_40_2$
9.591	2,3-Dimethyl-1,3-butadiene	C_6H_{10}
10.1	Triacetin	$C_9H_{14}O_6$
11.312	1-Chlorodecane	$C_{10}H_{21}Cl$
17.585	Tributyl acetylcitrate	$C_{20}H_{34}O_8$
18.044	1,2-Cyclohexanedicarboxylic acid, dinonyl ester	$C_{26}H_{48}O_4$
18.707	1,2-Cyclohexanedicarboxylic acid, cyclohexylmethyl nonyl ester	$C_{24}H_{42}O_4$
18.78	1,2-Cyclohexanedicarboxylic acid, dinonyl ester	$C_{26}H_{48}O_4$

Table 3. The composition of the acetonitrile fraction from the leaves of the forest apple tree

Retention time, min	Name of substance	Gross formula
2.16	Cyclopropane	C_3H_6
3.078	Propane, 2-cyclopropyl-	C_6H_{12}
3.575	Furan, 3-methyl-	C_5H_6O
3.66	Acetic acid	$C_2H_4O_2$
4.256	Furfural	$C_5H_4O_2$
8.863	5-Hydroxymethylfurfural	$C_6H_6O_3$
10.062	Triacetin	$C_9H_{14}O_6$
10.512	1-Octanol, 2-nitro-	$C_8H_{17}NO_3$
11.107	Bicyclo[5.2.0]nonane, 4-methylene-2,8,8-trimethyl-2-vinyl-	$C_{15}H_{24}$
11.188	*cis*-β-Farnesene	$C_{15}H_{24}$
11.308	Dodecane, 1-chloro-	$C_{12}H_{25}CI$
11.33	Cyclododecane	$C_{12}H_{24}$
11.544	1,3,6,10-Dodecatetraene, 3,7,11-trimethyl-, (Z,E)-	$C_{15}H_{24}$
11.676	Alpha-Farnesene	$C_{15}H_{24}$
15.385	E-9-Methyl-8-tridecen-2-ol, acetate	$C_{16}H_{30}O_2$
15.462	Estra-1,3,5(10)-trien-17β-ol	$C_{18}H_{24}O$
16.708	9,12-Octadecadienoic acid (Z,Z)-	$C_{18}H_{32}O_2$
16.896	Linoleic acid ethyl ester	$C_{20}H_{36}O_2$
17.551	8,11-Octadecadienoic acid, methyl ester	$C_{19}H_{34}O_2$
17.577	Tributyl acetylcitrate	$C_{20}H_{34}O_8$
18.181	Butyl 9,12-octadecadienoate	$C_{22}H_{40}O_2$
18.613	Isopropyl linoleate	$C_{21}H_{38}O_2$

CONCLUSIONS

In the course of the study, it was found that the content of substances extracted by acetonitrile is 4.7–5.4 and 2.3–2.8% for leaves and fruits of the apple tree, respectively. The obtained extracts were used to identify substances whose presence is specific to a given plant material and can be used to identify it both in crushed form and in powdered form.

FUNDING

The study was supported by the "Russian Academic Excellence Project 5-100" and the "Project to Improve the Competitiveness of Leading Russian Universities Among the World's Leading Scientific and Educational Centers."

COMPLIANCE WITH ETHICAL STANDARDS

The study was performed without the use of animals and without involving people as subjects.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

REFERENCES

1. Safiullin, R.S., Minnekeeva, K.A., and Shakirova, D.Kh., *Kazan. Med. Zh.*, 2006, vol. 87, no. 6, p. 462.
2. Aksenova-Sorokhtei, Yu.N., Novikova, V.E., Pozhilova, E.V., Pozhilova, E.A., Baranovskaya, E.A., and Klimkina, E.I., *Vestn. Smolensk. Gos. Med. Akad.*, 2016, vol. 15, no. 2, p. 102.
3. Razzhivin, R.V., Reshetnyak, V.Yu., Kuz'menko, A.N., Nesterova, O.V., and Popkov, V.A., *Moscow Univ. Chem. Bull. (Engl. Transl.)*, 2009, vol. 64, no. 2, p. 104.
4. Kurkin, V.A., Morozova, T.V., Pravdivtseva, O.E., and Kurkina, A.V., *Pharm. Chem. J.*, 2018, vol. 52, no. 10, p. 850.
5. Logvinova, E.E., *Extended Abstract of Cand. Sci. (Pharm.) Dissertation*, Moscow: Sechenov Univ., 2017.
6. Pisarev, D.I., Novikov, O.O., Skoropudov, V.I., Khalikova, M.A., Zhilyakova, E.T., and Ogneva, O.V., *Nauchn. Vedomosti, Ser. Med. Farm.*, 2010, vol. 22, no. 12/2, p. 123.
7. Beketov, E.V., Abramov, A.A., Nesterova, O.V., and Kondrashev, S.V., *Vestn. Mosk. Gos. Univ., Ser. 2: Khim.*, 2005, vol. 46, no. 4, p. 259.

Translated by M. Shulskaya