

ISSN 2518-1491 (Online),
ISSN 2224-5286 (Print)

ҚАЗАҚСТАН РЕСПУБЛИКАСЫ
ҰЛТТЫҚ ҒЫЛЫМ АКАДЕМИЯСЫНЫҢ

Х А Б А Р Л А Р Ы

ИЗВЕСТИЯ

НАЦИОНАЛЬНОЙ АКАДЕМИИ НАУК
РЕСПУБЛИКИ КАЗАХСТАН

NEWS

OF THE ACADEMY OF SCIENCES
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**ХИМИЯ ЖӘНЕ ТЕХНОЛОГИЯ
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**СЕРИЯ
ХИМИИ И ТЕХНОЛОГИИ**



**SERIES
CHEMISTRY AND TECHNOLOGY**

6 (426)

**ҚАРАША – ЖЕЛТОҚСАН 2017 Ж.
НОЯБРЬ – ДЕКАБРЬ 2017 г.
NOVEMBER – DECEMBER 2017**

**1947 ЖЫЛДЫҢ ҚАҢТАР АЙЫНАН ШЫҒА БАСТАҒАН
ИЗДАЕТСЯ С ЯНВАРЯ 1947 ГОДА
PUBLISHED SINCE JANUARY 1947**

**ЖЫЛЫНА 6 РЕТ ШЫҒАДЫ
ВЫХОДИТ 6 РАЗ В ГОД
PUBLISHED 6 TIMES A YEAR**

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ISSN 2518-1491 (Online),

ISSN 2224-5286 (Print)

Меншіктенуші: «Қазақстан Республикасының Ұлттық ғылым академиясы» Республикалық қоғамдық бірлестігі (Алматы қ.)

Қазақстан республикасының Мәдениет пен ақпарат министрлігінің Ақпарат және мұрағат комитетінде 30.04.2010 ж. берілген №1089-Ж мерзімдік басылым тіркеуіне қойылу туралы куәлік

Мерзімділігі: жылына 6 рет.

Тиражы: 300 дана.

Редакцияның мекенжайы: 050010, Алматы қ., Шевченко көш., 28, 219 бөл., 220, тел.: 272-13-19, 272-13-18,
www.nauka-nanrk.kz/chemistry-technology.kz

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Типографияның мекенжайы: «Аруна» ЖК, Алматы қ., Муратбаева көш., 75.

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«Известия НАН РК. Серия химии и технологии».

ISSN 2518-1491 (Online),

ISSN 2224-5286 (Print)

Собственник: Республиканское общественное объединение «Национальная академия наук Республики Казахстан» (г. Алматы)

Свидетельство о постановке на учет периодического печатного издания в Комитете информации и архивов Министерства культуры и информации Республики Казахстан №10893-Ж, выданное 30.04.2010 г.

Периодичность: 6 раз в год

Тираж: 300 экземпляров

Адрес редакции: 050010, г. Алматы, ул. Шевченко, 28, ком. 219, 220, тел. 272-13-19, 272-13-18,
<http://nauka-nanrk.kz/chemistry-technology.kz>

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News of the National Academy of Sciences of the Republic of Kazakhstan. Series of chemistry and technology.
ISSN 2518-1491 (Online),
ISSN 2224-5286 (Print)

Owner: RPA "National Academy of Sciences of the Republic of Kazakhstan" (Almaty)

The certificate of registration of a periodic printed publication in the Committee of Information and Archives of the Ministry of Culture and Information of the Republic of Kazakhstan N 10893-Ж, issued 30.04.2010

Periodicity: 6 times a year

Circulation: 300 copies

Editorial address: 28, Shevchenko str., of. 219, 220, Almaty, 050010, tel. 272-13-19, 272-13-18,
<http://nauka-nanrk.kz/chemistry-technology.kz>

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Editorial address: Institute of Organic Catalysis and Electrochemistry named after D. V. Sokolsky
142, Kunayev str., of. 310, Almaty, 050100, tel. 291-62-80, fax 291-57-22,
e-mail: orgcat@nursat.kz

Address of printing house: ST "Aruna", 75, Muratbayev str, Almaty

NEWS

OF THE NATIONAL ACADEMY OF SCIENCES OF THE REPUBLIC OF KAZAKHSTAN

SERIES CHEMISTRY AND TECHNOLOGY

ISSN 2224-5286

Volume 6, Number 426 (2017), 24 – 30

UDC 577.112.38

UDC 543.635.35

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INVESTIGATION OF CHEMICAL CONSTITUENTS OF *BERGENIA CRASSIFOLIA*

Abstract. The roots of *Bergenia crassifolia* collected in Kazakhstan, were investigated for chemical constituent. The quantitative and qualitative analysis of the medicinal plant have been made. Biological active constituents such as organic acid (1.49 %), flavonoids (0.14%), and polysaccharides (1.74 %) together with moisture content (8.6%), total ash (8.4%), and extractives content (34.8%) of plant *B. crassifolia* were determined. In The Institute of Combustion Problems using the method of multi-element atomic emission spectral analysis in the ash of the plant was identified 7 macro-micro elements, in which the main contents were calcium (2054.10 mg/ml) and potassium (1050 mg/ml). In addition, twenty amino and eight fatty acids were analyzed from *B. crassifolia* extract. The results showed that the major contents of amino acids were glutamate (2705 mg/100g), aspartate (1216 mg/100g) and alanine (958 mg/100g), as well as fatty acids were linoleic (56.8%) and oleic acids (28.4%), respectively.

Key words: *Bergenia crassifolia*, bioactive constituents, macro-micro elements, amino-, fatty acids, GC-MS.

Introduction

Bergenia is flowering plant of the family Saxifragaceae which named in commonly as elephant-eared saxifrage, or elephant's ears. This genus contain of 10 species, which is widespread in area of Siberia regions (including Altai) and central Asia, from vastness's of Afghanistan to north-west in China and the Himalayan mountains [1]. In territory of Kazakhstan can be found two species *Bergenia crassifolia* and *Bergenia ugamica*, where second is listed in the Red Book of Republic of Kazakhstan [2]. *Bergenia crassifolia* (L.) is commonly known as badan, Siberian tea, Mongolian tea, leather bergenia. In Altai, *B. crassifolia* is popular as a subtea and called as "Chigir Tea". Brew the overwintered leaves that have undergone natural fermentation, taking dark colored drink with taste remotely like black tea, and has a number of beneficial properties and a tonic effect [3]. *B. crassifolia* has been used for treatments of bronchitis, gastroenteritis, diarrhea, hemostasia, and metrorrhagia in Kazakh Medicine [4]. The phytochemical constituents including bergenin, tannins, flavonoids, phenols, polysaccharide, amino acids and coumarins with some pharmacological actions such as antioxidant, antimicrobial, antiviral, anti-inflammatory, diuretic, immunostimulating, and lipase inhibiting activities have also been reported [5-10].

Proteins are large, complex molecules that are critical for the normal functioning of the human body. They are essential for the structure, function, and regulation of the body's tissues and organs. Proteins are made up of hundreds of smaller units called amino acids that are attached to one another by peptide bonds, forming a long chain. First point of investigation was on amino acids, which is organic (carboxylic) acids, the molecules of them contain one or more amino groups (NH₂-groups), the basic structural elements of protein molecules. In recent years, individual amino acids, their salts and mixtures have been increasingly used as highly effective, low-toxic drugs for children and adults. Drugs containing amino acids are used for a variety of diseases. So, glutamic acid is used to treat diseases of the central nervous system, methionine and histidine - the treatment and prevention of liver diseases, cysteine - eye diseases [11].

It is known that fatty acids - components of lipids exist in plants, animals, and microorganisms. Lipids are necessary for our body, because without them, metabolism cannot be carried out normally, and toxins and toxins accumulate in cells and tissues, as purification processes are slowed down. Many of fatty acids cannot be synthesized in human organism. Those fatty acids are required, however, for cellular processes and the production of other necessary omega-3 and omega-6 fatty acids. In addition fatty acids have a wide range of commercial applications, for example, they are used not only in the production of numerous food products but also in soaps, detergents, and cosmetics. Soaps are the sodium and potassium salts of fatty acids [12].

In the present study, the quantitative and qualitative analysis of bioactive constituents such as moisture, total ash, and extractives contents of *B. crassifolia* have been carried out, as well as amino-fatty acid contents were determined. In all this study, the quantitative and qualitative analysis of bioactive constituents and fatty acid contents of *B. crassifolia* which growing in Altay region of Kazakhstan were reported for the first time.

Materials and Methods

The root of plant material *Bergenia Crassifolia* was collected at Altay region Kazakhstan in July, 2015. The botanical identification was made by pharmacist Bahargul Konirhan, Institute of Medicine Inspection Department of Altay City, Xinjiang, China. The air dried roots of *B. crassifolia* were cut into small pieces and stored at room temperature.

The quantitative and qualitative analysis of biologically active constituents of the plant were made according to methods reported in the State Pharmacopeia XI edition techniques.

In the "Center of Physico-Chemical methods of research and analysis", Republican State Enterprise Kazakh National Al-Farabi University, MON RK using the method of multi-element atomic emission spectral analysis in the ash of *B. crassifolia* was analyzed elemental constituents. To determine the mineral composition of ashes was used Shimadzu 6200 series spectrometer.

To determine the amino acid composition was made anew [13] of the raw material used GC/MS device. GC-MS analysis: the aerial part of *B. crassifolia* were analyzed by Gas Chromatograph coupled to Mass Spectrometer using polar mixture of 0.31% carbowax 20 m, 0.28% silar 5CP and 0.06% lexan in chromosorb WA-W-120-140 mesh., column (400 x 3mm). The column temperature was programmed from 110°C (held for 20min), at 6°C/min from 110°C to 180°C, at 32°C/min from 185°C to 290°C. when it reaches to 250°C, it should stay constant till finishing of exit of all amino acids.

Results and discussion

The quantitative and qualitative analysis of biologically active constituents together with moisture content, total ash, extractives contents were determined from roots of *B. crassifolia*. The results shown in Table-1.

The ash of plant raw materials is the balance of inorganic substances obtained after burning the raw material and then calcining the residue to a constant mass. The ash of plants consists of a mixture of various inorganic substances characteristic of the plant, and mineral impurities that can get into the raw material during collection and drying. The moisture of plant materials is the loss in the mass due to hygroscopic moisture and volatile substances detecting when plant material is dried till constant weight. The moisture content in medicinal plant raw materials serves as one of the numerical indicators characterizing its quality. Medicinal plant raw materials should not contain moisture above the permissible standards, because with high humidity, during storage conditions are created that contribute to a decrease in its quality. For most types of medicinal plant raw materials, the permissible moisture limit is usually 12-15%.

Table 1 - Quantitative and qualitative analysis of *Bergenia Crassifolia*

Content, %					
Moisture content	Ash	Extractives	Organic acids	Flavonoids	Polysac-charides
8.6	8.4	34.8	1.49	0.14	1.74

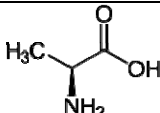
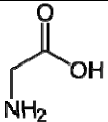
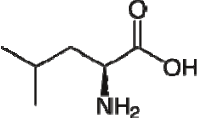
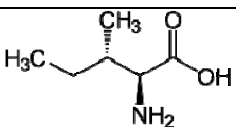
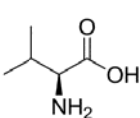
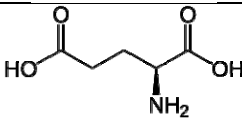
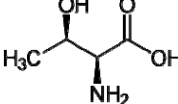
In The Institute of Combustion Problems using the method of multi-element atomic emission spectral analysis in the ash of *B. crassifolia* were determined seven macro- and microelements, showed in Table-2 and major of them are Calcium (2054.10 mg/ml) and Potassium (1050.10mg/ml). One of the main factors of nutrition, affecting health, working capacity and active longevity, are micronutrients - macro- and microelements. The body does not produce microcircuits and should receive them in ready form, for example, with food. The ability to store these substances in the body is absent. Calcium is participating on formation of bone tissue, the formation of teeth, the process of blood coagulation, neuromuscular conduction. Potassium is the most important component of intracellular fluid, controlling acid-base balance, muscular activity, and synthesis of proteins and glycogen [14].

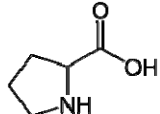
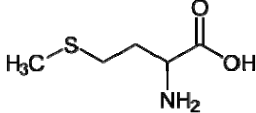
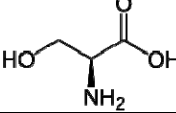
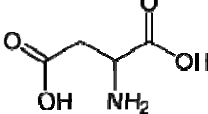
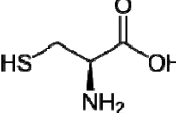
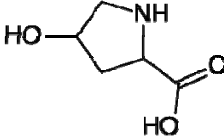
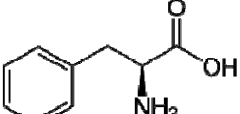
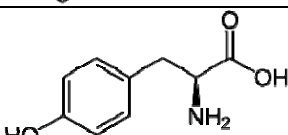
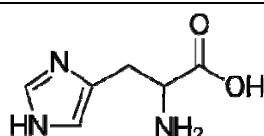
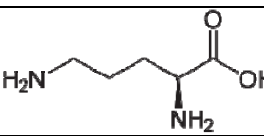
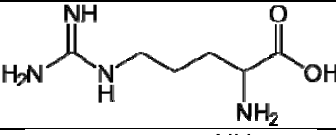
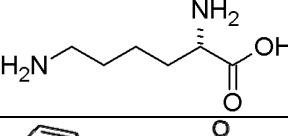
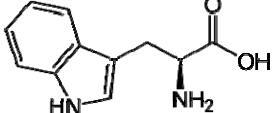
Table 2 - Composition of macro-micro elements in ash of *Bergenia crassifolia*

Element	K	Mg	Ca	Mn	Fe	Zn	Cu
µg/ml	794.5750	107.73	440.63	1.3556	4.5005	1.6911	0.6098

Determination of fatty acid composition of raw material, and dried plant *B. crassifolia* extracted with a chloroform-methanol mixture (2: 1) for 5 minutes, the extract is filtered through a paper filter and concentrated to dryness. Then, to taked extract add 10 ml of methanol and 2-3 drops of acetyl chloride and further methylation at 60-70° C in a special system for 30 minutes. The methanol is removed by rotary evaporation and the samples are extracted with 5 ml of hexane and analyzed using a gas chromatograph "CARLO-ERBA-420" allocated the Kazakh Academy of Nutrition for 1 hour. As a result, chromatograms of methyl esters of fatty acids were obtained. By comparison with reliable samples by the time of exit from the column, eight fatty acids were identified.

Table 3 - Amino acids contents of *B. crassifolia*

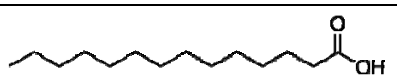
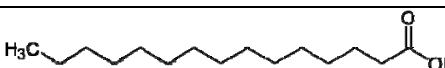
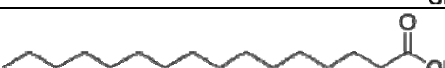
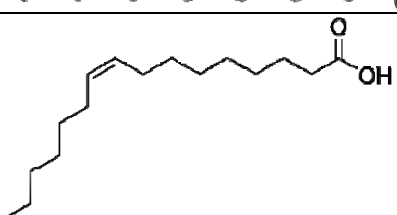
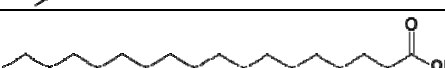
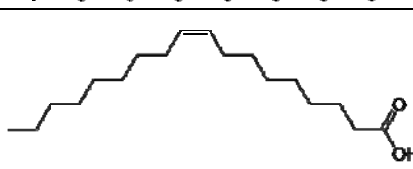
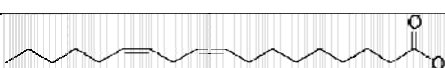

№	Amino acids	Molecular formula	Structure	MW	Amount in plant, mg/100g
1	Alanine	C ₃ H ₇ NO ₂		89	958
2	Glycine	C ₂ H ₅ NO ₂		75	405
3	Leucine	C ₆ H ₁₃ NO ₂		131	462
4	Isoleucine	C ₆ H ₁₃ NO ₂		131	344
5	Valine	C ₅ H ₁₁ NO ₂		117	220
6	Glutamate	C ₅ H ₉ NO ₄		147	2705
7	Threonine	C ₄ H ₉ NO ₃		119	232

8	Proline	C ₅ H ₉ NO ₂		115	806
9	Methionine	C ₅ H ₁₁ NO ₂ S		149	125
10	Serine	C ₃ H ₇ NO ₃		105	502
11	Aspartate	C ₄ H ₇ NO ₄		133	1216
12	Cysteine	C ₃ H ₇ NO ₂ S		121	46
13	Oxyproline	C ₅ H ₉ NO ₃		131	3
14	Phenylalanine	C ₉ H ₁₁ NO ₂		165	382
15	Tyrosine	C ₉ H ₁₁ NO ₃		181	394
16	Histidine	C ₆ H ₉ N ₃ O ₂		155	248
17	Ornithine	C ₅ H ₁₂ N ₂ O ₂		132	3
18	Arginine	C ₆ H ₁₄ N ₄ O ₂		174	365
19	Lysine	C ₆ H ₁₄ N ₂ O ₂		146	260
20	Tryptophan	C ₁₁ H ₁₂ N ₂ O ₂		204	92

Quantitative composition of fatty acids in *B. crassifolia* mostly contained in linoleic acid (56.8%) and Oleic acid (28.4%). Linoleic acid has received much attention in recent years because of its interesting

biological benefits. The main health effects described for linoleic acid include reduction of carcinogenesis, atherosclerosis, inflammation, obesity, diabetes, as well as growth promoting and bone formation-promoting properties [15]. Oleic acid can inhibit the progression of diseases affecting the brain and adrenal glands, as well as improve memory and reduce blood pressure, but there is evidence that the substance can provoke cancer, in particular breast cancer [16].

Table 4 - Fatty acids contents of *Bergenia crassifolia*

№	Fatty acids	Molecular formula	Structure	MW	Amount in plant, %
1	Meristic acid C _{14:0}	C ₁₄ H ₂₈ O ₂		228	1.2
2	Pentadecanoic acid C _{15:0}	C ₁₅ H ₃₀ O ₂		242	0.4
3	Palmitic acid C _{16:0}	C ₁₆ H ₃₂ O ₂		256	7.2
4	Palmitoleic acid C _{16:1}	C ₁₆ H ₃₀ O ₂		254	1.5
5	Stearin acid C _{18:0}	C ₁₈ H ₃₆ O ₂		284	3.4
6	Oleic acid C _{18:1}	C ₁₈ H ₃₄ O ₂		282	28.4
7	Linoleic acid C _{18:2}	C ₁₈ H ₃₂ O ₂		280	56.8
8	Linolenic acid C _{18:3}	C ₁₈ H ₃₀ O ₂		278	1.1

In the composition of amino acids mainly were glutamate (2705 mg/100g), aspartate (1216 mg/100g) and alanine (958 mg/100g). Glutamate is replaceable amino acid, which plays the role of a neurotransmitter with high metabolic activity in the brain, stimulates redox processes in the brain, the exchange of proteins. Normalizes the metabolism, changing the functional state of the nervous and endocrine systems [17]. Aspartic acid increases immunity, metabolism, deactivates ammonia, participates in the formation of ribonucleic acids, promotes the removal of chemicals, including drugs, restores working capacity. Studies conducted by scientists have proved the effectiveness of taking asparaginic acid preparations for increasing testosterone levels. Aspartic acid is taken as an additive by bodybuilding athletes to improve strength, increase libido and testosterone in the blood [18]. Alanine plays a significant role in metabolic processes, as well as to regulate the level of sugar in the bloodstream. This amino acid protects against the development of cancer of the pancreas and prostate gland, it is an important part of sports nutrition, increases physical endurance and allows to build muscle mass [19].

Conclusion

Quantitative and qualitative analysis of bioactive constituents and the moisture, total ash, and extractives contents of roots *B. crassifolia* were determined. Besides, macro-micro elements in the ash of the medicinal plant were investigated, and total seven macro-micro elements were identified by the method of multi-element atomic emission spectral analysis. Meanwhile, twenty amino and eight fatty acids were

determined from *B. crassifolia*. The results showed that the major contents of amino acids were glutamate (2705 mg/100g), aspartate (1216 mg/100g) and alanine (958 mg/100g, and fatty acids were linoleic (56.8%) and oleic acid acids (28.4%), respectively.

The main health effects described for *B. crassifolia* constituents include reduction of carcinogenesis, atherosclerosis, inflammation, obesity, diabetes, as well as growth promoting and bone formation-promoting properties, allows to build muscle mass, normalizing the metabolism, changing the functional state of the nervous and endocrine systems regulate the level of sugar in the bloodstream. The research work on investigation of chemical constituents of the medicinal plant to be continuing.

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BERGENIA CRASSIFOLIA ХИМИЯЛЫҚ ҚҰРАМЫН ЗЕРТТЕУ

Аннотация. Қазақстанда жиналған *Bergenia crassifolia* тамырының химиялық құрамы зерттелді. Дәрілік өсімдіктің сандық және сапалық талдауы жүргізілді. Өсімдіктің ылғалдылығы (8.6%), жалпы күлділігі (8.4%) және экстрактивтілігі (34.8%), сонымен бірге органикалық қышқыл (1.49%), флавоноидтар (0.14%) және полисахаридтер (1.74%) сияқты биологиялық активті компоненттер құрамы анықталды. Жану проблемалары институтында, атомдық эмиссия спектральды талдау әдісін қолдана отырып, өсімдіктің күліндегі 7 микро және макро элементтері анықталды және оның негізгі құрамы кальций (2054.10 мг / мл) және калий (1050 мг / л) элементтерінен тұратыны анықталды. Бұдан басқа, *B. crassifolia*-ден жиырма амин және сегіз майлы қышқыл анықталды. Алынған нәтижелер бойынша амин қышқылдардың негізгі құрамы глутамат (2705 мг / 100 г), аспарат (1216 мг / 100 г) және аланин (958 мг / 100 г) және май құрамында линол (56.8%) және олеин қышқылдары (28.4%).

Тірек сөздер: *Bergenia crassifolia*, биоактивті құрамдастар, макро-микроэлементтер, амина-майлықшы-кылдар, GC-MS.

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ИССЛЕДОВАНИЕ ХИМИЧЕСКОГО СОСТАВА BERGENIACRASSIFOLIA

Аннотация. Был исследован химический состав корней *Bergeniocrassifolia*, собранного в Казахстане. Сделан количественный и качественный анализ лекарственного растения. Определены биологически активные компоненты, такие как органические кислоты (1.49%), флавоноиды (0.14%) и полисахариды (1.74%), а также содержание влаги (8.6%), общая зольность (8.4%) и экстрагенты (34.8%) растения *B. crassifolia*. В Институте проблем сжигания с использованием метода многоэлементного атомно-эмиссионного спектрального анализа в золе растения было найдено 7 макро-микроэлементов, основными веществами из которых были кальций (2054.10 мг / мл) и калий (1050 мг / мл). Кроме того, из корней *B. crassifolia* были идентифицированы двадцать аминокислот и восемь жирных кислот. Результаты показали, что основным содержанием аминокислот являются глутамат (2705 мг / 100 г), аспарат (1216 мг / 100 г) и аланин (958 мг / 100 г), а среди жирных кислот - линолевые (56.8%) и олеиновые кислоты (28.4%) соответственно.

Ключевые слова: *Bergeniocrassifolia*, биоактивные компоненты, макро-микроэлементы, амина-, жирные кислоты, GC-MS.

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ISSN 2518-1491 (Online), ISSN 2224-5286 (Print)

Редакторы: *М. С. Ахметова, Т. А. Апендиев*
Верстка на компьютере *А.М. Кульгинбаевой*

Подписано в печать 03.12.2017.

Формат 60x88¹/₈. Бумага офсетная. Печать – ризограф.

7,8 п.л. Тираж 300. Заказ 6.