Chemical Composition of the Essential Oil of the Boreal Relict of *Pyrola rotundifolia* L. from Northern Kazakhstan

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Abstract: In Kazakhstan *Pyrola rotundifolia* L. is the plant-relict in the flora of insular pine forests of the region of low hillocks and declivities in Kazakhstan - a group of insular pine forests of Kokshetau, Bayanaul and Karkaralinsk. In this study, the essential oils from dried aerial parts of *P. rotundifolia*, collected in natural habitats of the State National Natural Park “Burabay” (Akmola oblast, Northern Kazakhstan), were extracted by hydrodistillation and analyzed by gas chromatography - mass spectrometry. The yield of the essential oil amounted to 0.057% in relation to the mass of the air-dry raw material. The major components in dried plant oil were 2,6-dimethyl-1,4-naphthoquinone (12.99–93.49%) and dibutyl phthalate (4.42–40.48%), depending on the growth conditions.

Key words: *Pyrola rotundifolia* L., *Pyrolaceae*, essential oil composition, 2,6-dimethyl-1,4-naphthoquinone, dibutyl phthalate.

1 INTRODUCTION

*Pyrola rotundifolia* L. – it is a perennial herbaceous plant of the family Pyrolaceae Dum.

*P. rotundifolia* is a boreal species typical of coniferous taiga and sphagnum bogs. It is widely distributed in the Caucasus, Western and Eastern Siberia, Arctic Siberia, the Far East, Russian Central Asia (mountains), Scandinavia, Central and Atlantic Europe, the Mediterranean, Asia Minor, Mongolia, the Himalayas, Japan, China, North America.

In Kazakhstan *P. rotundifolia* is the plant-relict in the flora of insular pine forests of the region of low hillocks and declivities in Kazakhstan - a group of insular pine forests of Kokshetau, Bayanaul and Karkaralinsk. It penetrated into the region of low hillocks and declivities in Kazakhstan, mainly, in Pleistocene when the climate was cooler and more humid than now. The pine forests of Turgay Hollow (such as Aman-Karagay) in the west, the valley of the Ishim in the north, the pine forests of Priirtyshie in the east could serve as the connecting links between the upland pine forests of Kazakhstan and the forests of South Ural, Western Siberia and Altai.

*P. rotundifolia* contains flavonoids (quercetin, kaempferol), phenols and their derivatives (hydroquinone, ursine, homoarbutin, methylarbutin, isohomoarbutin), quinones (renifolin, chimaphilin), essential oil, tannin, mucilage, resin, saponins, hyperoside, quinic acid, a bitter substance - urson, amyrin, ericolin, trioxynbenzoic acid, natural gums, andromedoxin, quinic acid, vitamin C, carbohydrates, microelements (magnesium, zinc, barium and others)\(^1\)\(^,\)\(^2\).

Anisoles (methoxybenzenes) and phenylpropanoids are the main components of the flowery odor of *P. rotundifolia* L.\(^3\)\(^,\)\(^4\). *P. rotundifolia* derives a great deal of carbon out of its fungic mycorrhizal bonds. It was found that *P. rotundifolia* has a high carbon (C) heterotrophy level. *P. rotundifolia* acquires 67.5% of its C from fungi\(^5\). The scientists have also identified fungi in *P. rotundifolia*, mainly these are ectomycorrhizal basidiomycetes, including *Tomentella, Cortinarius, Russula, Hebeloma*, as well as some ectomycorrhizal and/or endophytic ascomycetes\(^6\). An aqueous ethanol extract of *P. rotundifolia* induced AMP-activated protein kinase (AMPK) phosphorylation in C2C12 myotubes\(^6\). This experiment studied the methods, conditions and concentrations using *P. rotundifolia* for preserving

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1065
the pickle. The results showed that the use of *P. rotundifolia* can extend the preserving time of the low-salt pickle over two days in ordinary temperature. The experiments of the bacteriostatic circle showed the solution of 100 mg/l of *P. rotundifolia* by decocting had alike or better results compared with the same concentration of potassium sorbate and propionic calcium.7

Thanks to the content of various bioactive substances *P. rotundifolia* is widely used in homeopathy and medicine. In traditional Chinese medicine it is used under the treatment for neuralgia, gastric hemorrhage, pulmonary hemorrhage and arthritic diseases. The water extract of the plant inhibits the growth of many kinds of human pathogenic bacilli in vitro. The extract of *P. rotundifolia* efficiently regenerates the normal microflora of the oral cavity and selectively inhibits the growth of putrefactive microorganisms.8 In homeopathy and folk medicine of Russia *P. rotundifolia* is used under the diseases of the urogenital system. In Tibetan medicine it is used as the antipyretic, under the treatment for osseous tuberculosis, diseases of liver. In Mongolian medicine *P. rotundifolia* is used as the diuretic and hemostatic remedies. *P. rotundifolia* is a substitute for tea. In spring it is eaten around by wild animals.

The properties and the chemical composition of *P. rotundifolia* are studied enough, but in the official scientific literature there is no information about the chemical composition of the essential oil. The given work provides information about the results of the study of the chemical composition of the essential oil of *P. rotundifolia* growing in Northern Kazakhstan.

2 EXPERIMENTAL

2.1 Plant material

Collection of the material was carried out in places of natural growth of *Pyrola rotundifolia* L. in Northern Kazakhstan in Akmola oblast in the State National Natural Park “Burabay”. Specimens for the study were collected in the first ten-day period of July of 2014 in the stage of full blooming (Fig. 1). Identification and documentation (certificates of the specimens) of the plant species were made by Dr. Tamara Stikhareva with the use of the key to the plants “Kazakhstan Flora”. The herbarium of the identified plants was placed at the Department of Breeding of Kazakh Research Institute of Forestry and Agroforestry in Shchuchinsk. Collection and preparation of the raw material (drying up to the air-dry condition) were carried out with the use of generally accepted methods. Characteristic of the places of collection and the percent of moisture content in the plant material are given in Table 1 (hereinafter the studied specimens are named according to their characteristics and conditions of the habitat).

The climate of Northern Kazakhstan is mainly sharply continental. Winter in the north of the country is cold and long. Summer is moderately warm. An average temperature of the coldest month (January) varies from –25 – 35°C and the temperature of the hottest month (July) varies from +25 + 35°C. An average amount of precipitation is 300-600 mm.

2.2 Essential oil isolation

In order to get the essential oil, the leaves were steeped in water for 24 hours. The essential oil of all the air-dried samples (100 g) was isolated by hydrodistillation for 6 hours with the use of a Clevenger-type apparatus, which consists of evaporating flask with a capacity of 1000 mL, whole brazed cooling system, and laboratory funnel. Nominal capacity measuring tube is 1 mL, the price of division of the scale is 0.02 mL, and permissible error is ±0.02 mL. The isolated essential oil was collected by ethyl acetate and then it was isolated from the solvent and weighed.

2.3 Gas chromatography - Mass spectrometry

The qualitative and quantitative compositions of the specimens of essential oils were analyzed by the method of chromatography - mass spectrometry on an Agilent Technologies 7890 A GC System gas chromatograph with an Agilent Technologies 5975 C mass selective detector. An HP-5MS capillary column (5% Phenyl Methyl Silox, 30 m x 0.25 mm) at the flow rate of gas-carrier of helium 1 mL/min was used. The temperature of the vaporizer was 230°C. For 10 minutes the temperature of the column has been 40°C, with the programming of the temperature up to 240°C at...
the rate of the change of the temperature \(2^\circ\)C/minute, and then this column has been set into isometric mode of operation for 20 minutes. The mode of injection was without the division of the stream. The volume of the sample was 0.2 microliters. Conditions of the recording of mass spectra were 70 eV, the range of mass was m/z 10-350. The percentage of the components was calculated automatically starting from the areas of peaks of the total chromatogram of ions. The components were identified on mass spectra and on retention time with the use of library Wiley 275.l, National Institute of Standards and Technology V.2.0 GC/MS and literature\(^{10}\). The injections were carried out in three replications.

### Table 1 The places of collection of the plant material.

| Number of the specimen | Characteristic of the habitat | Growth conditions | Percent (%) of moisture content in the plant material |
|------------------------|------------------------------|-------------------|---------------------------------------------------|
| I                      | A pine-birch forest (6 pines, 4 birches), the age – 80-90 years, the stand density – 0.8. Mixed herbs and short-footed community with the predominance of *Brachypodium pinnatum* (L.) Beauv., as well as *Rubus saxatilis* L., *Filipendula ulmaria* (L.) Maxim. and others. The total projecting cover of the grass stand – 50-60%, the projecting cover of *Pyrola rotundifolia* L. – 3% | Fresh growth conditions: a fairly moist pine forest | 66.67 |
| II                     | A pine forest (10 pines), the age - 80-90 years, the stand density - 0.5. A small reed community with the predominance of *Calamagrostis epigeios* (L.) Roth., here also present *Rubus saxatilis* L., *Brachypodium pinnatum* (L.) Beauv. and others. The total projecting cover of the grass stand – 50-60%, the projecting cover of *Pyrola rotundifolia* L. – 7% | Growth conditions transforming from fresh to dry: a fairly moist pine forest transforming to a dry pine forest | 66.05 |
| III                    | A birch-pine forest (6 birches, 4 pines), the age - 60-70 years, the stand density - 0.8. Short-footed and mixed herbs community with the predominance of *Rubus saxatilis* L., *Brachypodium pinnatum* (L.) Beauv., *Fragaria vesca* L. and others. The total projecting cover of the grass stand –80-90%, the projecting cover of *Pyrola rotundifolia* L. – 7% | Fresh growth conditions, in immediate proximity to the forest brook: an aboriginal moist birch forest | 70.34 |
| IV                     | A birch forest (10 birches), the age - 50-60 years, the stand density - 0.7. A reed – meadowsweet community with the predominance of *Filipendula ulmaria* (L.) Maxim., *Phragmites communis* Trin., there also present *Rubus saxatilis* L., *Equisetum arvense* L. and others. The total projecting cover of the grass stand — 90-95%, the projecting cover of *Pyrola rotundifolia* L. – 5% | Growth conditions transforming from fresh to moist: an aboriginal moist birch forest transforming to an aboriginal wet birch forest | 68.35 |

### 3 RESULTS AND DISCUSSION

The yield of the essential oil amounted to 0.057% in relation to the mass of the air-dry raw material. The essential oil has a yellow color with red specks. At distillation of the solvent the essential oil crystallizes.

The conducted study of the chemical composition of the essential oil of *P. rotundifolia*, gathered in the State National Natural Park “Burabay” (Northern Kazakhstan, Akmola oblast) in 4 different growth conditions in the period of mass flowering on the 8\(^{th}\) and 10\(^{th}\) June, 2014, allowed us to single out its 2 main components. 2,6-Dimethyl-1,4-naphthoquinone (12.99-93.49%) and dibutyl phthalate (4.42-40.48%) were the major components of the oil (Fig. 2). In all there were identified 13 components (Table 2). Gas chromatograms of the essential oil of the aerial parts of *P. rotundifolia* from Samples I – IV are rep-
resented in Figs. 3-6.

2,6-Dimethyl-1,4-naphthoquinone exhibits anti-hemorrhagic action\(^{11}\). The content of the given naphthoquinone in the essential oil of *Pyrola rotundifolia* explains its using under the gastric and pulmonary bleedings.

However, the essential oil of *Pyrola rotundifolia* contains dibutyl phthalate (1,2-benzenedicarboxylic acid, dibutyl ester). Dibutyl phthalate is a phthalate ester extensively used in industry in polyvinyl chloride products as plasticizer, in various varnishes and lacquers, safety glass, nail polishes, paper coatings, dental materials, pharmaceutics and plastic food wrap. Dibutyl phthalate is widely used as a repellent. Dibutyl phthalate does harm to the endocrine and nervous systems of a human being, causes oncological diseases and toxic hepatitis\(^{12-15}\).

While getting into the soil and water, the products, con-

Table 2 The main composition of the specimens of the essential oil of *Pyrola rotundifolia* L. according to the data of chromato-mass-spectrometry.

| No. | Compounds (look through Table 1): | Content of components in specimens (%) | RI calc. |
|-----|----------------------------------|----------------------------------------|----------|
|     | The number of the specimens | I (RT, min %) | II (RT, min %) | III (RT, min %) | IV (RT, min %) |
| 1   | 1,3-dimethylbenzene | 9.749 2.64 | 9.767 5.47 | 9.740 3.79 | − − |
| 2   | p-cymene | − − − − | 14.498 3.05 | − − − − | 1023 |
| 3   | 1,8-cineole | − − − − | 14.744 2.86 | − − − − | 1046 |
| 4   | γ-terpinene | − − − − | 15.663 3.00 | − − − − | 1200 |
| 5   | dodecane | − − − − | 20.038 1.50 | − − − − | 1238 |
| 6   | 4-(1-methylethyl)-benzaldehyde | − − − − | 25.533 1.07 | 25.524 3.70 | − − − − | 1400 |
| 7   | tetradecane | − − − − | 30.382 3.57 | − − − − | 1600 |
| 8   | hexadecane | − − − − | 38.062 23.77 | − − − − | 1960 |
| 9   | 2,6-dimethyl-1,4-naphthoquinone | 31.437 23.77 | 31.486 23.77 | 31.475 23.77 | 93.49 6.51 |
| 10  | benzyl benzoate | − − − − | 34.125 1.29 | − − − − | 1742 |
| 11  | octadecane | − − − − | 34.749 1.72 | − − − − | 1800 |
| 12  | hexahydrofarnesyl acetone (6,10,14-trimethyl-2-pentadecanone) | 35.699 2.37 | 35.699 2.96 | − − − − | 1846 |
| 13  | dibutyl phthalate (1,2-benzenedicarboxylic acid, dibutyl ester) | 38.064 23.77 | 38.064 4.42 | 38.065 40.48 | 38.051 6.51 |
|     | Total identified | 95.58 94.41 83.40 100.00 |

RT: retention time
%: calculated from TIC data
RI calc: calculated retention index

V. Kirillov, T. Stikhareva and G. Atazhanova et al.

*J. Oleo Sci.*, 64, (10) 1065-1073 (2015)

Fig. 2 Structure of 2,6-dimethyl-1,4-naphthoquinone (*a*) and dibutyl phthalate (*b*).
Chemical Composition of the Essential Oil of the Boreal Relict of Pyrola rotundifolia L. from Northern Kazakhstan

J. Oleo Sci. 64, (10) 1065-1073 (2015)

Detecting phthalates, in particular dibutyl phthalate, in the essential oil reveals that these contaminations can be absorbed from water and soil into the plant roots and the aerial parts of the plants. Penetration of phthalates into the human and animal organisms occurs while using these plants as the food and medicinal plants\(^\text{13, 14}\). From Table 3 one can see that dibutyl phthalate can be accumulated in different parts of the plants and can be one of the main components of the essential oil.

Dibutyl phthalate was present in 8 samples of essential oils out of 35 lemon oils, 31 orange oils and 21 mandarin oils, produced in the crop years of 1994-1996 in Sicilia and Calabria\(^\text{25}\). Residues of dibutyl phthalate were found in samples of bergamot essential oils produced in Calabria in the crop years of 1999 and 2000, the mean content was 1.51 and 1.65 mg/l respectively\(^\text{26}\).

2,6-Dimethyl-1,4-naphthoquinone and dibutyl phthalate, the main components in our study, accumulated at \(P. \text{rotundifolia}\) not equally under the different growth conditions (Table 2). Figure 7 shows the percentage of 2,6-dimethyl-1,4-naphthoquinone and dibutyl phthalate in the essential oil of \(P. \text{rotundifolia}\) depending on the growth conditions.
conditions.

So, *P. rotundifolia* under number IV, is the richest in 2,6-dimethyl-1,4-naphthoquinone (93.49%). Its samples were gathered in the birch forest (the aboriginal moist birch forest transforming to the aboriginal wet birch forest) of the State National Natural Park "Burabay"; the growth conditions were transforming from fresh to moist. The sample under number II had a high content of 2,6-dimethyl-1,4-naphthoquinone (83.45%) too. The plants of *P. rotundifolia* of the given sample grew in the pine forest (the fairly moist pine forest transforming to the dry pine forest); the growth conditions were transforming from fresh to dry. The sample under number III had the minimum level of accumulation of 2,6-dimethyl-1,4-naphthoquinone (12.99%). In this case, the plants of *P. rotundifolia* grew in the birch-pine forest (the aboriginal moist birch forest); the growth conditions were fresh, in immediate proximity there flowed the brook. In the very sample of the essential oil III there was revealed the maximum content of dibutyl phthalate (40.48%) of all the samples. One can suppose that the maximum content of dibutyl phthalate in the given sample is explained by the fact that *P. rotundifolia* grew in immediate proximity to the brook, the water of which, apparently, contained dibutyl phthalate. It is necessary to pay attention to the place where Sample III of *P. rotundifolia* was gathered, there was no rubbish which could contain dibutyl.

**Fig. 5** Gas chromatogram of the essential oil of the aerial parts of *P. rotundifolia* from Sample III.

**Fig. 6** Gas chromatogram of the essential oil of the aerial parts of *P. rotundifolia* from Sample IV.
Chemical Composition of the Essential Oil of the Boreal Relict of Pyrola rotundifolia L. from Northern Kazakhstan

J. Oleo Sci. 64, (10) 1065-1073 (2015)

As one of the reasons of the presence of dibutyl phthalate in the essential oil, there could be the brook’s water which got into the soil and then from the soil into the root system and the aerial part of the plant. Besides dibutyl phthalate, in Sample III there were revealed such components as 1,3-dimethylbenzene, p-cymene, 1,8-cineole, γ-terpinene, dodecane, 4-1-methylethyl-ʣ-benzaldehyde, tetradecane, hexadecane, benzyl benzoate, octadecane, hexahydrofarnesyl acetone. The total content of the given components in Sample III formed 33.72 ′. The essential oil of Sample I contained a large amount of dibutyl phthalate (23.77 ′) too. Sample I also contained 2,6-dimethyl-1,4-naphthoquinone (66.80 ′), 1,3-dimethylbenzene (2.64 ′) and hexahydrofarnesyl acetone (2.37 ′). The samples of this plant grew in the pine-birch forest; the growth conditions were fresh. In this case one can suppose that the groundwater could serve as the source of contamination with dibutyl phthalate. We are inclined to think that the brook’s water and the groundwater can contain phthalate, in particular dibutyl phthalate, since the State National Natural Park is situated not far from the town of Shchuchinsk and the resort settlement “Burabay”.

There is another version of the presence of dibutyl phthalate in the essential oil of P. rotundifolia; to be exact it is due to the presence of mycorrhizal bonds. According to literary sources, phthalates, including dibutyl phthalate, can be synthesized by endophytic fungi and streptomycetes from the decay products of cellulose and lignin. P. rotundifolia is a micotrophic plant and, according to French and Estonian scientists, it does not have a strict preference for any fungal taxon. One can assume that mycorrhizal fungi produced dibutyl phthalate which penetrated into the aerial parts of P. rotundifolia through the root system and then appeared in the extracted essential oil.

It should be noted that the typical habitats of Pyrola rotundifolia L. in Kazakhstan there are: mossy bogs along the banks of the brooks flowing out of the springs; banks of the brooks, particularly in deep shady ravines; sphagnum bogs, raised bogs, swamp-subors; mossy pine forests, mossy and grass pine forests; black alder forests.

### Table 3

| Plant species                  | Part of plant | Content of dibutyl phthalate in essential oil, % | Reference |
|-------------------------------|---------------|-----------------------------------------------|-----------|
| Reseda lutea L.               | aerial parts  | 2.3                                           | 16)       |
| Rumex chalepensis Miller.     | aerial parts  | 6.5                                           | 17)       |
| Lelea indica (Burm.f.) Merr.  | flowers       | 7.18-7.48                                     | 18)       |
| Anchusa italicita Retz.       | aerial parts  | 9                                             | 19)       |
| Trigonella monantha C. A. Mey. Subsp. monantha | aerial parts  | 10.3                                          | 20)       |
| Lobelia pyramidalis Wall.     | aerial parts  | 10.66                                         | 21)       |
| Viola tianshanica Maxim.      | aerial parts  | 15.19                                         | 22)       |
| Stachys inflata Benth.         | aerial parts  | 0.47-34.66                                    | 23)       |
|                              | twig parts    | 34.22                                         | 24)       |
| Clerodendrum inerme (L.) Gaerth. | leaf parts  | 59.28                                         |           |
|                              | root parts    | 44.27                                         |           |

**Fig. 7** Percentage (%) of 2,6-dimethyl-1,4-naphthoquinone and dibutyl phthalate in the essential oil of P. rotundifolia depending on the growth conditions.
4 CONCLUSION

Chemical composition of the essential oil of the boreal relict of *Pyrola rotundifolia* L. from Northern Kazakhstan is studied for the first time. The major components of oil were 2,6-dimethyl-1,4-naphthoquinone (12.99-93.49%) and dibutyl phthalate (4.42-40.48%), depending on the growth conditions. The results suggested that *Pyrola rotundifolia* L. essential oil and its major constituent - 2,6-dimethyl-1,4-naphthoquinone has potential use in medicine. The results can be used in future investigations of *P. rotundifolia* L., to improve the new knowledge about *P. rotundifolia* L., and to provide a venue to develop and debate new ideas.

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