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RESEARCH ARTICLE

Fatty acid profile and biochemical properties of *Dracocephalum palmatum* Steph. ex Willd in extreme climate conditions

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Abstract

The composition of fatty acids in the general lipid balance of *Dracocephalum palmatum* Steph. Ex Willd that is found in extreme climatic conditions (Northern Pole of Cold) was researched by thin-layer and gas-liquid chromatography. This research aims to study the fatty acid profile of the perennial species *Dracocephalum palmatum* used as a medicinal herb in traditional medicine. We established that polyunsaturated fatty acids [FA], and more specifically linoleic and alpha-Linoleic acids, are prevalent in the lipidic constitution. The exceptional resilience of arctic and boreal plants is attributed to their flexible energy system that includes carbohydrates, proteins, and lipids. The latter are crucial in the energy balance of plants because they function as the main accumulator of spare energy and can create optimal conditions in cell membranes, unlike carbohydrates and proteins. Polyunsaturated fatty acids [PFA] present in the lipid layer allow the membranes to stay in the liquid state. Fat oxidation releases an amount of water that is considerably greater than that released by the combustion of carbohydrates and proteins. This research reveals that the fluidity of membranes in the arctic plant in question is optimal due to a high level of unsaturated lipids. The high amount of unsaturated FA in *Dracocephalum palmatum* lipids is attributed to the plant adapting to its poor growing conditions. We assume that late flowering plants with a higher than average level of PFA (linoleic and linolenic acids) higher than average play an important role in the conservation of reaction energy resources of animals in the northern environment.

Keywords

Fatty acids, Pole of Cold, lipids, saturated fatty acids, unsaturated fatty acids, gas-liquid chromatography, *Dracocephalum palmatum*, fatty acid methyl esters

Introduction

The Republic of Sakha (Yakutia, Russia) is located between 105°32′ – 162°55′ E and 55°29′ – 76°46′ N, occupies an area of 3,103 thousand square kilometers and has all its territory covered by permafrost. According to long-term average temperature data, in the north-eastern part of the Sakha Republic, the period when the temperature is between 10 and –67 °C is approximately 9 months a year (270 days a year) (Petrov 2016). About 40% of the Republic of Sakha (Yakutia) lies above the Arctic circle. *Dracocephalum palmatum* Steph., an arctic endemic species, is native to such peculiar conditions. It is a perennial multistemmed rhizome plant with ovoid pinnate leaves and magenta flowers on short stems gathered in verticillasters, endemic species to the arctic tundra of Chukotka, Anadyr, Eastern Siberia and the Russian Far East (Malyshev et al. 2006). *Dracocephalum palmatum* is found in rocks and sandy sediments and on slopes with stony and gravel-filled soils. The plant in question is used as a medical herb by the indigenous population.

Up until 2013, there was no scientific data on the chemical constituents and biological activity of *Dracocephalum palmatum*. As a result of a comprehensive chromatographic study of *Dracocephalum palmatum*, phenolic compounds and two triterpenes were isolated from the aerial part (Olennikov et al. 2013; 2017).

We isolated two new glycosides of eriodictyol and luteolin from the aerial part of *Dracocephalum palmatum*, identified using UV, NMR, CD and mass spectrometry data as (S)-eriodictyol-7-O-(6''-O-malonyl)-β-D-glucopyranoside (pyracanthoside-6''-O-malonate), (S)-eriodictyol-7-O-(4''-O-malonyl)-β-D-glucopyranoside (pyracanthoside-4''-O-malonate) and luteolin-7,4'-di-O-α-L-rhamnopyranosyl-(1→6)-β-D-glucopyranoside (luteolin-7,4'-di-O-rutinoside), which was given the name dracopalmaside (Olennikov and Chirikova, 2015).

However, research on the composition of the components of fatty acids in *Dracocephalum palmatum* has not yet been carried out yet. This study aims to examine the fatty acid profile of the perennial arctic species *Dracocephalum palmatum*, noted for its use in traditional medicine.

Materials and methods

The leaves of the *Dracocephalum palmatum* were used as research objects. Samples of wild-growing *D. palmatum* was collected in the vicinity of the village of Tomtor, Oymyakon, the Republic of Sakha, the Russian Federation (63°26′34″N, 143°10′24″E, July 2018). Then we froze the leaves in liquid nitrogen and transported them to a laboratory using the Dewar vessel. The samples were lyophilized using the VirTis lyophilizer (USA) and preserved at a temperature of 70 C ° until we performed the tests.

Total lipid extraction [TL] was performed using a modified extraction method (Imbs 2012). The amount of TL in the lyophilized samples was specified in accordance with the average mass of the extract. In addition to this procedure, we cleaned the samples from pigment impurities using thin layer chromatography [TLC]. We vaporized the hexane extract on the rotor and redissolved it in 100 mcl chloroform, then applied it to the TLC plate in a thin longitudinal stripe (silica gel served as an adsorbent material) with a 1.0–1.5 cm indent from the lower edge. Once the solvent had fully dried, the plate was dipped into TLC glass filled with benzol that served as the eluent. After the eluent reached the upper edge of the plate, it was removed and dried. We sprayed the edge of the plate with 10% sulfuric acid solution to determine the exact position of the fatty acid methyl esters [FAME] and heated it on a stove. The FAMEs were taken from an area of the plate that had not been sprayed or prewarmed. They were placed in a funnel with a pre-cleaned cotton filter to remove 10-20 ml of chloroform. The chloroform extract was placed in a bulb, vaporized on a rotor, and redissolved in 1 ml of hexane to be chromatographed later. We used the Clarus 590 gas chromatography and a flame ionization detector to analyze the resulting FAME (Perkin Elmer, USA) (Christie 1993). The degree of the unsaturation of lipids was estimated using the unsaturation rate (k) and double bond index [DBI]. We conducted the experiment three times.

Result

The fatty acid profile of the leaves of *Dracocephalum palmatum* is demonstrated in Table 1. An analysis of the general lipid fraction of the fatty acid composition of the *Dracocephalum palmatum* leaves shows that the leaves contain 13 individual components. Of all saturated fatty acids present in the samples, the palmitic acid content is the highest and amounts to 14.4% of the FA sum. Myristic acids (C14:0) and (C18:0) have the same relative abundance of 1.7% of the FA sum. The behenic acid (C22:0) content in the general lipid constitution of the samples in question amounts to 1.3% of the FA sum. Acids C15:0, C17:0-a, C17:0, and C20:0 are the least present in the saturated FA sum. It should be noted that all fatty acids formed aliphatic chains as part of lipids, with the only exception being the C17:0-a acid with a branched structure. The C17:0-a content amounts to 0.2% of the FA sum. The presence of this acid in the lipid composition of *Dracocephalum palmatum* can be attributed to the influence of a prokaryotic organism. Branched FAs are rare in embryophytes, but can be seen in prokaryotes such as bacteria and algae (Kirichenko et al. 2019).

Polyunsaturated alpha-linolenic C18:3(ω-3) acid was the most present unsaturated fatty acid (58.6% of the FA sum) and prevalent in the sample tissue. The alpha-linolenic acid content was 18.8 mg per 1 g of lyophilized dry mass. We suppose that this acid allows plants to adapt to low-temperature stress by increasing the fluidity of the membranes. This is the reason behind its growth both absolutely and relatively (Los 2014). The content of C18:2(ω-6) linoleic acid was significantly lower and was 14.2% of the FA sum. It is important to note that these polyunsaturated acids (C18:2 and C18:3) are essential as they are not synthesized in animals and humans. Among other unsaturated fatty acids present in the leaves of *Dracocephalum palmatum*, two C16:1 isomers were found. Furthermore, the content was 6.7 higher than the content and amounted to 2% of the FA sum. The oleic acid content was 4.1% of the FA sum and 1.3 mg per 1 g of dry mass. This acid preceded the biosynthesis of linoleic (C18:2ω-6) and alpha-linoleic (C18:3ω- 3) in plant cell membranes. The absolute fatty acid content in the leaves of *Dracocephalum palmatum* was 32 ± 3.0 mg per 1 g of dry mass.

Table 1. The composition and content of fatty acids in the leaves of *Dracocephalum palmatum* native to Northeast Yakutia (Pole of Cold)

Fatty acids	% of FA sum	FAME, mg/g of dry mass
C14:0	1.7 ± 0.2	0.5 ± 0.1
C15:0	0.3 ± 0.1	0.1 ± 0.0
C16:0	14.4 ± 1.8	4.8 ± 0.6
C16:1(ω-9)	0.3 ± 0.1	residue
C16:1(ω-5)	2 ± 0.3	0.7 ± 0.1
C17:0-a	0.2 ± 0.0	residue
C17:0	0.4 ± 0.1	0.1 ± 0.0
C18:0	1.7 ± 0.5	0.5 ± 0.2
C18:1(ω-9)	4.1 ± 0.5	1.3 ± 0.1
C18:2(ω-6)	14.2 ± 0.7	4.5 ± 0.3
C18:3(ω-3)	58.6 ± 4.2	18.8 ± 2.6
C20:0	0.8 ± 0.3	0.2 ± 0.0
C22:0	1.3 ± 0.3	0.4 ± 0.1
∑	100	32 ± 3.0
Saturated ∑	20.9 ± 3.2	6.7 ± 1.3
Unsaturated ∑	78.4 ± 2.9	25.3 ± 2.6
SDR	0.7 ± 0.1	-
ODR	0.9 ± 0.0	-
LDR	0.8± 0.1	-
DBI	2.1	-
k	3.8	-

The total saturated FA percentage was 20.9% of the FA sum. The total amount of unsaturated FA exceeded that of saturated FA 3.7 times, influencing the unsaturation rate (k - 3.8). The high rate of unsaturated FA in the lipid constitution of *Dracocephalum palmatum* is due to plants adapting to unfavorable growing conditions. More precisely, it is attributed to the reduction in luminance (photoperiod reduction) and the decrease in air temperature in the fall.

Discussion

In previous research conducted by D. N. Olennikov, 23 individual components were identified in a *Dracocephalum palmatum* tincture. These were mainly coumarins, flavonoids, phenylpropanoids, and triterpenoids, as was prevopisly suggested (Olennikov et al. 2013).

The effect of dried leaves of *Dracocephalum palmatum* on tumors was studied using human prostate cancer cells (PC–3) (Lee et al. 2020). In the study in question, we reported that *Dracocephalum palmatum* extract could be a potential resource for the development of promising chemotherapeutic drugs against prostate cancer. The studies mentioned in this document confirm the metabolic variety of the plant in question, as well as the pharmacological activity of its metabolites.

An analysis of the fatty acid profile of *Dracocephalum palmatum* revealed that palmitic acid (C16:0) was one of the saturated acids most prevalent. C16:0 FA is a dominant acid in the general lipid composition of many eukaryotes, since it participates in the metabolism of essential fatty acids (Luckey 2014).

The high lipid rate in herbaceous plants that grow in harsh environments suggests the structural and functional resilience of their photosynthetic apparatus [PSA]. The ability of the pigment system to change at low temperature is one of essential parts of the PSA adaptive reactions (Sofronova et al. 2019). The fatty acids C18:2(ω-6) and C18:3(ω-3) is essential in the process of converting light energy into photosynthetic pigments and lipid compounds. In addition, they have antioxidant and photoprotective properties that tend to increase under extreme conditions (Jahns et al. 2012). We established that polyunsaturated fatty acids, and more specifically linoleic (14.2% of the FA sum) and alpha-linoleic acids (58.6% of FA sum), are prevalent in the lipid content. In another species, *Dracocephalum moldavica* L., the alpha-linoleic acid (C18:3ω-3) in aqueous seed extracts reached 55.1% of the FA sum (Song et al. 2021).

In the summer period, an increased content of saturated FAs is observed in the composition of lipids in the cell membranes of herbaceous plants, which is probably associated with a decrease in the activity of desaturases. Our results show that *Dracocephalum palmatum* is exposed to stress factors and adapts to the harsh natural and climatic conditions of the Pole of Cold even in summer in the high- mountain phytocenoses of Oymyakonya.

Conclusion

We showed that the arctic species *Dracocephalum palmatum* contains a significant amount of unsaturated fatty acids in its lipid composition. The most important are the polyunsaturated FA C18:2 and C18:3 that are responsible for energy and protection. Plant lipids are known to be the most important component of the animal diet, as they cover an essential part of the total energy demand and serve as a source of essential polyunsaturated fatty acids. Although the total nutritional value of plants decreases during the fall, many of them show an increase in proteins, soluble sugars, lipids, antioxidants, and carotenoids. This is especially prominent in autumn vegetable species and is accompanied by an increase in the total number of lipids and polyunsaturated FAs in plant tissues. The unsaturation rate of fatty acids in membrane lipids can change when exposed to cold. This adaptive mechanism is intended to keep membranes functional by changing the composition of fatty acid composition in lipids at different temperatures. For example, exposure to an increase in cold leads to the unsaturated fatty acid content in the membranes of frost-tolerant plants. This keeps the lipids from hexagonal packing and thus allows them to maintain the liquid crystal state. The increase in lipids in plant tissues during late vegetation and their natural cryopreservation in autumn and winter are key to northern animals successfully surviving winters. Stockpiling large amounts of FA-rich fat, as seen in an aboriginal Yakut horse, is one of the most important mechanisms of adaptation to severe weather conditions at the Pole of Cold during winter.

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