The study of biologically active substances of blue honeysuckle (Lonicera caerulea L.) leaves

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Abstract. The article presents the results of studying the chemical composition of the leaves of blue honeysuckle, naturally growing in the southern taiga of the Krasnoyarsk Territory. It was found that along with fiber and lignin, the studied biomass contains a significant amount (about 70 wt.%) of extractive substances. Using fractional extraction with solvents of increasing polarity, it was possible to extract from the studied raw materials 9.5 wt.% of substances soluble in non-polar solvents and about 60 wt.% of compounds soluble in isopropanol and water. A spectrophotometric study showed the presence in the leaves of blue honeysuckle (Lonicera caerulea L.) such classes of biologically active compounds that provide a wide range of pharmacological action of the plant, such as chlorophyll-containing derivatives, a complex of bioflavonoids, carbohydrate components, anthocyanins, tannins, organic acids and others. This circumstance opens up broad prospects for the practical usage of the biomass under study in the production of food additives, pharmaceutical and perfumery and cosmetic preparations.

1. Introduction

Long-term studies of Siberian flora plants have shown that the vast majority of them have a unique chemical composition and high biological activity. One of the promising plants in this regard is the blue honeysuckle - Lonicera caerulea L., which fruits, leaves, stems and roots are widely used in scientific and folk medicine. A number of works are devoted to the pharmacological evaluation of drugs based on honeysuckle fruits, in particular, as anti-inflammatory and bactericidal agents [1, 2], there are indications of their usage as an anesthetic drug [3], for the treatment of heart [4] and some other diseases [5]. In addition, the possibility of using the fruits of blue honeysuckle in the production of soft drinks [6], in the bakery [7] and other food industries [8] is indicated.

A study of the chemical composition of honeysuckle fruits, as well as the isolation and establishment of the nature of the active principles of the indicated plant materials, was carried out by a number of researchers [9 - 11]. It was shown that the biological activity of blue honeysuckle fruits is due to the presence of vitamin C (45-48 mg%), vitamin A (0.08-0.12 mg%), thiamine (28-39 mg%), riboflavin (25-38 mg %) and folic acid (72-102 mg%). The presence of P-active polyphenols in the fruits (rutin, catechins, leukoanthocyanins, anthocyanins, etc.) is one of the valuable qualities, since these substances interfere with the oxidation of vitamin C and enhance its general strengthening effect on the body [11]. Among biologically active flavonols and flavones, rutin (48 mg / 100 g), isoquercetin (up to 12 mg / 100 g), quercetin (up to 10 mg / 100 g) luteolin (up to 14 mg / 100 g) and diosmin (up to 5 mg/ 100 g) were found [12]. No toxic substances - alkaloids and glucosides - were
found in the fruits of blue honeysuckle. All this turns the latter into the most valuable raw material for the production of food products for medical purposes.

However, despite the availability of the plant and a wide range of pharmacological properties, only the chemical composition of the fruits of blue honeysuckle is quite fully described in the literature. Data on the chemical composition of the vegetative parts of this plant are very limited or not available in the open literature.

In this study, we focused on studying the chemical composition of the leaves of blue honeysuckle (Lonicera caerulea L.), naturally growing in the southern taiga of the Krasnoyarsk Territory, in order to establish the content of various classes of biologically active substances.

2. The experimental part

As the raw material, the plant's leaf plates, collected at the end of May 2019, during the budding period, were used. It is believed that during this period the plant accumulates the largest amount of biologically active substances necessary for successful flowering and fruiting in the conditions of a short Siberian summer.

Immediately after harvesting, the leaves were dried in an oven at a temperature of 40-500C. An analytical sample was taken by the method of quartering from raw materials in accordance with GOST NKVT 14143-69, GF-IX, article 231 [13]. The ash content in the analyzed sample was determined in accordance with GOST 24027.2-80, and the moisture, lignin, polysaccharides were determined according to generally accepted methods [14].

To study the chemical composition of the extractives of the studied biomass, we used the method of fractional extraction of the dried plant preparation with organic solvents of increasing polarity: diethyl ether, ethyl acetate, isopropanol, and also water, followed by separation of the extracts in solvents that are not miscible with water into acidic, neutral, and phenolic parts. This method makes it possible to determine both the total amount of extractive substances contained in plant materials and the amount of resinous substances, phenolic and carbohydrate components separately. The content of extractives was determined by the loss of mass of the sample after exhaustive extraction in a Soxhlet apparatus for 20 or more hours, followed by drying of the sample at 1050C. The presence of various classes of chemical compounds was determined by the absorption of electromagnetic radiation by extracts in the UV and visible spectrum [15].

3. Discussion of the results

The results of the study of the chemical composition of the leaves of blue honeysuckle (Lonicera caerulea L.), naturally growing in the southern taiga of the Krasnoyarsk Territory, are presented in table 1.

Table 1. The chemical composition of the leaves of blue honeysuckle (Lonicera caerulea L.).

| Defined indicator     | Content,% of a.s.n. |
|-----------------------|---------------------|
| Moisture              | 6,1                 |
| Lignin                | 5,0                 |
| Polysaccharides       | 7,7                 |
| Essential oil         | traces              |
| Extractives           | 68,4                |
| Extracted from them:  |                     |
| diethyl ether         | 5,9                 |
| ethyl acetate         | 3,6                 |
| isopropanol           | 26,3                |
| water                 | 32,6                |

The significant content of extractives in the analyzed biomass (about 70 wt.%) made us turn to the question of their qualitative and quantitative composition. Despite the fact that the content of
biologically active substances extracted from the leaf plates of *Lonicera caerulea* L. with diethyl ether is low - 5.9 wt. % (table 1), the extract contains numerous classes of organic compounds (figure 1, curve 1): of these, chlorophyll (absorption maximum at $\lambda \sim 667$ nm) and auron ( $\lambda \sim 395$ - 405 nm) are represented to a greater extent, and to a lesser extent anthocyanins ($\lambda \sim 500$-530 nm), catechins ($\lambda \sim 365$ nm), fatty acids, phenolcarboxylic acids (probably n is hydroxybenzoic and protocatechuic acid ( $\lambda \sim 305$ nm)), and also simple phenols (265 nm).

The ethyl acetate extract of blue honeysuckle leaf does not contain substances that absorb UV and visible radiation (figure 1, curve 2).

The highest content of biologically active substances is extracted from the leaves of honeysuckle with polar solvents - 58.9 weight % (table 1). Extractive substances extracted by isopropanol (figure 2, curve 1) give absorption maxima at $\lambda \sim 260$-280 nm.

This circumstance indicates a significant content of leucoanthocyanins, aglycones and flavonoids: rutin - quercetin-3-glycoside, campferol-3-soforazide, campferol-7-diglycoside, apigenin-5-glycoside, quercetin, kemperfol, saponaretin, daringenerigideroin oligidroidenolignene, daringenoiden, daringenhydroiden oligidroidenolignin isouqueretin, luteolin-7-glucoside [16].

Obviously, due to the relatively high content of a complex of various flavonoids - compounds with P-vitamin activity, blue honeysuckle leaf preparations have antioxidant, anti-inflammatory and capillary-strengthening properties.
The weakly expressed absorption maximum at $\lambda$ - 340 nm is explained by the low content of coumarins and hydroxyphenol carboxylic acids in the extract. The absorption maximum at $\lambda$ - 405-410 nm indicates a relatively low content of aurons in the extract, and at $\lambda$ - 265 nm simple phenols. The preparation aqueous extract (figure 2, curve 2) contains organic water-soluble acids: malic, oxalic, citric (absorption in the field of $\lambda$ - 230-250 nm), isoflavones, xanthones, flavones: apigenin, luteolin, genquanine (absorption at $\lambda$ - 265 - 270 nm), hydroxycinnamic acids ($\lambda$ - 330 nm), flavanones ($\lambda$ - 285 nm and 310-330 nm), tannins ($\lambda$ - 350-360 nm).

4. Conclusions
The chemical composition of the leaves of the blue honeysuckle Lonicera caerulea L, naturally growing in the southern taiga of the Krasnoyarsk Territory, was studied.

It was established that the studied plant biomass contains 5 wt.% lignin, 7.7 wt.% carbohydrates and about 70 wt.% extractives.

It has been shown that the following classes of organic compounds are extracted with nonpolar solvents from blue honeysuckle leaves: chlorophyll, aurons, anthocyanins, catechins, coumarins, fatty acids, phenolcarboxylic acids, and also simple phenols. The presence of these classes of substances provides antioxidant and biocidal activity of preparations from the studied plant biomass.

Extractives substances extracted by polar solvents are represented with leukoanthocyanins, aglycones, and flavonoids. The presence of compounds with P-vitamin activity in the leaves of blue honeysuckle determines the antioxidant, anti-inflammatory and capillary-strengthening properties of the latter.

The investigated aqueous extract of blue honeysuckle leaves contains organic water-soluble acids: malic, oxalic, citric; isoflavones; xanthones; flavones: apigenin, luteolin, genquanine; hydroxycinnamic acids, flavanones, tannins. Their presence provides antibacterial, antiviral and anti-inflammatory activity of drugs from the studied raw materials, as well as astringent effect.

Thus, a spectrophotometric study showed the presence in the leaves of blue honeysuckle (Lonicera caerulea L.), naturally growing in the southern taiga of the Krasnoyarsk Territory, of various classes of biologically active compounds that provide a wide spectrum of pharmacological action of the plant. This fact opens up wide prospects for the practical use of the studied raw materials in the production of food additives, pharmaceutical, perfumery and cosmetic preparations.

This will reduce the use of valuable food raw materials - fruits of blue honeysuckle - for non-food purposes and will ensure a steady supply of raw materials to the pharmaceutical, perfumery and cosmetic industry enterprises, regardless of the seasonality and yield of the fruits of the plant under study.

References
[1] Sayakova G M, Parkhatkyzy N and Zhumashova G T 2015 Development of a new therapeutic ointment “Stop pus” based on domestic LRS - edible honeysuckle Vestnik KazGMU 3 264-6
[2] Sayakova G M and Ablaeva L R 2014 Development of a new soft dosage form based on domestic plant materials Vestnik KazGMU 5 190-96
[3] Sultana S, Kamil H, Ilyas M 1984 Chemical investigation of Lonicera maackii J Indian Chem. Soc. 61 (8) 730
[4] Plekhanova M N, Streltsina S A and Rostova N C 1993 Phenolic compounds of fruits of the genus Lonicera subsect. caerulea species Plant Resources 29 16-25
[5] Malysheva S K 2013 Medicinal properties of some types of honeysuckle Natural and technical sciences 6 118-22
[6] Menyaylo LN, Baturina I A, Veretnova O Yu et al. 2015 Scientific principles of the formation of an assortment of food products with desired properties. Technologies for the production and processing of plant materials (Krasnoyarsk) p 212
[7] Soboleva E V, Sergacheva E S, Smertina E S, Fedyanina L N, Lyakh V A and Gladyschchuk O S 2018 Use of honeysuckle extract (Lonicera edulis) in the technology of bakery products
Vestnik MAX 1 26-32

[8] Kharitonenko A G 2003 Commodity and technological aspects of the use of honeysuckle fruits and products of its processing in food production (Kemerovo) p 176

[9] Kuklina A G, Skvortsova A K 1990 To the introduction of Honeysuckle honeysuckle Bul. GBS 157 3-9

[10] Timoshin A V 2010 Qualitative composition of honeysuckle fruits of various varieties (introduced into the culture in the Krasnoyarsk Territory) Vestnik KrasGAU 7 52-6

[11] Chepeleva G G and Chirkova E S 2014 Differences in the biochemical composition of honeysuckle varieties (Lonicera introduced in the Krasnoyarsk Territory) Vestnik KrasGAU 2 178-81

[12] Chen X et. al. 2006 Separation and determination of compounds in Honeysukle by integration of ion-exchange chromatography fractionation with reversed-phase liquid chromatography-atmospheric pressure chemical ionization time-of-flight mass spectrometry analysis J of Pharmaceutical and Biomedical Analysis 40 (3) 559-70

[13] 1989 State Pharmacopoeia of the USSR (Moscow) 2 389

[14] Poznyakovsky V M 2000 Expert examination of fresh fruits and vegetables (Novosibirsk)

[15] Zaprometov M N 1991 Fundamentals of the biochemistry of compounds (Moscow) p 320

[16] Antipova E A, Kudrikova L E, Tikhomirova L I, Bazarnova N G, Cheprasova M U and Harnutova E P 2019 Assessment of the polyphenols content in biotechnological raw materials Iris Sibirica L. Sterkh variety in comparison with intact plants Plant chemistry 2 239-50