Original Research Article
Chemical Composition of Lipophilic Fractions of Raw Material Crataegus Rivularis Nutt

ABSTRACT

Aims: study of the composition of lyophilic substances in the raw materials of Crataegus rivularis Nutt.

Study design: experimental laboratory studies of dry plant materials.

Place and Duration of Study: Bashkir State Medical University, Izhevsk State Medical Academy, Volga Research Medical University and Ural Branch of the Russian Academy of Sciences from August 2021 to May 2022.

Methodology: Using gas chromatography with a mass-selective detector, lipophilic fractions of the fruits of shoots, flowers, leaves and fruits of riverine hawthorn, harvested in 2021 in the South Ural Botanical Garden-Institute, were studied.

Results: In general, the shoots and leaves of Crataegus rivularis accumulate compounds of the class of steroids, phytosterols, terpenoids, saponins and phenolic compounds, which suggests that the raw material may have additional pharmacological properties, such as hypocholesterolemic and antioxidant.

Conclusion: The results obtained show the good prospects for further studies of the composition of hawthorn extracts to expand the possibilities of phytotherapy.

Keywords: hawthorn, gas chromatography, plant extract, lipophilic fractions.

1. INTRODUCTION
Crataegus L., a genus of plants known as hawthorn, belongs to the family Rosaceae, is a shrub and tree, including about 280 species. Hawthorns grow in temperate zones and are widely distributed in Europe, East Asia, and North America [1,2]. Hawthorn has been used since ancient times in folk medicine, and its positive effect on the cardiovascular system was described by Dioscorides in the first century [3]. In the Russian Federation, the use of hawthorn fruits and flowers is allowed for medical use, while a number of foreign countries also use hawthorn shoots and leaves in medical practice. Thus, a number of European countries, including China, contain some types of hawthorn and raw materials based on them in their pharmacopoeias [4,5]. Each type of plant material is characterized by the accumulation of a large number of groups of biologically active substances with a variety of biological properties. Having identified the component composition of each of the groups of biologically active substances, establishing their chemical structure, it seems possible to predict their biological activity, and after isolation and purification, to investigate the pharmacological properties. One of the groups of biologically active substances contained in hawthorn and of practical interest are lipophilic substances (essential oils, sterols, fatty acids, pigments, and others). It is worth noting a wide range of their pharmacological characteristics, such as anti-atherosclerotic, hypolipidemic, wound healing, antibacterial and antioxidant effects [6-13]. All this confirms the expediency of isolation, identification and further studies of lipophilic substances contained in plant materials in order to expand the range of herbal products.
2. MATERIAL AND METHODS / EXPERIMENTAL DETAILS / METHODOLOGY

The objects of the study were shoots, leaves, flowers and fruits of Crataegus rivularis Nutt. – hawthorn riverine from the Rosaceae family [14]. The raw materials were harvested during various periods of the plant's vegetation in 2020 at the South Ural Botanical Garden-Institute, a separate structural subdivision of the Federal State Budget Scientific Institution of the Ural Federal Research Center of the Russian Academy of Sciences. The raw materials were harvested by hand during the daytime in dry warm sunny weather at outdoor temperature +20 - + 25 °C, since temperature is an important physical-chemical interaction factor [15,16]. The collected raw materials were dried by air-shadow drying, and then were packed in paper bags. Studies of the composition of plant raw materials were conducted from August 2021 to May 2022. The storage of raw materials was carried out in a dry and dark room at an air temperature not higher than 20 °C and at a humidity not higher than 50% [17,18].

Lipophilic fractions were obtained from shoots, leaves, flowers and fruits of the plant. The extraction of lipophilic substances was carried out by a single maceration of 5.0 g of crushed raw materials (accurately weighed with a particle size passing through a sieve with a hole diameter of 2 mm) with petroleum ether in a ratio of 1:10 by heating in a boiling water bath under reflux for 2 hours. The composition of the lipophilic fractions of the obtained extracts from the raw materials of Crataegus rivularis was identified using the method of gas chromatography with a mass selective detector (GC-MS). The fractions were differentiated into individual components on a Maestro GC7820 gas chromatograph with a G4513A autoinjector, an Agilent Technologies 5975 mass selective detector. 1 ml/min, temperature gradient: 60°C–2 min, then the heating rate was 5°C/min. up to 290 °C, holding time at the initial temperature - 2 minutes, holding time at the final temperature - 5 minutes; injector temperature 280 °C, injected sample volume 1 µl. The detector was used in the electron impact mode (70 eV), the spectra were recorded in the ion current scanning mode. Analysis of the extracted compounds of lipophilic extracts was performed in comparison with library mass spectra. The publication presents data of identified compounds with a coefficient of similarity to library spectra of at least 80%.

3. RESULTS AND DISCUSSION

As a result of the analysis of the lipophilic fraction of the shoots of Crataegus rivularis by gas chromatography with mass spectrometric detection, 13 substances were found, 9 compounds were identified by coincidence with library mass spectra. The vast majority of identified compounds are hydrocarbons - alkanes (tricosane, pentacosane, eicosan, heptacosan. In addition, the presence of saponins (α-amirin), phytosterols (β-sitosterol), celidoniol, phenolic compounds (Table 1), (Fig. 1)

Table 1. Components of lipophilic fractions of Crataegus rivularis.

<table>
<thead>
<tr>
<th>№ of the compound</th>
<th>Components of lipophilic fractions</th>
<th>Retention time, min</th>
<th>Quantitative content of compounds in the sample, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.</td>
<td>2-(4-methyl-3-cyclohexen-1-yl)-2-propanol</td>
<td>6.358</td>
<td></td>
</tr>
<tr>
<td>3.</td>
<td>Geraniol</td>
<td>7.087</td>
<td></td>
</tr>
</tbody>
</table>
4. Thymol  |  7.531 |  6.181  
5. α-terpinolene  |  8.251 |  4.602  
6. Geraniol  |  8.583 |  3.592  
7. Dibutyl phthalate  |  14.126 |  14.125 |  0.696  
8. Genikosan  |  15.152 |  0.978  
10. Glycine  |  18.356 |  1.003  
11. Octadecan  |  19.877 |  0.189  
12. Tricosan  |  19.958 |  2.676 |  1.049 |  3.119  
13. Squalene  |  20.128 |  1.769  
14. δ-tocopherol  |  21.042 |  2.552  
15. Hexacosene  |  21.479 |  4.373  
16. Pentacosan  |  21.584 |  6.379 |  42.145  
17. Hexadecane  |  22.296 |  15.066  
18. Eikosan  |  22.708 |  3.091 |  4.989 |  0.468  
19. Heptacosan  |  23.097 |  5.188 |  4.303 |  0.171  
20. Octabenzene  |  23.687 |  4.087  
21. Celidoniol  |  24.553 |  36.826 |  37.585 |  0.260  
23. β-amirin  |  25.912 |  1.871  
24. β-D-dihydrofucosterol  |  29.350 |  12.273  
25. α-amirin  |  30.693 |  7.215 |  10.132  

**Fig. 1.** GC-gramma of lipophilic fractions from shoots of Crataegus rivularis.
In the lipophilic fraction of the leaves of Crataegus rivularis, 13 compounds were also found, 11 were identified (Table 1). It has been established that the leaves also contain hydrocarbons, phytosterols (β-stigmasterol, δ-sitosterol), aliphatic amino acids (glycine), triterpene hydrocarbons (squalene), tocopherols (δ-tocopherol) (Fig. 2).

**Fig. 2. GC-gramma of lipophilic fractions from leaves of Crataegus rivularis.**

In the flowers of Crataegus rivularis, the presence of 18 volatile compounds was detected, of which 13 were recognized. The presence of a number of hydrocarbons (eicosan, tricosan, octadecane, etc.), essential oil components - esters (2-(4-methyl-3-cyclohexene-1-yl)-2-propanol), monoterpenes hydrocarbons (α-terpinolene), aliphatic monoterpenic alcohols (geraniol), aromatic alcohols (thymol), as well as celidoniol, phytosterols (δ-sitosterol), triterpene saponins (α-amirin and β-amyrin) (Fig. 3) (Table 1)
The lipophilic fraction of the fruits of Crataegus rivularis contains about 31 compounds, of which only 5 were identified. Benzene derivatives, hydrocarbons, and celidoniol were found (Fig. 4) (Table 1)
Analyzing the data obtained, it might be noted that the greatest accumulation of hydrocarbons, saponins and steroid compounds is observed in the shoots and leaves of Crataegus rivularis. The flowers mainly accumulate volatile aromatic compounds, mainly components of the essential oil, while the method of extracting the lipophilic fraction by maceration with petroleum ether turned out to be the most suitable for the flowers of Crataegus rivularis, since the highest extraction of volatile substances and a high signal intensity are observed. The fruits of Crataegus rivularis with this method of extracting lipophilic compounds do not show an active accumulation of such substances, therefore, in further studies, it is necessary to consider other organic solvents and extraction modes.

4. CONCLUSION

The lipophilic fractions of shoots, leaves, flowers and fruits of Crataegus rivularis isolated by maceration with petroleum ether were studied by gas chromatography with mass spectrometric detection. It has been shown that the shoots and leaves of Crataegus rivularis accumulate compounds of the class of steroids, phytosterols, terpenoids, saponins and phenolic compounds. This suggests that vegetable raw materials may have hypocholesterolemic and antioxidant effects [19,20]. The obtained results testify to the expediency of further research of this species both in order to expand the scope of hawthorns and to introduce a new species into medical practice. This search direction is promising due to the fact that hawthorn extract has low toxicity and high safety [21].

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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