STUDY OF PLANTS OF GENUS STACHYS ON THE EXAMPLE OF BETONICAOFFICINALISL. WITHIN THE SCIENTIFIC COURSE “PHARMACEUTICAL REMAKE”

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Abstract

The paper describes a retrospective analysis of the use of common betony in traditional and modern medicine. The Stachys plants have not been used in modern domestic medicine yet. Therefore, based on the historical experience of the use of common betony in traditional medicine, we have emphasized its value as a plant with high therapeutic potential.

In this study, we used the so-called "Pharmaceutical remake", intended to revive interest in the now-forgotten objects of flora, which were previously widely used in medicine. The polyphenol structure of betony grass was studies with the use of the reverse-phase and graduate elution high-performance liquid chromatography. The presence of glycosides of diosmin, acacetin, luteolin and apigenin was established. The grass also contains hydroxycinnamic acids such as chlorogenic and rosemary acids, with the latter dominating in the polyphenol complex. The composition of plant terpenes in a hexane extraction has been studied by gas-liquid chromatography with mass spectrometric detection. It was determined that the terpenoid composition of betony is mainly represented by sesquiterpenes: aromadendrene and germacrene, and monoterpenes: 3-carene and dihydrocarveol.

Keywords: Common betony, Pharmaceutical remake, High performance liquid chromatography, Flavonoids, hydroxycinnamic acids, Gas-liquid chromatography, Terpenoids.

Introduction

In the XIX century, medicine had drugs of natural origin only in its arsenal. There were virtually no synthetic drugs. Later, there was a very rapid transition to synthetic drugs, which now dominate in the consumer market. However, the accumulated over the centuries phytotherapy experience have been lost. It has been suggested to understand the
The term "pharmaceutical remake" as a complex of traditional and innovative technologies, analytical and pharmaceutical operations (models), leading to a revival of the formerly known and unused now medicinal compositions and forms [1, 10].

The Stachys plants have come in our sight. A genus Stachys includes only 20 species, 7 of which grow in Russia. Many of them have been historically used in official and non-official medicine [2]. Traditional medicine has usually used a common betony in its arsenal (Figure 1).

![Figure 1 – The common betony appearance](image)

A common betony (*Betonica officinalis* L.) (common hedgenettle, purple betony, wood betony, bishopwort, or bishop's wort) is a perennial herb of the Lamiaceae family.

The use of betony in medicine is known since ancient times. Dioscorides and Galen believed betony to be a powerful medicinal herb for many diseases. It is known that Anthony Muse, a court physician of Augustus, cured the Emperor with the help of betony, for which he was greatly rewarded and got his marble statue reared during his life [4]. Numerous handwritten medical manual and herbalists of XV-XVIII centuries described betony as a wonderful drug.

In Russia, betony has not been used in official medicine, but applied in traditional medicine for a wide range of diseases [4].

Recently, interest in betony has been renewed. A famous herbalist N.G. Kovaleva in her book "Treatment with plants" (Moscow, "Medicine", 1971) recommended to use an aerial part of betony as a mean of reducing the excitability of the central nervous system, improving metabolism, as well as for hypertension, atherosclerosis and kidney diseases [5].

In traditional medicine the plant is used for high acidity of gastric juice, bronchitis, asthma, whooping cough, pyelonephritis, cystitis, hemorrhoids, nervous exhaustion, rhinitis, migraine, epilepsy, rheumatism, gout, jaundice, as well
as an astringent and wound-healing agent. Its rhizomes and roots – as purgative and emetic agent. Their decoction is used for nervous disorders, general weakness, reduced appetite, kidney and liver diseases, gastritis, colitis. Aerial part is used as antihypertensive, excitability-reducing and metabolism-promoting agent for hypertension, atherosclerosis, and kidney diseases. Its tincture and extract is used for various gynecological bleeding and uterine subinvolution after childbirth and abortion. Oral infusion – for liver diseases, jaundice, poor digestion, as a mean of reducing blood pressure in hypertension, as a sedative agent for various nervous disorders, fainting, poor blood circulation, as a hemostatic agent for lung and uterine bleeding, as an astringent and expectorant agent for colds, bronchitis, tracheitis, persistent cough with purulent sputum, asthma, pulmonary tuberculosis; for external use – for wounds washing, for tumors, convulsions, rheumatism; its infusion and decoction – for hysteria, anemia, scrofula. Fresh minced leaves are applied onto the non-healing wounds and ulcers. Powder of dried leaves is snuff like tobacco for headaches of various origins and for prolonged cold. The infusion of the leaves and flowers is used for stroke, headache, eye diseases, diarrhea, gout, etc. [6].

In France and Bulgaria, betony is included in the State Pharmacopoeia. The plant contains a volatile oil (0.058%), the bitter, resinous and tannin substances, organic acids, carotenoids, flavonoids, vitamin K, ascorbic acid, sugar, and calcium salts. The seeds contain up to 42% of fatty oil, which comprises palmitic, stearic, oleic, linoleic, and linolenic acid [6, 11]. However, the chemical composition of the plant has been studied fragmentarily.

Objective of this study was to confirm the prospectivity of *Betonica officinalis* L. as the official medicinal plant.

**Materials and methods**

To study the chemical composition of common betony we used alcohol extraction of raw materials. We used 70% ethyl alcohol as extragent. An extraction was obtained by percolation in the battery of three diffusers according to traditional manufacturing scheme for liquid extracts at a ratio of 1:1.

Chromatographic studies were carried out on a chromatographic device «Agilent Technologies 1200 Infinity», USA, with autosampler Agilent 1200, vacuum microdegasser, gradient pump and thermostat of the same series. Electronic absorption spectra were recorded using a spectrophotometric diode-array detector Agilent 1200 (a wavelength range of 190 to 950 nm, a 10 mm variable-path cell; volume of 13 μl), and the scanning pitch – 2 nm.

Recording and processing of spectral data and chromatograms was conducted with “Agilent Chem Station” software.

The amount of betony polyphenol complexes was chromatographed under the following conditions:
In our study, we used gradient elution mode for betony polyphenols, because, as we know, this group of compounds is characterized by substantially variable polarity. This variant of elution has contributed to a more complete and uniform output of components, which is clearly illustrated by the chromatogram below. Elution started with the aqueous phase, with further gradual increase in alcohol concentration [9].

The mobile phase composition was programmed as described in Table 1.

**Table 1 - Betony polyphenols gradient elution conditions.**

<table>
<thead>
<tr>
<th>Time, min</th>
<th>A%</th>
<th>B%</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>90</td>
<td>10</td>
</tr>
<tr>
<td>10</td>
<td>80</td>
<td>20</td>
</tr>
<tr>
<td>20</td>
<td>70</td>
<td>30</td>
</tr>
<tr>
<td>30</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>40</td>
<td>10</td>
<td>90</td>
</tr>
</tbody>
</table>

Detection was performed: 325 nm for hydroxycinnamic acids, 350 nm for flavonoids.

Identification of the components was carried out by matching the retention times of analytes with a CO retention time recorded under similar experimental conditions and based on the results of diode array detection.

The relative content of flavonoids and hydroxycinnamic acids was defined as the ratio of the chromatographic peak area and the total area of peaks of all identified flavonoids, hydroxycinnamic acids, according to formula 1:

\[
X_i = \frac{S_i \times 100}{\sum S},
\]

Where \(S_i\) – mean value of the component peak area in the chromatograms;

\(\sum S\) – mean value of the sum of all peak areas in the chromatograms.

The effectiveness of the chromatographic system was evaluated according to the criteria calculated by the following formulas.
Column efficiency was determined by calculating the theoretical plate number \( N \). The higher the efficiency, the greater this value and lower the extension of the peak of the originally narrow band as it moves through its column, and the narrower the peak at the column exit. The value of no less than 5000 was used as optimal criterion of column efficiency.

The number of theoretical plates was calculated by the formula 2:

\[
N = 16 \times \left( \frac{t}{\mu} \right)^2
\]

(2)

Where \( t \) – retention time of the analyte, mm;

\( \mu \) – baseline peak width, mm.

The main evaluation criterion of the adequate separation of adjacent peaks was the separation coefficient \( R_s \), which should not be less than 1.5, according to the European Pharmacopoeia [7]. The peaks should be separated by a baseline.

Peaks separation coefficient \( R_s \) was calculated by formula 3:

\[
R_s = \frac{\Delta l}{\mu_{0.5(1)} + \mu_{0.5(2)}}.
\]

(3)

Where \( \Delta l \) – the distance between the tops of two adjacent peaks, mm;

\( \mu_{0.5(1)}, \mu_{0.5(2)} \) – width at half height of the peaks of two components, mm.

A chromatographic peak shape characterizing the overload of chromatographic column was determined by calculating the peak asymmetry coefficient (\( T_f \)) by the formula:

\[
T_f = \frac{\mu_{0.05}}{2 \times f}.
\]

(4)

Where \( \mu_{0.05} \) – peak width at a height of 5.0% of the base line, mm;

\( f \) – distance between the peak base at a height of 5.0% of the base line and the perpendicular drawn from its top, mm.

The optimum value of the asymmetry coefficient \( T_f \) was index – less than 2.

Terpenoids were isolated through the hexane extraction in “Soxlet” apparatus.

Hexane extraction was further tested for the presence of terpenoids by gas-liquid chromatography with mass spectrometric detection.
The measurement was carried out on the chromatography-mass spectrometer GCMS-QP2010 Ultra by “Shimadzu”, Japan.

**Separation was performed on the column:**
- **Zebron ZB-5MS** 30 m L × 0.25 mm ID × 0.25 μmdf;
- Liquidphase: 5%-polysilarylene-95polydimethylsiloxane;
- Temperatureranges: -60°C – +325/350°C;
- SerialnumberNo. 238059.

**Chromatographic conditions:**
- Carrier gas – helium, constant flow rate – 0.7 ml/min;
- The analysis was performed in a programmable temperature mode:
  - Column temperature was programmed in the range 70°C (2 min. isotherm) - 230°C (5 min. isotherm). Temperature rise rate 3°C/min;
  - Evaporator temperature – 240°C;
  - Ion source temperature – 250°C;
  - Interfacetemperature – 250°C;
- Sample input – split mode (Splitratio 1/50) – 1.5 min;
- Detectorvoltage – 0.84kV;
- Emissionflow – 60 µA;
- Injectionvolume – 1µl.

Detection was carried out under total ion current (SCAN) in the range of m/z 70 - 350 Da, with a scan rate of 769 and resulting time of 0.4 seconds. Identification of individual components was carried out according to the comparative analysis with NIST 11 database.

**Results and discussion**

The chromatogram of 70% ethanol extract of the herb *Betonica officinalis* L. is shown in Figure 2.
Chromatography results (Table 2) indicate that the polyphenolic complex of the herb *Betonica officinalis* L. includes glycosides of diosmin, acacetin, luteolin and apigenin, as well as hydroxycinnamic acids – chlorogenic and rosmarinic acid, with the latter dominating in the polyphenol complex [8].

**Table 2 - Chromatography results of 70% ethanol extract of the herb *Betonica officinalis* L.**

<table>
<thead>
<tr>
<th>Retention time, min</th>
<th>UV-spectrum</th>
<th>Total relative content, %</th>
<th>Identified component</th>
</tr>
</thead>
<tbody>
<tr>
<td>5.416</td>
<td><img src="image1" alt="UV-spectrum" /></td>
<td>3.65</td>
<td>Chlorogenic acid</td>
</tr>
<tr>
<td>11.702</td>
<td><img src="image2" alt="UV-spectrum" /></td>
<td>0.72</td>
<td>Isoorientin</td>
</tr>
<tr>
<td>12.554, 13.372, 13.628</td>
<td><img src="image3" alt="UV-spectrum" /></td>
<td>13.68</td>
<td>Rosmarinic acid</td>
</tr>
</tbody>
</table>
The calculation results of performance criteria of the used chromatographic system are shown in Table 3.

Table 3 - Chromatographic system suitability for the determination of *Betonica officinalis* L. polyphenols.
According to data in Table 3, the main criteria (N>5000, R_s>1.5, T_f<2) correspond to standard values. Thus, the used chromatographic system can be considered suitable for the determination of polyphenols of common betony.

Since common betony is an aromatic plant, an important part of the study was to determine the terpenoid composition of the plant.

Terpenoid chromatography results are shown in Figure 2.

![Figure 3 - Separation chromatogram of the common betony terpenoids.](image)

Interpretation of the chromatographic results is shown in Table 4.

**Table 4 - Component composition of terpenoids of common betony.**

<table>
<thead>
<tr>
<th>Retention time, min</th>
<th>Mass-spectrum</th>
<th>Identified component</th>
</tr>
</thead>
</table>
Thus, we have determined that the terpenoid composition of betony is mainly represented by sesquiterpenes: aromadendrene and germacrene, and monoterpenes: 3-carene and dihydrocarveol.

**Conclusion**

It is known that the number of medicinal plants reaches 20 thousand, however, the official medicine has yet used about 300. In recent decades, due to the emergence of a new nosological entity – "drug disease"– the relevance of the use of plant drugs has increased enormously.

Introduction of medicinal herbs previously known to official and traditional medicine into officinal compounding of medicinal forms on the basis of their biologically active compounds provides virtually unlimited opportunities to developers. This way of extending the range of medicinal products appears to be quite rational and low-cost, since it eliminates the need to carry out a full-scale scientific search.

The information on *Betonica officinalis* L. presented in this paper serves as illustration to the presented inferences.


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