PROCEDURES USED FOR ISOLATION OF COMPLEX BIOLOGICAL ACTIVE CONSTITUENTS FROM POLYGONUM L.

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ABSTRACT

This work deals with the development of a procedure for the isolation of a complex of biologically active substances from the aerial parts of some plants of Polygonum L. grown up in Kazakhstan and the determination of a possibility of their application to officinal medicine. The effect of various factors (the temperature, the extraction time, the raw material: solvent ratio) on the chemical composition of the extracts of a high yield of biologically active substances from Polygonum minus, P. aviculare, P. hydropiper is studied.

Keywords: Polygonum, antimicrobial activity, complex of BAS, flavonoids.

INTRODUCTION

Over 1,000 plants of genus of Polygonum L. grow up around the whole world. 48 species and the endemic plant Polygonum betpakdalense are grown in Kazakhstan. Among them 8 species have pharmacopoeial properties, although many other have industrial reserves. Almost all species of the genus are weeds so that they are extremely widespread [1 - 3].

29 species are widely used in folk medicine (because of their antitumor, fixing, tonic, anti-inflammatory, diuretic, wound healing properties), 23 species find an application in everyday life in tanning, tissues coloring, in perfumery and distillery industry, while 17 species provide fodder for farm animals and birds [2 - 5].

P. aviculare and P. hydropiper are pharmacopoeial plants finding application in therapy. These plants have a long history of use in folk medicine as remedies for the treatment of inflammation, rheumatoid arthritis, colic pain, fever, epilepsy, headache, chill, joint pain and infectious diseases. They are also used as diuretic and anthelmintic remedies, CNS stimulants, for insomnia, kidney diseases, hemorrhoids, hypertension and angina treatment [3, 6, 7]. P. minus has powerful antioxidant, anti-microbial and anti-inflammatory activity and is used in ulcer, hepatic abnormalities, genotoxicity, cytotoxicity and protozoa infections treatment [8].

Due to its geographical position, Kazakhstan is a promising base for production of medicines from plant raw materials. In addition, it is experimentally proved that the Kazakh plant’s analogues are distinguished by their high content of polyphenolic compounds and, in particular, flavonoids, and polysaccharides. They are known and recognized in the field of antioxidants and immunomodulators. In this regard, the study of local plant materials is an important subject [3 - 10].

The extraction of plant raw material having a cellular structure is a complex physical and chemical process whose course is influenced by a number of factors. It is the main stage of obtaining extracts from plant raw materials. The content of extractive substances is one of the important characteristics determining the extract quality. It is known the extraction process depends on a number of factors such as the techniques used, the
solvents, the duration and temperature of extraction [11].

The bioactive compounds can be extracted from the corresponding plant materials by various classical extraction techniques. Most of them are based on the extracting power of the different solvents used and the application of heat and/or mixing. The existing classical techniques used in this field refer to Soxhlet extraction, maceration, percolation, hydrodistillation and intensive extraction methods [12].

The extraction efficiency of any conventional method depends mainly on the choice of the solvents. The polarity of the targeted compound is the most important factor in choosing a solvent. The molecular affinity between the solvent and the solute, the mass transfer, the environmental safety, the human toxicity and the financial feasibility should also be considered in selecting a solvent for bioactive compound extraction. Methanol and acetone are commonly used as solvent in Soxhlet extraction of flavonoids [13, 14].

The quantity of the substance diffusing through the raw material layers is directly proportional to the duration of the process. Not only biologically active substances, but also ballast substances pass into the extract. Therefore, the end of the extraction process should not be attributed to the amount of the substances extracted and the active compounds yield [15].

The extraction efficiency at any temperature depends on the nature of the active compounds. The heating increases the diffusion rate of the substances extracted from plant raw materials. Some volatile components may be lost at a high extraction temperature. This drawback limits the application to thermo labile compounds extraction [12, 15]. The aim of the present communication is to study the process of extraction of some plants of the genus Polygonum L. for BAS complex isolation.

**EXPERIMENTAL**

**Materials**

Three plants of Polygonum L. (Polygonum minus, P. aviculare, P. hydropiper) were collected in their flowering phase during July-August 2015 in Almaty region. The plant raw materials were dried at 50°C to the minimum permissible percentage of moisture (less than 7 %) [3]. The analysis of the content of heavy metals and radioactive isotopes showed no deviation from the limiting values verifying their ecological purity [3, 16].

**Methods**

The selection of the optimal technological parameters of raw plant materials processing aimed at the identification of those affecting directly the composition and the quality of the final complex of biologically active substances (BAS) [12]: 1) the nature of the solvents; 2) raw material:solvent ratio; 3) the temperature; 4) the duration of the extraction; 5) the extraction frequency. The selection of the optimal solvent was carried out on the basis of the qualitative analysis of the main BAS groups and the quantitative extraction of extractive substances by pharmacopoeial methods [3, 12]. The amount of the extracted substances was characterized by the amount of the dry residue (W, mass %) determined by the gravimetric method [11].

The chemical composition was studied by paper and thin-layer chromatography (PC and TLC) using the specific reactions for each group of BAS and comparing with the taps and the standard samples of the compounds different groups on the ground of the color and the chromatographic mobility observed in the various solvent systems.

**RESULTS AND DISCUSSION**

The solvents selection aims at the determination of the whole complex of active compounds present in the plant raw materials and the preferential content present. The changes of BAS amounts are listed in Table 1. The quantitative analysis of the extractive substances from the plant raw material shows that 50 % ethanol was the optimal solvent for P. minus and P. hydropiper, while 50 % acetone – for P. aviculare.

It is obvious that with in case of an unchanged amount of the plant material, the increase of the extragent amount in the extraction process results in increase of the substance dissolved and transported from the cell to the intercellular space. At the same time, the increase of the volume of the extragent leads to decrease of BAS concentration in the extract.

The ground raw material is introduced to a five-, seven-, nine- and twelve-fold amount of the solvent for studying the dependence of the raw material: solvent ratio on the BAS amount in the plants studied. The results are shown in Fig. 1. The raw material: solvent ratio has a positive effect. In fact, the increase of the solvent in the ratio considered brings about a greater amount of the
extractive substances obtained despite the solvent used. This is consistent with the mass transfer principle as the concentration gradient between the solid and most of the liquid is the driving force. As P. aviculare raw material is fluffy, the initial raw material to a solvent ratio is chosen to be equal to 1:7.

In accordance with the mass transfer law the difference between the concentration of the extract and the solvent increases the transition of the soluble substances into the solvent and continues until the establishment of equilibrium. It is so because the concentration difference is the driving force of the diffusion process.

However, the increase of the solvent consumption is limited by the cost of concentrating the extract obtained. It follows that the cost of the extract evaporation is an important factor requiring consideration. Therefore, the smaller the raw material: solvent ratio, the more efficient from an economic point of view is the extraction process.

The extraction is carried out at a plant raw material: solvent ratio of 1:7 for P. minus, of 1:10 – for P. aviculare and of 1:7 – for P. hydropiper.

The effect of the extraction temperature on the process of maximum extraction of BAS complex is studied (Fig. 2). The temperature increase leads to an increase of the solubility of the various polyphenolic constituents, as well as to an increase of the rate of their diffusion. Since the boiling point of the solvents used does not exceed 80ºC no significant change of the composition

<table>
<thead>
<tr>
<th>Solvent</th>
<th>Amount of the extractive substances, %</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>P. minus</td>
</tr>
<tr>
<td>Water</td>
<td>19.12</td>
</tr>
<tr>
<td>10 % ethanol</td>
<td>14.66</td>
</tr>
<tr>
<td>30 % ethanol</td>
<td>15.81</td>
</tr>
<tr>
<td>50 % ethanol</td>
<td><strong>24.19</strong></td>
</tr>
<tr>
<td>70 % ethanol</td>
<td>18.55</td>
</tr>
<tr>
<td>96 % ethanol</td>
<td>16.01</td>
</tr>
<tr>
<td>acetone</td>
<td>3.64</td>
</tr>
<tr>
<td>50 % acetone</td>
<td>19.82</td>
</tr>
<tr>
<td>benzene</td>
<td>7.39</td>
</tr>
<tr>
<td>ethyl acetate</td>
<td>3.52</td>
</tr>
<tr>
<td>chloroform</td>
<td>7.39</td>
</tr>
</tbody>
</table>

Table 1. A quantitative analysis of the extractive substances from plant raw materials with bipolar solvents, %.
of the extracted substances is expected. The temperature regime is varied from 20°C to 80°C. It is found that the optimal temperature of extraction of *P. minus* is 40°C, while it amounts to 60°C for *P. aviculare*.

The changes of BAS amount depend on the extraction time as shown in Fig. 3. The extraction is carried out in the presence of the chosen solvent and the predetermined values of the plant raw material: solvent ratio and temperature. The results in Fig. 3 show that the optimal duration of *P. minus* extraction is 5 h, while that of *P. aviculare* and *P. hydropiper* is 3 h. The increase of the time of extraction from 1 h to 5 h leads to increase of the yield of the extractive substances. However, the further prolongation has a negative effect on the extractives yield.

An important indicator of the exhaustive isolation of BAS complex from medicinal plant materials refers to the number of extractions. The concentration of the extracted substances equals their concentration in the isolated phytocomplex, i.e. not all extracted substances are transferred to the crude extract. That is why a secondary extraction is required. It is reported [15] that the optimal number of successive extraction equals two [15].

The solvent is evaporated at 40°C under vacuum to dryness and the dry residue is weighed. The solvent evaporation has to be done under reduced pressure and at a low temperature to minimize the extracted polyphenolics degradation. The evaporation process temperature should be maintained at 40°C or below [17] due to the possible proceeding of hydrolysis, isomerization, and polymerization at higher temperatures.

The data obtained provide to summarize the main parameters of BAS complex isolation. They are shown in Table 2. According to the developed block-scheme of processing *Polygoum* L. plants, three complexes of BAS are obtained in quantities of 3.5 g (*P. minus*), 6.0 g (*P. aviculare*) and 6.2 g (*P. hydropiper*).

A quantitative analysis of BAS main groups of flavonoids, tannins, amino- and phenolic acids, coumarins, polysaccharides is carried out to control [18] the group compositions of the plant raw materials and their complexes. The results obtained are presented in Table 3. Flavonoids and their glycosides prevail in the phytocomplexes of *P. minus* and *P. hydropiper*, while polysaccharides, tannins and phenolic acids are predominantly found in *P. aviculare*. Some examples of bioactive substances are shown in Fig. 3.

Table 2. Procedure parameters of BAS complex isolation.

<table>
<thead>
<tr>
<th>Procedure conditions</th>
<th><em>P. minus</em></th>
<th><em>P. aviculare</em></th>
<th><em>P. hydropiper</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Degree of crushing raw materials, mm</td>
<td>5</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Solvent</td>
<td>50 % ethanol</td>
<td>50 % acetone</td>
<td>50 % ethanol</td>
</tr>
<tr>
<td>Plant raw material: solvent ratio</td>
<td>1:7</td>
<td>1:10</td>
<td>1:7</td>
</tr>
<tr>
<td>Temperature, °C</td>
<td>60</td>
<td>40</td>
<td>60</td>
</tr>
<tr>
<td>Time, h</td>
<td>5</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Amount of extraction</td>
<td>2</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Conditions for concentrating the extract</td>
<td>t &lt;40°C, p &lt;1 atm.</td>
<td>t &lt;40°C, p &lt;1 atm.</td>
<td>t &lt;40°C, p &lt;1 atm.</td>
</tr>
</tbody>
</table>
compound extracted with different solvents are given by Cowan [14]. Tannins, polyphenols, flavonols, terpenoids and alkaloids are isolated by ethanol, while flavonoids – by acetone. Aqueous solvents increasing the polarity of the isolated constituents are used in present study. The component composition of the extracts is studied by PC and TLC. n-Butanol: acetic acid: water (40: 12.5: 29) is used as a mobile phase (Table 4).

**CONCLUSIONS**

The study focused on the determination of the conditions of extraction of natural antioxidants from herbal plants leads to the following conclusions:

A polyphenolic phytocomplex is extracted from the plant raw materials by repeated extraction using 50 % ethanol or 50 % acetone as solvents at their boiling point. That provides a maximum quantity of the extractive substances;

The values of the raw material: solvent ratio refer to 1:7 for *P. minus*, *P. hydropiper* and 1:10 for *P. aviculare*. A maximum quantity of the extractive substances is obtained at minimum expenses;

The maximum yield of polyphenols is achieved by extraction within 5 h in case of *P. minus* and 3 h in that of *P. hydropiper, P. aviculare* followed by further concentration of the extract at 40ºC under vacuum. The increase of the time extraction is impractical because no further increase of the extractive substances quantity is reached while the expenses increase.

The results indicate that the choice of the solvent, the temperature value, the time extraction and the raw materials: solvent ratio affect the physical and chemical properties of the extracts and which is why these parameters are important for processing *Polygonum* L. plants.

**REFERENCES**

13. L. Wang, C.L. Weller, Recent advances in extraction of nutraceuticals from plants. Trends in Food Science and Technology, 17, 6, 2006, 300–312.
14. M.M. Cowan, Plant products as antimicrobial agents, Clinical Microbiology Reviews, 12, 4, 1999, 564-582.