Study of nitrates accumulation in herbs of *Thymus pulegioides* L. for flora of Ukraine

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A – research concept and design; B – collection and/or assembly of data; C – data analysis and interpretation; D – writing the article; E – critical revision of the article; F – final approval of the article

*Thymus pulegioides* L. syn. (*Thymus ovatus* Mill.) of family, *Lamiaceae* is a fairly common species in Ukraine. Given the literature data, the studied species needs a more thorough study of nitrates accumulation due to the ecological situation in Ukraine, as well as the search for herbal medicinal products with sufficient raw material base and a minimum amount of carcinogenic and harmful substances.

**The aim of the research:** To determine the quantitative content of nitrates in the infusion of grass (1:10) *Thymus pulegioides* L. flora of Ukraine, using the ionometric method of analysis.

**Materials and methods.** Infusion of *Thymus pulegioides* L. (1:10) harvested in different regions of Ukraine was studied with the help of the ionometric method of analysis.

**Results.** The accumulation of nitrates was quantified for *Thymus pulegioides* L. depending on the growing region, using the ionometric method of analysis. The data obtained during the study of the nitrates accumulation in the grass of the species varied from 62.55 ± 7.31 to 232.44 ± 24.11 which do not exceed the generally accepted sanitary norms. Summarizing the study, it was found that the minimum accumulation of nitrates in HRM was found in Poltava and Dnipropetrovsk regions (environment).

**Conclusions.** Given the data obtained, it can be concluded that the study of species of the genus *Thymus* L. by ionometric method of analysis for nitrates content to control the quality of plant raw materials and drugs based on it was relevant. The central part of Ukraine is a promising and ecologically safe region for the growth of the studied species.

**Key words:** *Thymus pulegioides* L., herb, ionometric method of analysis, nitrates.
At present, the main problem in ecology is the state of the environment and the impact of anthropogenic harmful factors on environmental pollution. Every year the level of chemical pollution in Ukraine is exacerbated due to scientific and technological progress and careless attitude of people to the environment. It is known that the level of accumulation of harmful substances depends on many factors: the type, location of the plant, soil moisture, and the use of mineral fertilizers. It should be noted that the high level of environmental pollution leads not only to the accumulation of nitrates but also significantly increases the number of pesticides in different parts of plants. It is known in the scientific literature that nitrates have a negative effect on the cardiovascular and excretory systems. Information on the permissible content of nitrates in herbal raw material (HRM) and extracts from it is insufficient, so it is an important aspect in modern pharmacy and phytotherapy to review the accumulation of nitrates in herbal raw material (1:10) Thymus pulegioides L. flora of Ukraine.

Aim
To determine the quantitative content of nitrates in the infusion of grass (1:10) Thymus pulegioides L. flora of Ukraine, using the ionometric method of analysis.

Materials and methods of research
For the experimental studies, we used the herb plant material Thymus pulegioides L. which was harvested in different regions of Ukraine during the flowering phase (June–October 2017–2018) in accordance with commonly accepted requirements. During experimental studies, the accumulation of nitrates in plant medicinal raw materials was documented.

The qualitative composition of nitrate compounds was determined by pharmacopoeial reaction with diphenylamine in concentrated sulphatic acid [4]. The quantitative content of
nitrates was determined by ionometric method on the device EV-74 (Republic of Belarus, JSC “Gomel Plant of Measuring Instruments”) with nitrate-selective electrode type EI-NO3− (reference electrode – silver chloride EVL-1 MZ). In the phytochemical laboratory of the department, a standard solution of potassium nitrate (CP) with a concentration of 0.1 mol/l was used to prepare working solutions with concentrations of C1 = 0.01 mol/l, C2 = 0.0001 mol/l in 1 % solution of potassium alum.

The weighted amount of the test sample of 10 g (exact amount) was ground to a powdery state and transferred to a 100 ml volumetric flask. Then 50 ml of 1 % solution of potassium alum was added, carefully stirred for 3 min, the electrode potential (mV) was measured, and with the help of a calibration graph the nitrate content was calculated. For the analysis of infusions (1:10) with the herbal raw material (HRM) 10 ml of the pharmaceutical form was used. Due to the absence of standardizing documentation regulating the presence of these substances, we used the maximum permissible concentration (MPC) regulations for agricultural plant products (up to 350 mg/kg) to determine safety. For the study of infusions (1:10) from raw materials, we used 10 ml of the medicinal form.

**Results**

The research results are given in Table 1. According to the pharmaceutical regulatory documents on analytical method validation, which have clearly established sanitary norms that regulate the content of nitrates in drinking water for human consumption, where the maximum permissible concentrations (MPC) should not exceed 10 mg/l; in herbaceous agricultural plants 300–370 mg/kg; rhizomes and roots 400–450 mg/kg [10–12]. During the study, it was found that the accumulation of nitrates in the grass *Thymus L.* ranges from 62.55 ± 7.31 to 232.44 ± 24.11. The content of nitrates did not exceed the existing sanitary norms of the MPC.

**Discussion**

The obtained data support the perspective of using the ionometric method of analysis to control the quality of potential medicinal raw materials.

**Conclusions**

1. With the help of experimental studies of the HRM the level of nitrate accumulation was estimated as ranging from 62.55 ± 7.31 to 232.44 ± 24.11.

2. The presence and content of nitrates indicate that the accumulation of substances depends on the conditions of growth of the species and the territorial location of *Thymus pulegioides* L.

3. Deterioration of the ecological condition of the environment is the main problem for finding safe plant raw materials that would accumulate a minimum amount of harmful substances during the growing season.

4. Summarizing the study, it was found that the minimum accumulation of nitrates in HRM was found in Poltava and Dnipropetrovsk regions (environment).

**Prospects for further research.** The data obtained by the experimental study can be used for further phytochemical and ecological studies of *Thymus pulegioides* L. syn. (*Thymus ovatus* Mill.), as well as for the standardization of HRM of the studied species of the genus *Thymus L.* for nitrate content. The central part of Ukraine is a promising and ecologically safe region for the growth of the studied species.

**Conflicts of interest:** authors have no conflict of interest to declare.

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**Table 1. The results of the quantitative determination of the number of nitrates in herbs and infusions of herbs (1:10) of the genus *Thymus L.* (June–October 2017–2018), (x ± ∆ x), % µ = 6**

<table>
<thead>
<tr>
<th>Plant species</th>
<th>Harvest location</th>
<th>Content in HRM, mg/kg</th>
<th>Content in infusions (1:10), mg/l</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Thymus pulegioides</em> L.</td>
<td>Zaporizhzhia region, Zaporizhzhia</td>
<td>232.44 ± 24.11</td>
<td>112.61 ± 12.36</td>
</tr>
<tr>
<td></td>
<td>Poltava region, Hlobyne</td>
<td>126.76 ± 13.55</td>
<td>64.34 ± 7.22</td>
</tr>
<tr>
<td></td>
<td>Kherson region, Henichesk</td>
<td>218.23 ± 19.64</td>
<td>129.77 ± 13.94</td>
</tr>
<tr>
<td></td>
<td>Mykolaiv region, Olexandria</td>
<td>224.35 ± 21.82</td>
<td>118.18 ± 12.79</td>
</tr>
<tr>
<td></td>
<td>Zaporizhzhia region, Volodymyrivka village</td>
<td>167.28 ± 17.39</td>
<td>85.45 ± 9.12</td>
</tr>
<tr>
<td></td>
<td>Dnipropetrovsk region, Apostolove</td>
<td>115.75 ± 12.72</td>
<td>62.55 ± 7.31</td>
</tr>
</tbody>
</table>

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