PHYTOCHEMICAL STUDY OF SALVIA GRANDIFLORA AND SALVIA OFFICINALIS LEAVES FOR ESTABLISHING PROSPECTS FOR USE IN MEDICAL AND PHARMACEUTICAL PRACTICE

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1. Introduction
The pharmaceutical market for synthetic drugs is showing rapid growth. At the same time, there remains high level of topicality of herbal medicines research. New drugs based on biologically active substances of plant origin are emerging every year [1, 2]. The market of medicinal herbal raw materials in Ukraine is rapidly developing, as evidenced by the development and increase in the number of enterprises of all units from the cultivation and harvesting of medicinal products to the production of finished products. However, in today's conditions, the market of medicinal plants of Ukraine is dominated by imported raw materials, which exacerbates the issue of import substitution to ensure the medical safety of the country [3, 4].

About 23 % of all pharmacopoeial species of Ukraine have to be imported from abroad for the needs of the domestic pharmaceutical industry. In conditions of import dependence and scarcity of domestic medicinal plant raw materials, the search for new sources of biologically active substances among the representatives of the flora of Ukraine is an urgent task of modern pharmaceutical science [1, 5].

Particular attention is drawn to the study of representatives of the Salvia genus in Ukraine [6, 7]. It is the largest genus in the Lamiaceae family. 21 species
grow in Ukraine [8–10]. The pharmaceutical market of Ukraine presents about 40 drugs, the components of which have biologically active substances (BAS) of Salvia leaves [11, 12].

Previous chemotaxonomic studies of representatives of the Salvia in Ukraine have shown the prospect of using raw materials of S. grandiflora in the pharmaceutical industry [1, 9, 13]. Therefore, it is advisable to conduct a comparative study of the leaves of this species and the galenic drug on its basis in comparison with the pharmacopoeial species - leaves of S. officinalis [14, 15].

The aim of the study was to conduct a comparative phytochemical study of S. grandiflora and S. officinalis leaves to determine the possibility of using non-pharmacopoeial species in pharmaceutical and medical practice.

2. Planning (methodology) of research

To achieve the aim it was necessary to solve the following problems:

– to analyse the macro- and micro-elemental, amino acid composition of S. grandiflora and S. officinalis leaves;

– to study the qualitative composition and quantitative content of the main groups of biologically active substances in the leaf of the studied species;

– to carry out a comparative analysis of the obtained results to determine the possibility of using non-pharmacopoeial species in pharmaceutical and medical practice.

3. Materials and methods

The object of the study was the leaves of S. grandiflora and S. officinalis, which were harvested at the Botanical Garden of Lviv National University named after Ivan Franko, under the guidance of a senior researcher, Ph.D Skibitska M. I. [16, 17].

Macro- and micronutrient composition studies in the leaves of S. officinalis and S. verticillata were carried out by atomic emission spectrography method on a DFS-8 spectrophotograph at State Scientific Institution “Institute for Single Crystals” of NAS of Ukraine. The AC arc was obtained with the help of the IBC-28 generator. The spectra were recorded on PFS-02 photographic plates [18, 19].

The study of the amino acid composition of the plant raw materials of the studied species was performed by HPLC on a chromatograph firm Agilent Technologies (model 1100). For the chromatography we used column AA 200 × 2.1 mm and protective column; as mobile phase – solution A (20 mM sodium acetate and 0.018 % triethylamine, adjusted to pH 7.2 with 1–2 % acetic acid, with the addition of 0.3 % tetrahydrofuran, and solution B (40 % CH3CN, 40 % MeOH and 20 % 100 mM sodium acetate, adjusted to pH 7.2 with 1–2 % acetic acid); volumetric flow rate – 0.45 ml / min; column temperature – 40 °C; detection was carried out with a UV detector after pre-column dewatering first with o-phthalic aldehyde (OPA reagent) and then with 9-fluorenylchlororformate (FMOC reagent) for display of proline [20, 21]. The identification of amino acids was performed by the retention time of the standards of the corresponding amino acids (TC 6-09-3147-83) [18, 22, 23].

Study of the saponin composition of the leaves of the studied species was performed by HPLC on a Shimadzu LC20 Prominence chromatograph in a modular system equipped with a four-channel pump LC20AD, thermostat ST020A columns, SIL20A automatic sampler, Bridge CST2020 diode-matrix detector and SPD-20A detector 150 mm * 4.6 mm with a grain size of 5 microns (Waters); column temperature – 30 °C; detection wavelength – 205 nm; the flow rate of the mobile phase is 1.0 ml / min; the volume of the sample injected is 20 µl. Mobile phase: methanol for HPLC: 0.2 % ammonium acetate solution (pH 6.75) (80:20). Elution mode: isocratic. The identification of the components was carried out by the retention time and the compliance of the UV spectra with the substance standards. The spectra of triterpene saponins have a maximum absorption at (200–210) nm, so detection of this group of compounds was carried out at 205 nm [24, 25].

The study of the composition of phenolic compounds was performed by HPLC on a Shimadzu LC20 Prominence chromatograph in a modular system equipped with a four-channel pump LC20AD, thermostat ST020A column, automatic SIL20A sampler, diode-matrix detector SPD-M20A and ChemStation LC18 and ChemStation LC 250 mm x 4.6 mm, particle size 5 µm; column temperature – 35 °C; detection wavelength – 330 nm (for hydroxycinnamic acids, flavonoid glycosides), 370 nm (for flavonoid aglycones), 280 nm (for tannins), 340 nm (coumarins); the flow rate of the mobile phase is 1 ml / min; sample volume injected – 5 µl; mobile phase: eluent A: 0.1 % trifluoroacetic acid solution in water; eluent B: 0.1 % trifluoroacetic acid solution in acetonitrile. The identification of the components was performed by the retention time and the compliance of the UV spectra with the substance standards [23, 26–28].

The quantitative determination of phenolic compounds was also performed by spectrophotometric method. The optical density was measured on an Evolution 60S spectrophotometer (USA) at the appropriate wavelength. The content of the sum of hydroxycinnamic acid derivatives was determined in terms of rosmarinic acid at 505 nm, the content of flavonoids in terms of luteolin – at a wavelength of 360 nm, the content of the amount of phenolic compounds in terms of per gallic acid – at 270 nm. For statistical accuracy, the experiments were performed at least five times [20, 29, 30].

4. The results of the study

Analysis of macro- and micronutrient composition of leaves of S. officinalis and S. grandiflora. As a result of the analysis, the content of 15 macro- and micronutrients was identified and established (Tab. 1).

Analysis of the content of amino acids in the objects of the study. The results of the study of the amino acid composition of leaves of S. officinalis and S. grandiflora are shown in Tab. 2.

Analysis of the content of saponins in the raw materials of the studied species. The HPLC method identified and quantified the content of 8 saponins in the leaf of the studied species of the Salvia genus (Tab. 3).
Table 1

| Element | Element content, mg / 100 g | Salvia officinalis | Salvia grandiflora |
|---------|-----------------------------|-------------------|-------------------|
| Fe      | 65.7                        | 78                |
| Si      | 330                         | 1040              |
| P       | 145                         | 220               |
| Al      | 32.8                        | 130               |
| Mn      | 4.7                         | 7.8               |
| Mg      | 290                         | 390               |
| Pb      | <0.03                       | 0.13              |
| Ni      | 0.03                        | 0.065             |
| Mo      | 0.04                        | 0.1               |
| Ca      | 730                         | 1300              |
| Cu      | 0.43                        | 0.65              |
| Zn      | 14.6                        | 28.6              |
| Na      | 290                         | 117               |
| K       | 2050                        | 3250              |
| Sr      | 2.9                         | 6.5               |
| Total content | 3956.21                   | 6568.85           |

Table 2

| No. | Amino acid    | Quantitative content, % |
|-----|---------------|-------------------------|
|     |               | S. officinalis | S. grandiflora |
| 1   | Aspartic acid | 0.97 | 1.44 |
| 2   | Threonine     | 0.41 | 0.61 |
| 3   | Serine        | 0.34 | 0.55 |
| 4   | Glutamic acid | 1.08 | 1.84 |
| 5   | Proline       | 0.69 | 0.53 |
| 6   | Glycine       | 0.49 | 0.77 |
| 7   | Alanine       | 0.53 | 0.86 |
| 8   | Valine        | 0.55 | 0.87 |
| 9   | Isoleucine    | 0.42 | 0.66 |
| 10  | Leucine       | 0.67 | 1.16 |
| 11  | Tyrosine      | 0.28 | 0.52 |
| 12  | Phenylalanine | 0.50 | 0.75 |
| 13  | Histidine     | 0.22 | 0.32 |
| 14  | Lysine        | 0.49 | 0.74 |
| 15  | Arginine      | 0.37 | 0.72 |

Table 3

| No. | Compound          | Retention time, min | Quantitative content of mg / g of raw material |
|-----|-------------------|---------------------|---------------------------------------------|
|     |                   |                    | S. officinalis | S. grandiflora |
| 1   | Ursolic acid      | 17.45               | 7.74 | 4.25 |
| 2   | Euscatic acid     | 8.53                | 0.83 | 2.48 |
| 3   | Tormentic acid    | 12.68               | 1.09 | 0.07 |
| 4   | Uvaol             | 22.80               | 0.15 | 1.1 |
| 5   | Oleanolic acid    | 16.34               | 2.46 | 0.8 |
| 6   | Erythrodiol       | 22.59               | 0.11 | 0.03 |
| 7   | Betulin           | 14.57               | 0.26 | 1.1 |
| 8   | Lupeol            | 48.13               | 0.81 | 0.81 |
|     | Total content     |                     | 13.46 | 10.64 |

Content analysis of phenolic compounds in leaves of S. officinalis and S. grandiflora. The HPLC method identified and quantified the content of 14 phenolic substances in leaves of S. officinalis and S. grandiflora (Tab. 4). Among them, 6 are substances of flavonoid nature, 8 are hydroxycinnamic acids. The spectrophotometric method was used to determine the quantitative content of phenolic compounds in the studied objects, including derivatives of hydroxycinnamic acids, flavonoids and the sum of phenolic compounds (Table 5).
Phenolic composition of leaves of *S. officinalis* and *S. grandiflora*

| No. | Compound                  | Retention time, min | Quantitative content of mg / g of raw material |
|-----|--------------------------|---------------------|-----------------------------------------------|
|     |                          |                     | *S. officinalis* | *S. grandiflora* |
|     |                          |                     | Quantitative | Quantitative |
| 1   | Rutin                    | 30.9–31.0           | 1.10          | 0.00          |
| 2   | Apigenin-7-glucoside     | 36.0–36.4           | 0.41          | 0.39          |
| 3   | Luteolin                 | 47.0                | 0.41          | 0.11          |
| 4   | Apigenin                 | 52.3–52.4           | 0.00          | 0.19          |
| 5   | Luteolin-7-glucoside     | 33.1                | 2.75          | 0.21          |
| 6   | Catechin                 | 19.4                | 0.22          | 0.18          |
| 7   | Chlorogenic acid         | 20.0–20.4           | 0.04          | 0.04          |
| 8   | Caffeic acid             | 21.8–22.0           | 0.20          | 0.19          |
| 9   | Rosmarinic acid          | 37.8–38.2           | 1.02          | -4.26         |
| 10  | Lithospermic acid ~      | 38.4                | 0.34          | 0.00          |
| 11  | Salvianolic acid F       | 23.1                | 0.03          | 0.00          |
| 12  | Salvianolic acid C       | 30.1                | 0.03          | 0.00          |
| 13  | Salvianolic acid B       | 47.7                | 0.31          | 0.14          |
| 14  | Salvianolic acid A       | 56.1                | 0.06          | 0.00          |
|     | The total content of salvianolic acids | 0.77          | 0.14          |
|     | The total content of hydroxycinnamic acids | 2.03          | -4.49         |
|     | The total content of flavonoids | 4.90          | 1.08          |
|     | The total content of phenolic compounds | 6.93          | 5.71          |

**5. Discussion of research results**

The obtained data from the study of macro and microelement (tab. 1) indicate a significant content in both types of micro elements such as: silicon (330–1040 mg / 100 g), phosphorus (145–220 mg / 100 g), magnesium (290–390 mg / 100 g), calcium (730–1300 mg / 100 g), sodium (290–117 mg / 100g) and potassium (2050–3250 mg / 100 g). The total micro element content of *S. grandiflora* leaves is 1.67 times greater than that of the *S. officinalis* pharmacopoeial species. The content of toxic elements such as cobalt, cadmium, arsenic and mercury, lead and molybdenum are within the maximum permissible concentrations for raw materials and foodstuffs.

In the *S. officinalis* leaf, 15 amino acids were identified (tab. 2). The dominant ones are glutamic acid, aspartic acid, valine and leucine, with a total content of 43.07 %. Among the identified amino acids 8 are irreplaceable, their content is 47.93 % of the total number of amino acids. In the letter of *S. grandiflora*, 15 amino acids were also identified. Glutamic acid is dominant, aspartic acid, valine and leucine have a total content of 43.66 %. Among the identified amino acids 8 are indispensable. Their content is 48.33 % of the total number of amino acids.

In *S. officinalis* leaves, 8 saponins were identified (tab. 3). Ursolic and oleanolic acids were dominant, with a total content of 75.82 %. In *S. grandiflora’s* leaves, 8 saponins were identified. Ursolic and euscopic acids were dominant, with a total content of 63.25 %.

In the leaf of *S. officinalis*, 13 substances of phenolic nature were identified (tab. 4). Among them, 5 flavonoids: rutin, apigenin-7-glucoside, luteolin, luteolin-7-glucoside, catechin; 3 hydroxycinnamic acids: chlorogenic acid, caffeic acid, rosmarinic acid and 5 derivatives of caffeic acid: lithospermic acid, salvianolic acid F, salvianolic acid C, salvianolic acid B, salvianolic acid A. Routine, apigenin-7-glucoside, luteolin, luteolin-7-glucoside, rosemary, lithospermic and salvianolic B acids were dominant.

In the *S. grandiflora* leaves were identified 9 substances of phenolic nature. Among them were 5 flavonoids: apigenin-7-glucoside, luteolin, apigenin, luteolin-7-glucoside, catechin; 3 hydroxycinnamic acids: chlorogenic acid, caffeic acid, rosmarinic acid; 1 coffee acid derivatives: salvianolic acid B. Apigenin-7-glucoside and rosmarinic acid were dominant.

The total content of flavonoids is highest in leaves of *S. officinalis* and is 4.90 mg / g. The total hydroxycinnamic acid content is highest in the leaves of *S.
grandiflora and is 4.49 mg / g, which is 221.18 % (2.21 times) more than in the pharmacopoeial species of S. officinalis (2.03 mg / g). The overall highest content of coffee acid derivatives is dominated by leaves of S. officinalis (0.77 mg / g).

The highest content of the sum of all detected compounds of phenolic nature is characteristic of leaves of S. officinalis and is 6.93 mg / g.

According to the spectrophotometric study of the content of phenolic compounds (tab. 5) in the leaf of the studied species of the genus Salvia, it was found that the highest content of hydroxyxycinnamic acid derivatives is specific for S. grandiflora leaves, the highest content of flavonoid compounds, and the total content of phenolic compounds is specific for S. officinalis.

The content of phenolic compounds in the two species studied is practically at the same level, except for the hydroxyxycinnamic acid content. In the leaf of the non-pharmacopoeial species S. grandiflora, the content of the amount of hydroxyxycinnamic acids is 2.21 times higher. Particular attention is drawn to the high content of rosemary acid in leaves of S. grandiflora 4.18 times more than in leaves of S. officinalis. The results of qualitative and quantitative analysis of BAS in the leaf of the non-pharmacopoeial species of S. grandiflora indicate the prospect and possibility of its use in medical and pharmaceutical practice as a source of phenolic compounds, in particular hydroxyxycinnamic acids.

**Study limitations.** For the statistical significance of the study, it would be advisable to investigate, even wild samples of raw materials from different regions of Ukraine, and not only cultivated and harvested in the Botanical Garden of the National University of Lviv named after Ivan Franko. It was advisable to compare not only the chemical composition of the raw material, but also to compare the pharmacological activity of galenic and neogalenic agents from these raw materials.

**Prospects for further research.** According to the results of the studies, further screening of pharmacological studies, analysis of terpenoid composition and development of parameters for standardization of S. grandiflora leaves are planned.

**6. Conclusions**

Comparative pharmacognostic and pharmacological studies of S. grandiflora and S. officinalis leaves revealed that S. grandiflora is a promising species for introduction into medical and pharmaceutical practice as a source of phenolic compounds, in particular hydroxyxycinnamic acids.

In both studied species, was found the presence of 15 micro and macronutrients, of which dominant are silicon, phosphorus, magnesium, calcium, sodium and potassium. The total trace element content of S. grandiflora leaves is 1.67 times greater than that of the pharmacopoeial species of S. officinalis. In the leaves of S. officinalis and S. verticillata, 15 amino acids and 8 saponins were identified. HPLC determined the qualitative composition and quantitative content of phenolic substances in leaves of S. officinalis and S. grandiflora (13 and 9 compounds, respectively). The total content of flavonoids is highest in leaves of S. officinalis and is 4.90 mg / g. The total hydroxyxycinnamic acid content is highest in the leaf of S. grandiflora and is 4.49 mg / g, which is 221.18 % (2.21 times) more than in the pharmacopoeial species of S. officinalis (2.03 mg / g). The overall highest content of coffee acid derivatives is found in leaves of S. officinalis (0.77 mg / g). The content of phenolic compounds in the two studied species is practically at the same level, except for the hydroxyxycinnamic acid content. In the leaf of the non-pharmacopoeial species S. grandiflora, the content of the amount of hydroxyxycinic acids is 2.21 times higher. Particular attention is drawn to the high content of rosmarinic acid in leaves of S. grandiflora, which is 4.18 times more than in leaves of S. officinalis.

The results of the comparative phytochemical and pharmacological study of S. officinalis leaves and S. grandiflora leaves significantly extend the data on non-pharmacopoeial species and indicate the undoubted prospect of using S. grandiflora leaves in pharmaceutical and medical practice.

**Conflict of interests**

There are no conflicts of interest regarding this study.

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