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Determination of phenolic compounds from *Stachys sieboldii MIQ*. herb and tubers

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Abstract
This study was undertaken in order to determine the content of phenolic compounds (total polyphenols, tannins and catechins) in *S. sieboldii* herb and tubers. HPLC method was employed to determine the content of catechins. Using spectrophotometric method the content of tannins and total polyphenols was analyzed. The major component of catechins in the herb was epigallocatechin – 1.37 % in the herb; in subterranean organs the predominant components were catechin and epigallocatechin (0.18% and 0.19% respectively). Content of tannins in *S. sieboldii* herb was (1.67± 0.01) %; in tubers- (1.35± 0.05)%. Amount of total polyphenols expressed as pyrogallol showed that in *S. sieboldii* herb their content was (7.93±0.01) % and in subterranean organs − (11.08±0.05) %.

Keywords: total polyphenols, tannins, catechins, *Stachys sieboldii* MIQ, HPLC, spectrophotometry

1. Introduction
Stachys is a large genus consisting of about 300 species distributed in temperate and tropical regions [1, 2].

The genus Stachys biosynthesizes various secondary metabolites including flavonoids, iridoids, fatty acids, phenolic acids, and essential oils, which are associated with anti-inflammatory, cytotoxic, antitoxic, antibacterial, and antioxidant activities [3].

Although the genus Stachys is promoted as containing various biologically active substances, S. sieboldii was examined by relatively few studies, and most studies have focused on stachyose and acteoside [4-6]. Stachys has been extensively used in folk medicine in China. The tuber is used as a food item in Japan. S. sieboldii has been found to be effective against the common cold and heart disease, provides pain relief. The methanolic extract of S. sieboldii tubers containing phenylethanoid glycosides provides anti-inflammatory effect and protects against kidney diseases [7]. Shinichi Harada *et al* have examined antioxidative mechanism of stachys tuber extract on learning and memory dysfunction associated with ischemic brain injury [8].

The content of catechins and tannins in the herb and tubers of Stachys sieboldii MIQ. are limited studied, thus the aim of our research was to determine the composition and content of these compounds in the analyzed objects by HPLC.

2. Materials and Methods
2.1 Plant material
Tubers and herb of Japanese artichoke (*Stachys sieboldii* MIQ.) were collected on research grounds of Educational and Scientific Centre "Institute of Biology and Medicine", Taras Shevchenko National University of Kyiv in November 2016. The tubers and herb were dried using conventional methods and then stored in paper bags in dry place.

All chemical were of analytical grade (> 95% purity). Gallic acid, ellagic acid, catechin, gallocatechin, epigallocatechin, epicatechin, epicatechin gallate and pyrogallol were purchased from Sigma–Aldrich (USA). Quantification was done via a calibration with standards (external standard method). All standards were prepared as stock solutions in methanol. Working standards were made by diluting stock solutions in 60 % aqueous methanol. High performance liquid chromatography (HPLC) method was applied for separation and quantification of catechins. UV-Vis spectroscopy was used for analysis of tannins.

2.2 Determination of tannins and total polyphenols by spectrophotometric method
Carry out all extraction and dilution operations protected from light.

To the 0.5g of the powdered drug in a 250 ml round-bottomed flask add 150 ml of water R. Heat on a water-bath for 30 min. Cool under running water and transfer quantitatively to a 250 mL volumetric flask. Rinse the round-bottomed flask and collect the washings in the volumetric flask, then dilute to 250.0 mL with water R. Allow the solids to settle and filter the liquid through a filter paper 125 mm in diameter. Discard the first 50 mL of the filtrate.

Total polyphenols. Dilute 5.0 ml of the filtrate to 25.0 mL with water R. Mix 2.0 mL of this solution with 1.0 ml of phosphomolybdotungstic reagent R and 10.0 mL of water R and dilute to 25.0 mL with a 290 g/l solution of sodium carbonate R. After 30 min measure the absorbance at 760 nm (A_1), using water R as the compensation liquid.

Polyphenols not adsorbed by hide powder. To 10.0 mL of the filtrate, add 0.10 g of hide powder CRS and shake vigorously for 60 min. Filter and dilute 5.0 mL of the filtrate to 25.0 mL with water R. Mix 2.0 mL of this solution with 1.0 mL of phosphomolybdotungstic reagent R and 10.0 mL of water R and dilute to 25.0 mL with a 290 g/L solution of sodium carbonate R. After 30 min measure the absorbance at 760 nm (A_2), using water R as the compensation liquid.

Standard. Dissolve immediately before use 50.0 mg of pyrogallol R in water R and dilute to 100.0 ml with the same solvent. Dilute 5.0 mL of the solution to 100.0 mL with water R. Mix 2.0 mL of this solution with 1.0 mL of phosphomolybdotungstic reagent R and 10.0 mL of water R and dilute to 25.0 mL with a 290 g/L solution of sodium carbonate R. After 30 min measure the absorbance at 760 nm (A_3) (Fig.1), using water R as the compensation liquid.
Calculate the percentage content of tannins expressed as pyrogallol from the expression 1:

$$X = \frac{62{,}5 \times (A_1 - A_2) \times m_2}{A_3 \times m_1} \quad (1)$$

m_1 - mass of the sample to be examined, in grams,
m_2 - mass of pyrogallol, in grams.
Calculate the percentage content of total polyphenols expressed as pyrogallol from the expression 2:

$$X = \frac{62{,}5 \times A_1 \times m_2}{A_3 \times m_1} \quad (2)$$

m_1 - mass of the sample to be examined, in grams,
m_2 - mass of pyrogallol, in grams [9].

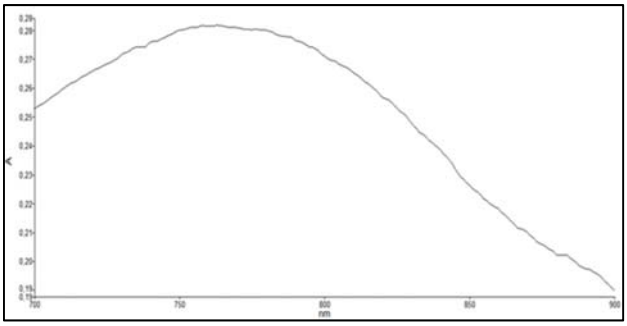

Fig 1: UV-Vis spectrum of pyrogallol standard

2.3 HPLC-analysis of catechins

Sample preparation. 2,00 g (accurately mass) of dried raw material were placed into a 50 mL flat-bottomed flask and extracted with 25 mL of water and the flask was joined to the reflux condenser and heated for 30 minutes with constant stirring. The obtained extracts were cooled and quantitatively transferred into the volumetric flask and the volume was restored to 100 mL with water. Solutions were filtered through a membrane filter with a pore size of 0.45 μm and placed into a vial.
The analytical HPLC system employed consisted of a high performance liquid chromatograph Agilent 1200 3 D LC System Technologies, USA coupled with a four-channel vacuum degasser G1354 A, autosampler G1329A, autosampler thermostat G1330 B, column thermostat G1316A, diode array detector (G1315C) in complex with PC software Agilent ChemStation (G2215 BA). The separation was achieved on a Discovery C 18, 250 mm x 4.6 mm x 5 μm (Supelco, № 505129) column with the precolumn of 20 mm at 25 °C temperature [10]. The mobile phase consisted of 0,1 % solution of trifluoroacetic acid, 5 % acetonitrile solution and water (solvent A) and 0,1 % solution of trifluoroacetic acid with acetonitrile (solvent B) – analysis of tannic substances components.
The flow rate was 0.1 ml/min and the injection volume was 20 μm. The monitoring wavelengths were 255 and 280 nm for catechins. The gradient elution parameters used were as given in the table 1.

Table 1: The gradient elution parameters for tannic substances

Tannic substances components (catechins)								
Time, min	**0**	**8**	**10**	**15**	**20**	**25**	**28**	**29-40**
Solvent B, %	100	12	12	25	25	75	75	100

The identification and quantation of each compound was based on a combination of retention time and peak area [11].

3. Results and Discussion

3.1 Total polyphenols and tannins determination

The determination of tannins was performed by the spectrophotometric method on Lambda 25 UV spectrophotometer (PerkinElmer, USA) with phosphomolybdotungstic reagent and hide powder at a wavelength 760 nm and calculated on pyrogallol. Content of tannins in *S. sieboldii* herb was (1.67± 0.01)% and in tubers - (1.35± 0.05)%.
Results of the total polyphenols determination by spectrophotometric method expressed as pyrogallol showed that in *S. sieboldii* herb their content was (7.93±0.01)% and in subterranean organs − (11.08±0.05)% (Fig.1).

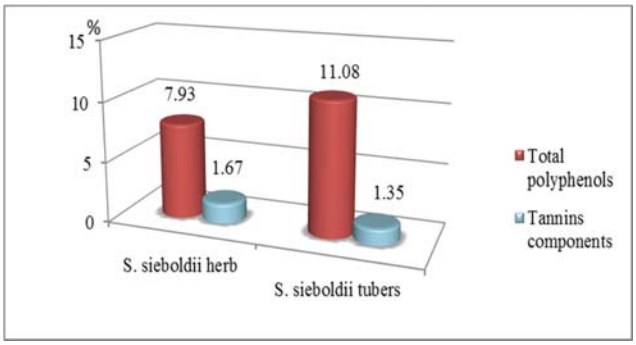

Fig 1: Total polyphenols and tannins components in S. sieboldii

3.2 Catechins determination

Using the aforementioned procedure, the catechins present in *S. sieboldii* herb and tubers were identified and quantified. Discovery C 18 (Supelco) stationary phase, which was used in this study to separate catechins in the above mentioned wavelengths showed satisfactory results (see figure 2 and 3). Table 2 summarizes the concentrations of catechins in the analyzed objects.

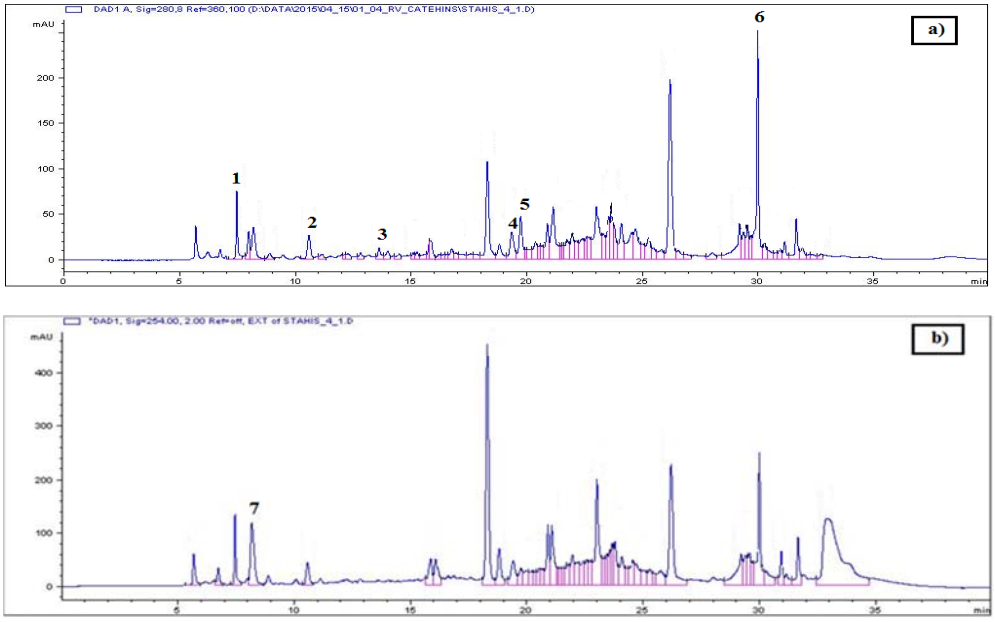

Fig 2: HPLC-chromatograms of catechins from *S. sieboldii* herb *(a - λ = 280 нм:* 1 – gallic acid, 2 – gallocatechin, 3 – catechin, 4 – epigallocatechin, 5 – epicatechin, 6 – epicatechin gallate*; b - λ = 255 нм:* 7 – ellagic acid*).*

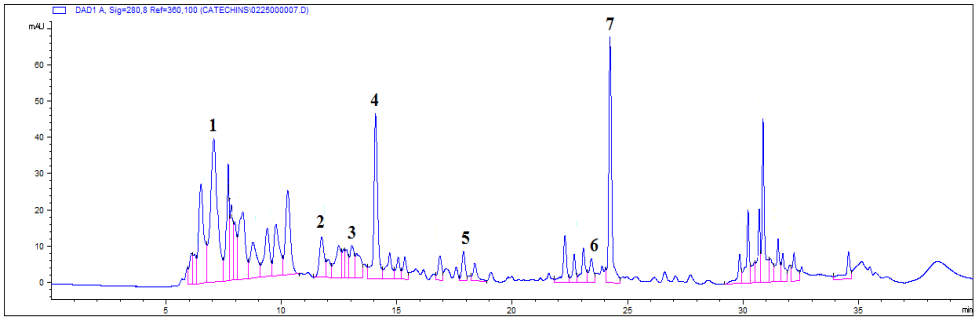

Fig 3: HPLC-chromatograms of catechins from *S. sieboldii* tubers *(λ = 280 нм):* 1 – gallic acid, 2 – gallocatechin, 3 – epigallocatechin, 4 – catechin, 5 – epicatechin, 6 – catehin gallate, 7 – epicatechin gallate.

Table 2: Content of tannic substances components in different parts of *S. sieboldii*

Compound	Content in dry raw material, %	
	***S. sieboldii* herb**	***S. sieboldii* tubers**
Gallic acid	0,03	0,06
Epigallocatechin	1,37	0,19
Gallocatechin	0,49	0,13
Catechin	0,05	0,18
Epicatechin	0,17	0,01
Epicatechin gallate	0,20	0,04
Ellagic acid	0,04	-
Catechin gallate	-	0,004

Using HPLC method we identified and quantified tannic substances components. The highest content was set for epigallocatechin – 1.37 % in the herb; in subterranean organs the predominant components were catechin and epigallocatechin (0.18% and 0.19% respectively) (Table 2).

Catechins possess powerful antioxidant properties. They have been found to possess antibacterial and antiviral as well as anticarcinogenic and antimutagenic properties [12]. Epigallocatechin has been shown to scavenge DPPH radicals with an EC_{50} value of 0.01 mM and to prevent the growth of several different AML cell lines at micromolar concentrations. Furthermore, at 30 µM epigallocatechin can

inhibit heregulin-β1-induced migration/invasion of MCF-7 human breast cancer cells [13].

4. Statistical analysis

The content of total polyphenols and tannins was evaluated in five independent analyses and data were expressed as means ± SD. Values were determined using Statistica v10.0 (StatSoft Inc., USA) program.

5. Conclusion

In this application note an HPLC method for the analysis and quantitation of eight catechins (gallic acid, epigallocatechin, gallocatechin, catechin, epicatechin, epicatechin gallate, ellagic acid and catechin gallate) from *S. sieboldii* was applied. In the herb catechin gallate was not identified and in tubers ellagic acid was not present. The total polyphenols and tannins in the herb and tubers were quantified by a spectrophotometry.

The phenolic profile of yacon leaves was established using HPLC method. Flavonoids, hydroxycinnamic acids, catechins and coumarines were identified and quantified in the analyzed object. The major components were rosmarinic acid (0.97 %), quercetin-3-D-glycoside (0.20%), gallocatechin (1.56 %) and umbelliferone (0.05 %). *P. sonchifolia* leaves represent a promising source of phenolic constituents, which may be used as natural antioxidants or as ingredients of functional foods.

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