

# Influence of development stage on the content of biologically active compounds of *Salvia miltiorrhiza* Bunge roots

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## Summary

Studies on contents of phenolic compounds and diterpenes in *Salvia miltiorrhiza* Bunge root during the vegetation period were carried out. In Polish climate and soil condition, the tanshinone II A occurred as the dominant compound from diterpenes group. Obtained results show that the most profitable period for harvesting *Salvia miltiorrhiza* raw material is fructification stage. However, it is very difficult to decide on the accurate harvest date in this period. After all, taking into account various weather conditions, harvest of the *Salvia miltiorrhiza* Bunge should be done at the end of the vegetation period (in November).

*Key words: Salvia miltiorrhiza, tanshinones, phenolic compounds*

## INTRODUCTION

*Salvia miltiorrhiza* Bunge (*Labiatae=Lamiaceae*) is widely used in Asia for the treatment of various cardiovascular diseases. *Salvia miltiorrhiza* root is a herbal raw material (*Salviae miltiorrhizae radix*). The chemical composition of the root has been extensively studied [1-19]. The major components of raw material are diterpenes with phenanthrenequinone structure (so-called tanshinones) and phenolic compounds. It is also necessary to mention the compounds in form of triterpenes, steroids, coumarins, flavonoids and tannins.

The aim of the study was to investigate the contents of tanshinones and phenolic acids during the vegetation period.

## MATERIAL AND METHODS

The experimental field cultivation was established on the lessivé soil developed from boulder clay of ground moraine. The upper layer was filled with clayey sand, the thickness of humus was 30 cm. Soil reaction (pH) was about 7.0. The contents of mineral components in the arable layer were:  $\text{NH}_4$  – 7.0;  $\text{NO}_3$  – 10.5; P – 172.3, K – 57.7; Ca – 2703; Mg – 192; Cl – 7.8;  $\text{SO}_4$  – 51.7; Na – 18.5 mg/l.

The field cultivation was established by vegetative propagation in autumn 2003 and 2004. The roots of two-year-old plants were collected every two weeks (from June to October) in 2005 and 2006.

Contents of tanshinones and rosmarinic acid were indicated using HPLC method with UV detector. For HPLC analysis 100 mg of samples were extracted with 5 ml methanol for 30 min in ultrasonic bath and filtered through a membrane filter (nominal pore size 0.45  $\mu\text{m}$ ).

HPLC analysis was performed on Agilent 1100 HPLC system equipped with photodiode array detector. For all separation a Lichrospher 100 RP18 column (125 x 4 mm, 5 $\mu\text{m}$ , Merck) was used. The mobile phase consisted of 0.1% trifluoroacetic acid (TFA) in water (A) and acetonitrile (B), applied in different gradient elution (tab.1)

Table 1.

Scheme of gradient elution

| time [min] | 0,1% TFA <sub>aq</sub> [%] | acetonitrile [%] |
|------------|----------------------------|------------------|
| 0.00       | 95.0                       | 5.0              |
| 5.00       | 95.0                       | 5.0              |
| 15.00      | 40.0                       | 60.0             |
| 30.00      | 25.0                       | 75.0             |
| 35.00      | 22.5                       | 77.5             |

The follow rate was adjusted to 0.5 ml/min., the detection wavelength set to DAD at  $\lambda=250.4$  nm, and 20  $\mu\text{L}$  of samples was injected. All separations were performed at the temperature of 25°C. Peaks were assigned by spiking the samples with standard compounds and comparison of the UV-spectra and retention times. The contents of biological active compounds are expressed in percentage value of dry weight.

The research of the contents of sum of phenolic compounds was carried out with the use of colorimetric method [20].

## RESULTS AND DISCUSSION

Significant seasonal changeability of researched compounds of *Salvia miltiorhiza* Bunge root was found.

The diterpenes level varied from 0.01 to 0.30% relevant of plant developmental stage (tab. 2, 3). During vegetative and flowering stage (in June and July) the contents of all tanshinones were low. From August to the end of September, when

the plants were fruiting, the amount of researched substances increased. A lower amount of tanshinones was noticed in October. The last increase of the amount of diterpenes was observed at the end of vegetation period (in November).

Table 2.

Contents of biological active compounds in *Salvia miltiorrhiza* Bunge roots during the vegetation period in 2005 [%]

| time of harvest | tanshinone I | tanshinone IIA | dihydrotanshinone | cryptotanshinone | rosmarinic acid | sum of phenolic compounds |
|-----------------|--------------|----------------|-------------------|------------------|-----------------|---------------------------|
| 02.06.05        | 0.07         | 0.09           | 0.06              | 0.07             | 6.20            | 8.71                      |
| 16.06.05        | 0.02         | 0.03           | 0.02              | 0.05             | 5.22            | 7.21                      |
| 30.06.05        | 0.01         | 0.02           | 0.03              | 0.04             | 3.96            | 6.01                      |
| 14.07.05        | 0.02         | 0.03           | 0.02              | 0.05             | 3.52            | 6.17                      |
| 28.07.05        | 0.04         | 0.07           | 0.06              | 0.06             | 3.25            | 4.45                      |
| 11.08.05        | 0.07         | 0.09           | 0.08              | 0.06             | 3.24            | 6.70                      |
| 25.08.05        | 0.14         | 0.26           | 0.11              | 0.11             | 7.27            | 11.67                     |
| 08.09.05        | 0.01         | 0.13           | 0.10              | 0.11             | 4.85            | 10.19                     |
| 22.09.05        | 0.08         | 0.16           | 0.12              | 0.06             | 3.95            | 7.21                      |
| 06.10.05        | 0.06         | 0.13           | 0.09              | 0.04             | 3.16            | 5.11                      |
| 20.10.05        | 0.04         | 0.05           | 0.04              | 0.06             | 2.55            | 4.73                      |
| 03.11.05        | 0.12         | 0.16           | 0.13              | 0.10             | 2.06            | 4.54                      |
| 17.11.05        | 0.14         | 0.25           | 0.18              | 0.06             | 3.63            | 5.49                      |
| mean            | 0.06         | 0.11           | 0.08              | 0.07             | 4.07            | 6.78                      |

Table 3.

Contents of biological active compounds in *Salvia miltiorrhiza* Bunge roots during the vegetation period in 2006 [%]

| time of harvest | tanshinone I | tanshinone IIA | dihydrotanshinone | cryptotanshinone | rosmarinic acid | sum of phenolic compounds |
|-----------------|--------------|----------------|-------------------|------------------|-----------------|---------------------------|
| 02.06.06        | 0.04         | 0.20           | 0.08              | 0.09             | 3.85            | 11.13                     |
| 14.06.06        | 0.02         | 0.05           | 0.06              | 0.11             | 2.28            | 7.87                      |
| 28.06.06        | 0.04         | 0.09           | 0.09              | 0.13             | 2.54            | 8.91                      |
| 12.07.06        | 0.04         | 0.08           | 0.10              | 0.09             | 2.17            | 5.15                      |
| 26.07.06        | 0.09         | 0.14           | 0.15              | 0.17             | 1.06            | 3.70                      |
| 09.08.06        | 0.06         | 0.21           | 0.22              | 0.12             | 1.33            | 5.50                      |
| 23.08.06        | 0.05         | 0.28           | 0.16              | 0.08             | 2.38            | 7.88                      |
| 06.09.06        | 0.04         | 0.12           | 0.19              | 0.11             | 2.11            | 5.34                      |
| 20.09.06        | 0.05         | 0.30           | 0.16              | 0.07             | 3.37            | 6.77                      |
| 04.10.06        | 0.04         | 0.15           | 0.11              | 0.07             | 4.02            | 7.87                      |
| 20.10.06        | 0.02         | 0.10           | 0.14              | 0.09             | 3.06            | 7.42                      |
| 03.11.06        | 0.02         | 0.07           | 0.06              | 0.05             | 3.25            | 8.48                      |
| 17.11.06        | 0.05         | 0.19           | 0.14              | 0.09             | 3.54            | 8.57                      |
| mean            | 0.04         | 0.15           | 0.13              | 0.10             | 2.69            | 7.28                      |

The content of rosmarinic acid varied from 1.06 to 7.27%, phenolic compounds sum from 3.70 to 11.67 (tab. 2, 3). During a vegetation period, the changeabilities of rosmarinic acid and phenolic compounds sum in *Salvia miltiorrhiza* Bunge were similar. In the first half of June (vegetative stage), the *Salvia miltiorrhiza* Bunge root had quite a large amount of phenolic acids, whereas in July the amount of these substances decreased. Afterwards, the plants started to fructificate and the level of researched

substances became higher again. In September and October, depending on the year of research, the contents of phenolic acids increased or decreased. However, in both years of study, the amounts of those compounds were increasing in November.

Analysing the results of the investigation it can be stated that tanshinone IIA was the dominating substance from diterpenes group. Comparing to the results obtained in 2005, the *Salvia miltiorrhiza* Bunge root harvested in 2006 had a higher amount of tanshinone IIA, dihydrotanshinone, cryptotanshinone and phenolic compounds sum, then little less of tanshinone I and rosmarinic acid. The above-described results differed due to various weather conditions. Lower temperature and sunless weather were observed in 2005, though, the amount of rain was similar to that noticed in 2006.

Obtained results gave the conclusion that the stage of fructification is the most profitable time for harvesting *Salvia miltiorrhiza* roots for healing properties. It is very difficult to make a decision on the accurate harvest date in this period due to various weather conditions in each year of the cultivation. Therefore, the raw material should be harvested at the end of vegetation period (in November). This is also a good time for accumulation of desired substances.

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## WPŁYW FAZY ROZWOJOWEJ NA ZAWARTOŚĆ ZWIĄZKÓW BIOLOGICZNIE CZYNNYCH W KORZENIACH *SALVIA MILTIORRHIZA* BUNGE

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### Streszczenie

Przeprowadzono badania zawartości diterpenów i polifenolokwasów w organach podziemnych *Salvia miltiorrhiza* Bunge. Dominującym związkiem z grupy diterpenów występującym w korzeniach roślin uprawianych w polskich warunkach klimatyczno-glebowych był tanshinon IIA. Otrzymane wyniki wskazują, że najkorzystniejszym okresem zbioru surowca na cele lecznicze jest faza owocowania roślin, jednak ze względów praktycznych surowiec należy zbierać w listopadzie po zakończeniu okresu wegetacji.

Słowa kluczowe: *Salvia miltiorrhiza*, tanshinony, związki fenolowe