Microbiological study of extracts of *Salvia miltiorrhiza* Bunge roots

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S u m m a r y

Roots of *Salvia miltiorrhiza* Bunge contain mainly fractions of water-soluble substances. Most tanshinones are found in ethyloacetate fractions, slightly less in hexane extract. Tanshinones in water extracts have not been found.
Ethyloacetate and hexane extracts from *Salvia miltiorrhiza* Bunge root are characterized by high microbiological activity (MIC range of 10–75 μg/ml using standard strain *Staphylococcus aureus* FDA 209P). The highest activity and maximum amount of cryptotanshinone was found in extracts obtained from roots of Polish origin. The microbiological activity of water fraction is quite low regardless of the source of raw material (MIC range 500-1000 μg/ml).
The results suggest that the microbiological activity of *Salvia miltiorrhiza* Bunge extracts depends on the quality and quantity of tanshinones composition.

Key words: Salvia miltiorrhiza Bunge; water, ethyloacetate and hexane extracts; tanshinones; minimal inhibitory concentration

INTRODUCTION

Salvia miltiorrhiza Bunge is a Chinese pharmacopoeial plant. The roots are used mainly in cardiovascular diseases [1, 2]. *Salvia miltiorrhiza* Bunge products are also noted for their antibacterial activity.

In vitro experiments have proved their inhibiting effect on *Staphylococcus aureus, Escherichia coli, Proteus vulgaris, Shigella flexneri* and *Salmonella typhi.* The same result was noticed using *in vitro* and *semi in vitro* methods in *Leptospira* [3].

Tanshinones, one of the main active substances of *Salvia miltiorrhiza* Bunge, have a very strong effect on *Mycobacterium tuberculosis var. hominis, Sarcina lutea, Bacillus polymyxa, Bacillus subtilis* and *Staphylococcus aureus* (drug-resistant strains) [3-5]. Tanshinones influence the virus of influenza type A increasing infected hen embryos survival from 20% to 80%, which depends on the titre of virus [6].

It has been shown that the flavonoid fraction of described species has also an antibacterial effect on many Gram-positive bacteria (e.g. extract of *Salvia miltiorrhiza* Bunge effects on *Streptococcus mutans* which consequently prevents dental plaque) [7-9].

Extract of raw materials and isolated tanshinones have inhibited effects on some pathogenic fungi. Moreover, *Salvia miltiorrhiza* Bunge inhibits the growth of *Vibrio cholerae* and *Trichomonas vaginalis in vitro* [3, 6].

The purpose of our experiments is to compare the microbiological activity of fractions of different polarity from *Salvia miltiorrhiza* Bunge roots cultivated in Poland with Chinese and Korean raw materials.

MATERIAL AND METHODS

Roots of *Salvia miltiorrhiza* Bunge originated from the cultivation in the Garden of Research Institute of Medicinal Plants were collected in autumn (October). Roots were cut into thick slices and dried in the room temperature. The samples of Asiatic commercial raw material (*Salviae miltiorrhizae radix*) were obtained from the Chinese Academy of Traditional Chinese Medicine (Beijing) and Natural Product Research Institute of Seoul National University.

In the phytochemical investigation there was an extraction conducted. Three fractions of different polarity (water, ethyloacetate and hexane) were obtained and used for each sample of raw material (fig. 1) [10]. Contents of tanshinones were indicated using HPLC method with UV detector elaborated and validated by the Research Institute of Medicinal Plants. Samples of 100 mg for HPLC analysis were extracted with 5 ml methanol for 30 min. in ultrasonic bath and filtered through a membrane filter (nominal pore size 0.45 μm).

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 μm, 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).

The follow rate was adjusted to 0.5 ml/min, the detection wavelength set to DAD at λ=250.4 nm, and 20 μL of samples was injected. All separations were performed at the temperature of 25^{0}C. Peaks were assigned by spiking 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.

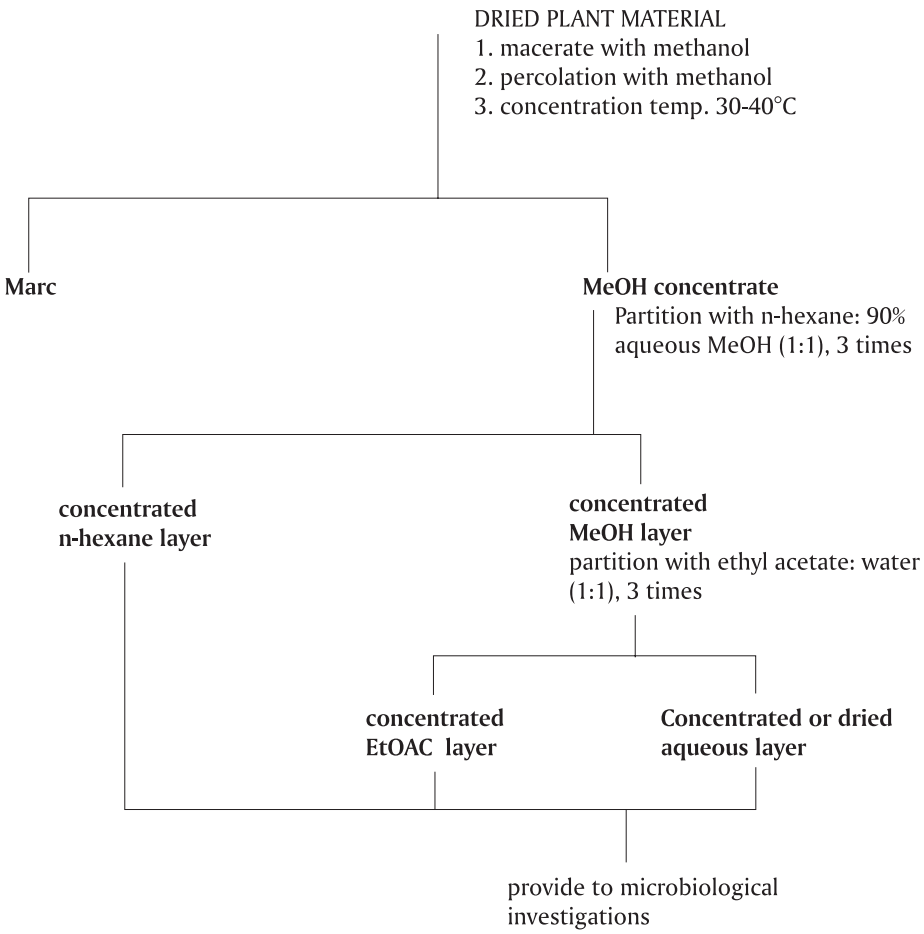

Figure 1 Procedure of plant material extraction

Table 1

Gradient elution used in HPLC analysis

time t [min.]	solvent A [%] acetonitryl	solvent B [%] 0,1 % CF_3COOH_{aq}
0	5.0	95.0
5	5.0	95.0
15	60.0	40.0
30	75.0	25.0
35	77.5	22.5

Microbiological research was carried out with the use of standard strain *Staphylococcus aureus* FDA 209P, sensitive to antibiotics. Dilutions were made in the liquid medium Antibiotic Broth (Merck) in the concentrations from 1 to 1500 μg/ml. Doses of 0.1 ml of 18-hour culture of standard strain containing 10^5 of cells in 1 ml

were added to the prepared dilutions. Incubation was carried out for 18 hours in the temperature of 37^{0}C. Afterwards, MIC (Minimal Inhibitory Concentration) of investigated extracts was determined.

RESULTS

Process production capacity is shown in table 2. As a result of applied extraction procedure, the highest amount of water-soluble substances (5.22-14.8%) was obtained. Productivity of other fractions was lower, from 0.74 to 2.12% for ethyloacetate fraction, and from 0.43 to 0.72% for hexane, respectively. Chinese commercial raw material contained the highest amount of all fractions.

Table 2.

Extraction capacity of particular fraction obtained from *Salviae miltiorrhizae radix*

origin of raw material	amount of raw material used [g]	extraction capacity [%]		
		water fraction	ethyloacetate fraction	hexane fraction
Poland	100	5.22	0.74	0.46
Korea	100	11.3	1.46	0.43
China	25	14.8	2.12	0.72

Content of tanshinones in particular extracts is shown in table 3. The highest amount of these substances was found in ethyloacetate fraction, while little less in hexane. Tanshinones did not occur in water fraction due to its chemical feature (water-insoluble) [11]. In ethyloacetate fraction the highest amounts of tanshinone I, tanshinone IIA and dihydrotanshinone were found in the raw material of Chinese origin. However, the highest level of cryptotanshinone was observed in extract obtained from plants of Polish origin. Among hexane fractions, the highest amount of cryptotanshinone and tanshinone IIA was found in Polish raw material extracts, while tanshinone I and dihydrotanshinone in Korean ones.

Table 3.

Tanshinones content in root *Salvia miltiorrhiza* Bunge extract

origin of raw material	contents of tanshinones [%]											
	water fraction				ethyloacetate fraction				hexane fraction			
	T I	T IIA	K	D	T I	T IIA	K	D	T I	T IIA	K	D
Poland	0	0	0	0	0.79	0.40	1.27	0.94	0.32	1.66	0.22	0.19
Korea	0	0	0	0	0.77	0.64	0.30	0.38	0.50	0.99	0.07	0.41
China	0	0	0	0	1.65	1.26	0.55	1.46	0.08	0.33	0.02	0.12

T I – tanshinone I, T IIA – tanshinone IIA, K – cryptotanshinone, D – dihydrotanshinone

Results of microbiological activity are shown in table 4. Fraction analysis proved that the highest microbiological activity was indicated in ethyloacetate extracts which contained maximum of tanshinones. The earlier research of microbiological

activity of particular tanshinones had shown that MIC for cryptotanshinone was 12.5–50.0 μg/ml and for dihydrotanshinone was 6.3–12.5 μg/ml [4, 5].

Table 4.

Microbiological activity of *Salvia miltiorrhiza* Bunge root extract (with regard to standard strain *Staphylococcus aureus* FDA 209P)

origin of raw material	MIC (μg/ml)		
	water fraction	ethyloacetate fraction	hexane fraction
Poland	1000	10	10
Korea	500	50	75
China	1000	25	50

This investigation has also shown that the highest activity of ethyloacetate and hexane fractions were found in Polish raw material (activity of 10 μg/ml). They contained maximum amount of cryptotanshinone, which is considered to be antibacterial substance, dealing especially with Gram-positive bacteria [5]. Quite high activity was also observed in the Chinese and Korean ethyloacetate and hexane extracts. Their microbiological activity oscillated form 25 to 75 μg/ml. Regardless of raw material origin, water extracts showed quite low microbiological activity (500–1000 μg /ml) which was caused by the tanshinones absence in the extracts. The earlier research of microbiological activity of other extracts had shown that MIC for methanol extract was 800.0 μg/ml [4], and for extract prepared by using the petroleum and chloroform 7.8 μg/ml [6].

The microbiological activity of *Salvia miltiorrhiza* Bunge extracts depends on the quality and quantity of tanshinones composition.

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BADANIA AKTYWNOŚCI MIKROBIOLOGICZNEJ WYCIĄGÓW Z KORZENI *SALVIA MILTIORRHIZA* BUNGE

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S t r e s z c z e n i e

Korzenie *Salvia miltiorrhiza* Bunge zawierają najwięcej frakcji z substancjami rozpuszczalnymi w wodzie. Największe ilości tanszinonów odnotowano we frakcjach etylooctanowych. Nieco mniejszą zawartością tych związków charakteryzowały się wyciągi heksanowe, a w ekstraktach wodnych nie stwierdzono ich obecności.
Wyciągi etylooctanowe i heksanowe z korzeni *Salvia miltiorrhiza* Bunge odznaczały się wysoką aktywnością mikrobiologiczną (MIC w granicach 10–75 μg/ml w odniesieniu do standardowego szczepu *Staphylococcus aureus* FDA 209P), przy czym.najaktywniejsze były frakcje pochodzące z upraw krajowych zawierające najwięcej kryptotanszinonu. Frakcje wodne, niezależnie od pochodzenia, wykazywały stosunkowo niską aktywność mikrobiologiczną (MIC w granicach 500–1000 μg/ml).
Stwierdzono, że aktywność mikrobiologiczna wyciągów z korzeni *Salvia miltiorrhiza* Bunge zależy od składu jakościowego i ilościowego tanszinonów.

Słowa kluczowe: Salvia miltiorrhiza Bunge, *wyciągi wodne, wyciągi etylooctanowe, wyciągi heksanowe, tanszinony, najmniejsze stężenie hamujące (MIC)*