

Extraction of flavonoids from garden (*Salvia officinalis* L.) and glutinous (*Salvia glutinosa* L.) sage by ultrasonic and classical maceration

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Abstract: Flavonoids were analysed in the extracts of garden (*Salvia officinalis* L.) and glutinous (*Salvia glutinosa* L.) sage. Ultrasonic extraction (20 minutes at 40 °C) and classical maceration (6 h at room temperature) of the extractable substances from dried herbs and dried residual plant materials from which the essential oil had previously been removed by hydrodistillation were performed with petroleum ether, 70 % aqueous solution of ethanol and water. It was found that the extracts from both plants contained flavonoids, but their compositions were dependent of the plant species, the polarity of the extracting solvent and the extraction technique applied. Apigenin and its derivatives (e.g., apigenin 4'-methyl ether), scutellarein 6-methyl ether, isoscutellarein 8-methyl ether, luteolin and 6-OH-luteolin-6-methyl ether were distinctive for *S. officinalis*. Apigenin, luteolin, 6-OH-luteolin-6-methyl ether, kaempferol 3-methyl ether, kaempferol 3,7-dimethyl ether, quercetin 3,7,3'-trimethyl ether and quercetin 3,7,3',4'-tetramethyl ether were distinctive for *S. glutinosa*. The flavonoids were also detected in considerable quantities in the plant material from which the essential oils had been already removed. Hence, this industrial waste plant material might be further used as a source of the flavonoids.

Keywords: garden sage, glutinous sage, *Salvia officinalis* L., *Salvia glutinosa* L., flavonoids, ultrasonic extraction, maceration.

INTRODUCTION

Garden sage (*S. officinalis* L.) is a valuable medicinal plant, which is used widely in traditional medicine. This plant species is very rich in biologically active compounds and many studies have indicated their increasing practical importance. The presence of flavonoids in the genus *Salvia* L. has already been confirmed.^{1,2} The therapeutic effects of many traditional drugs are attributed to this group of

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compounds because of their inhibitory effects on certain enzymes and antioxidative activity. They have been shown to possess antibacterial,³ antifungal,⁴ antiviral⁵ and anti-inflammatory⁶ activities. Their antiallergic,³ antioxidative⁷ and antimutagen⁸ activities have also been proven. Reduced risk of breast, prostate and colon cancers is related to isoflavonoid activity.⁹ Flavonoids have been studied in the prevention of menopausal symptoms and osteoporosis.⁹ It was shown that their biological activity depended on the location of the free hydroxyl groups on ring A, more so than that on ring B.^{10,11}

The technique of flavonoid isolation from a plant material, including the type of extracting solvent, depends generally on the type of flavonoid compound and the quantity of plant material. Less polar solvents, such as benzene and chloroform, were used for extraction of flavonoid aglycones, while more polar ones, such as acetone and ethanol, were used to extract flavonoid glycosides.¹² Isoflavonoids were extracted with a mixture of water and methanol,¹³ ethanol¹⁴ or acetonitrile.^{15,16} Ethanol, methanol, acetone and acetonitrile as extracting solvents with and without the addition of hydrochloric acid were also tested.¹⁷ Acetonitrile (60 %) showed a greater extraction efficiency than methanol (80 %).¹⁸ It was found that *S. officinalis* and *S. glutinosa* were rich with monoterpenes and sesquiterpenes, respectively.^{19–22}

Polar phenol acids are the main part of the hydrosoluble components of decoct, and the derivatives of caffeic acid, which are characteristic for sage, are the main part of phenolic acids.¹ Caffeic acid plays the main role in the family Lamiaceae and rosmarinic acid is the main phenolic component responsible for antioxidative activity. Flavones, apigenin and luteolin or their corresponding 6-hydroxy derivatives, are the most abundant components of flavonoids. Methyl ethers of flavones are located in sage leaves or herbal secretion products. Flavonols are mainly methyl ethers of kaempferol and quercetin. Retusine was also identified in *S. glutinosa*.¹

Different extraction techniques, such as hydrodistillation, maceration, Soxhlet extraction, ultrasonic extraction, *etc.*, are widely used for obtaining extractable substances from different parts of a number of plants. As a novel technique, ultrasonic extraction has recently been shown to be very promising and effective for obtaining bioactive substances from sage, ensuring higher yields of the extractable substances at much shorter times (approximately 20 min at 40 °C) than classical maceration (6 hours at 20 °C) but less efficient than Soxhlet extraction (6 h).^{23,24}

In the present work, the composition and yield of flavonoids extracted from herba of garden (*Salvia officinalis* L.) and glutinous (*Salvia glutinosa* L.) sage were studied. Also, the exhausted plant material remaining after hydrodistillation of the essential oil from the herba was used, since it was shown²⁵ that this material could be used as a valuable source of flavonoids. Two different techniques, namely ultrasonic (*i.e.*, ultrasound-assisted) and classical maceration, and three extracting

solvents of different polarity, namely petroleum ether, 70 % aqueous solution of ethanol and water, were applied in this study. The main goals were to estimate the efficiency of ultrasound application in the extraction of flavonoids and to compare the composition of the extracts and yield of flavonoids from different plant materials using extracting solvents of different polarity. In addition, the aim was to estimate the feasibility of using residual plant material from the hydrodistillation of the essential oils as a source of bioactive substances.

EXPERIMENTAL

Plant materials

The dry herba of garden (*Salvia officinalis* L.) and glutinous (*Salvia glutinosa* L.) sage from Serbia, as well as the dry exhausted plant material remaining after hydrodistillation of the essential oils from the herba were used to extract flavonoids. The plant materials were harvested, dried and kept as described elsewhere.²⁴ Before being used, the plant material was comminuted and sieved.²⁴ After hydrodistillation, the residual plant material was dried in a well-aired location in thin layer for 5 days, packed in paper bags and kept in a dark, dry and cool place. Moisture contents, determined by drying at 105 °C to constant mass, were about 12 % for all plant materials.

Extracting solvents

Petroleum ether, ethanol (aqueous solution: 70 vol. %) and distilled water were used as the extracting solvents, as previously.²⁴

Preparation of the samples

The dried plants or the residual plant material from which the essential oil had previously been isolated (10 g) and the extracting solvent were placed in an Erlenmayer flask (250 mL); the ratio of plant material and extracting solvent was 1:10 w/V. A series of flasks were immersed into an ultrasonic cleaning bath (Sonic, Niš, Serbia) operating at 40 kHz frequency and sonicated at 40±1 °C for 20 min, when nearly the maximum concentration of extractable substances in the liquid extracts was achieved.²⁴ Maceration was performed for 6 hours at room temperature.²³ The liquid extract was separated from the plant material by vacuum filtration, the solvent was evaporated under vacuum, and the extract was then dried under vacuum as described elsewhere.²⁴

The dry residues were dissolved in methanol (0.1 % w/V). The obtained extracts were filtered through filter paper (0.45 µm; Sartorius, Germany) and used for TLC and HPLC analyses. The identification of flavonoids was performed using standards, which were obtained from the Institute of Botany (Bulgarian Academy of Science, Sofia, Bulgaria). The standards were dissolved in methanol (0.01 %) before use.

Identification procedure

TLC: Two TLC sorbents and three mobile phase were used for the analysis of the flavonoid exudates. Toluene: dioxan: acetic acid (95:25:4, V/V/V) was used for the development of the exudates on silica gel plates silica gel 60 F₂₅₄ (10x20 cm, 0.2 mm layer). Toluene: 2-butanone: methanol (60:25:15, V/V/V) and toluene: petroleum ether: 2-butanone: methanol (60:30:10:5, V/V/V/V) were used for DC-Alufolien Polyamide 11 F₂₅₄ plates (10x20 cm, 0.15 mm layer). The solution of standards in methanol as well as the sample solutions (20 µL) were put on the start line. Detection with UV-light at 366 nm was performed before and after spraying with "Naturstoffreageenz A", 1 % solution of diphenylboric acid-ethanolamine complex in methanol.

HPLC: The chromatographic analyses were performed on a Hewlett Packard Series 1100 instrument. The separation was performed using a Hypersil ODS RP18, 5 µm, 250x4.6 mm I.D. column, 40 °C, 20 µL sample loop. The mobile phase comprised *t*-butanol, methanol and 20 mmol L⁻¹ potassium

dihydrogen phosphate buffer (adjusted to pH 3.15 with *ortho* phosphoric acid) at a volume ratio 11:37:52. It was filtered through a 0.45 µm filter (Millipore, Ireland) and degassed in an ultrasonic bath before use. The flow rate was 1 mL/min. The chromatograms were recorded at 360 nm selected on the specific UV absorption of the assayed compounds.²⁶ The concentrations of apigenin and luteolin were determined by the calibration curve method using standard solutions of the flavons: 1, 10, 20, 40 and 50 µg/mL and 1, 100, 200, 300 and 400 µg/mL for apigenin and luteolin, respectively.

RESULTS AND DISCUSSION

The flavonoid profiles of the species, as shown by HPLC, are given in Table I. The composition of the extracts depended on the polarity of the extracting solvent. The petroleum ether extracts of *S. officinalis* contained unpolar compounds (flavonoid aglycones), the aqueous ethanolic (70 % V/V) extracts contained polar and unpolar compounds, while the water extracts did not show visible spots of flavonoid aglycones. The petroleum ether extracts of the species *S. glutinosa* contained unpolar compounds in small quantities, while the aqueous ethanolic extracts (70 % V/V) were rich in flavonoid glycosides.

The species *S. officinalis* was featured by apigenin and its derivatives (ap 4'-OMe, scut 6-OMe, isoscut 8-OMe), luteolin and 6-OH-lut-6-OMe. The species *S. glutinosa* was characterized by apigenin, isoscut 8-OMe, luteolin, 6-OH-lut-6-OMe, kae 3-OMe, kae 3,7-diOMe, qu 3,7,3'-triOMe and qu 3,7,3'4'-tetraMe. Caffeic acid was found in both plant species in traces, mainly in the herbal materials. Obviously, the ethanolic extracts (70 %) were the richest in flavonoids. These extracts obtained by maceration of the herbal material contained more apigenin and luteolin in both plant species, compared to the corresponding residual plant material. In case of the residual plant material, the extracts obtained by ultrasonic extraction contained more flavonoids than those obtained by maceration. The petroleum ether extracts of *S. glutinosa* contained much more flavonoids than those of *S. officinalis*. Most of the flavonoids identified in this study were also found earlier in acetone extracts of *S. officinalis* and *S. glutinosa*.²⁵ In addition, several flavon and flavonol aglycones were also identified in both plants, as well as several phenolic, flavon and flavonol glycosides.¹

Interesting results were obtained with respect to the yields of two flavons, namely apigenin and luteolin, with the highest content in the extracts, as can be seen in Table II. The yields of flavons depended on the solvent polarity, the plant species, the type of plant material and the extraction technique applied. The two flavons were extracted only in traces from all plant materials using unpolar petroleum ether as well as from the residual plant materials using water, which indicated that the flavons had been completely isolated from the herba materials by the previous hydrodistillation. Aqueous ethanol solution (70 %) was the most favorable solvent for extracting both flavons from *S. glutinosa* using either ultrasonic or classical maceration. The highest yields of the two flavons from *S. officinalis* were achieved using water or aqueous ethanol (70 %). Higher yields of luteolin were obtained from *S. glutinosa* than from *S. officinalis*, independent of the extraction technique. Ultrasonic extraction was more successful than classical maceration in

TABLE I. Exudated flavonoids of *S. officinalis* and *S. glutinosa*

Components	<i>S. officinalis</i>												<i>S. glutinosa</i>																							
	Petroleum ether						Ethanol (70 %)						Water						Petroleum ether						Ethanol (70 %)						Water					
	H	M	US	RPM	H	M	H	M	US	RPM	H	M	H	M	US	RPM	H	M	H	M	US	RPM	H	M	H	M	US	RPM	H	M	H	M	US	RPM	H	M
Apigenin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Ap 4'-OMe	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Scut 6-OMe	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Isoscut 8-OMe	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Luteolin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
6-OH-Lut-6-OMe	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Kae 3-OMe	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Kae 3,7-diOMe	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Qu 3,7,3'-triOMe	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Qu 3,7,3',4'-tetraOMe	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Caffeic acid	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Plant material: H-herb, and RPM-residual plant material. Extraction technique: US-ultraasonic extraction; and M-maceration. Presence in the extract: (-) - not present and (+) - present.

extracting the flavons from the residual plant materials originating from both plant species using aqueous ethanol (70 %) and from both herba using water. Applying classical maceration, the highest yields of both flavons were obtained from the herba of both plant species using aqueous ethanol solution (70 %).

TABLE II. Yield of the flavons (%) in dry extracts obtained from *S. officinalis* and *S. glutinosa*

Plant species	Flavon	Solvent/Plant material/Extraction technique											
		Petroleum ether				Ethanol (70 %)				Water			
		H		RPM		H		RPM		H		RPM	
		US	M	US	M	US	M	US	M	US	M	US	M
<i>S. officinalis</i>	Apigenin	tr.	tr.	tr.	tr.	0.51	0.63	0.14	0.10	0.38	0.10	nd	nd
	Luteolin	tr.	tr.	tr.	tr.	2.61	4.22	1.31	1.01	8.16	1.91	nd	nd
<i>S. glutinosa</i>	Apigenin	0.03	tr.	tr.	tr.	0.28	0.81	5.32	3.48	0.19	0.17	0.02	0.01
	Luteolin	0,10	tr.	tr.	tr.	4.82	13.75	38.77	16.68	1.36	0.76	0.06	0.02

Plant material: H – herb and RPM – residual plant material. Extraction technique: US – ultrasonic extraction and M – maceration. Presence in the extract: tr. – present in traces and nd – not detected.

CONCLUSIONS

The composition of the extracts and the yield of flavonoides were found to depend on the plant species (*S. officinalis* or *S. glutinosa*), the type of plant material (herba or residual plant material), the polarity of the extracting solvent and the extraction technique (ultrasonic or classical maceration). Both plant species contained two characteristic flavons, namely apigenin and luteolin. *S. officinalis* was richer in flavons, while *S. glutinosa* contained flavonols in a greater amount, independent of the extraction technique. Higher yields of flavonoides were achieved using polar solvents (70 % aqueous solution of ethanol and water) than the non-polar one (petroleum ether), independent of the plant species and the type of plant material. In the case of the polar solvents, higher yields of flavonoides was obtained from the herba than from the residual plant material. The effect of ultrasound on the yield of flavonoids generally depended on the type of plant material. The extracts obtained from the residual plant material using ultrasonic extraction contained more flavonoids than those obtained by maceration. However, the extracts from the herba contained higher amounts of flavonoids if they were obtained by classical maceration.

Hydrodistillation is normally used to isolate essential oils from aromatic and medicinal plants, thereby creating a huge solid waste in the form of residual, exhausted plant material. This residual plant material has recently been shown to contain valuable bioactive substances, such as terpenes²⁴ and flavonoids.²⁵ The present results also show that this "waste" plant material has to be considered as a secondary raw material and a rich source of bioactive substances, with possible technological, economics and ecological justification.

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ИЗВОД

ЕКСТРАКЦИЈА ФЛАВОНОИДА ИЗ ОБИЧНЕ (*Salvia officinalis*) И ЛЕПЉИВЕ (*Salvia glutinosa* L.) ЖАЛФИЈЕ УЛТРАЗВУЧНОМ И КЛАСИЧНОМ МАЦЕРАЦИЈОМ

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Због значајних биолошких ефеката флавоноида (превентивних, антиоксидантних, антибиотских и др.), испитано је њихово присуство у екстрактима обичне (*Salvia officinalis* L.) и лепљиве (*Salvia glutinosa* L.) жалфије. Екстракција суве хербе, као и осушеног резидуалног биљног материјала, из којег је претходно хидродестилацијом изоловано етарско уље, извршена је петролетром, етанолом (70 %) и водом, коришћењем ултразвучне (20 минута на 40 °C) и класичне мацерације (6 сати на собној температури). Независно од екстракционе технике, поларности растварача и врсте биљног материјала, екстракти обе биљне врсте садрже флавоноиде. За врсту *S. officinalis* су карактеристични апигенин и његови деривати (на пример, апигенин-4'-ОМе), скутелареин-6-ОМе, изоскутелареин-8-ОМе, лутеолин и 6-ОН-лутеолин-6-ОМе, док врсту *S. glutinosa* карактерише апигенин, лутеолин, 6-ОН-лутеолин-6-ОН, кемферол-3-ОМе, кверцетин-3,7-диОМе, кверцетин-3,7,3'-триОМе и кверцетин-3,7,3',4'-тетраОМе. Присуство флавоноида је доказано у резидуалном биљном материјалу из којег је претходно издвојено етарско уље. Овај резултат упућује на могућност коришћења овог отпадног материјала у преради жалфије, као секундарне сировине за добијање флавоноида.

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