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The Influence of Microwave-Assisted Extraction on the Isolation of Sage (*Salvia officinalis* L.) Polyphenols

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Summary

In order to determine the optimal conditions for the extraction of sage polyphenols, the influence of extraction solvent (water, 30 % (by volume) aqueous ethanol and 30 % (by volume) aqueous acetone), extraction time (3, 5, 7, 9 and 11 min) and microwave power (500, 600 and 700 W) on the composition and concentration of phenolic compounds of dry wild sage (*Salvia officinalis* L.) during microwave-assisted extraction (MAE) was studied. Optimized MAE method was compared with conventional extraction (CE). Based on the amount of total phenols, microwave power of 500 W and extraction time of 9 min were selected as optimal extraction conditions, resulting in higher content of polyphenols when compared with CE. Ethanol and acetone solutions were equally effective extraction solvents, both producing higher extraction capacity than water. Using HPLC coupled with UV/PDA, fourteen polyphenols were identified (caffeic and rosmarinic acid derivatives, luteolin- and apigenin-glycosides) with rosmarinic acid and luteolin glycosides at the highest concentrations. The mass fractions of all individual polyphenols were higher in the MAE extracts than in the CE ones.

Key words: sage, phenolic compounds, conventional solvent extraction, microwave-assisted extraction

Introduction

Sage (*Salvia officinalis* L.) is an aromatic herb belonging to the Lamiaceae family, and was previously considered mainly for its essential oil content. However, in the past few decades sage has been the subject of an intensive study for its phenolic content, especially due to strong antioxidant and antimicrobial properties of its extracts (*1–3*). Sage polyphenols comprise a great diversity of structures, ranging from rather simple molecules to complex polymers among which flavonoids and phenolic acids are usually distinguished. The most abundant polyphenols in sage are phenolic acids, mainly caffeic acid derivatives. Caffeic acid dimmer, rosmarinic acid is present at the highest level and is reported to be responsible for high antioxidant activity of sage (*4*). The majority of flavonoids in sage are flavones of apigenin and luteolin and their derivatives with glycosides present at the highest concentration. Compared to apigenin, luteolin glycosides appear to be more common in sage, with luteolin-3'-glucuronide being the most abundant (*5*).

Considering the diversity of the structures of polyphenols present in sage, it is of great importance to select the optimal method for their simultaneous determination. Extraction is the critical step in the isolation of biologically active compounds from plant material. Conventional extraction (CE) methods are usually based on selecting the proper solvent and using heat in order to increase the solubility of targeted compounds. The polarity of the solvent plays an important role in the extraction of phenolic compounds (*6*). Polyphenols range from

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polar to non-polar ones, thus a wide range of solvents has been used for their extraction. They are extracted from the matrix using organic solvents such as water, ethanol/water and acetone/water mixtures (*7*,*8*). These methods often demand longer extraction time, larger amount of solvent, and carry the risk of thermal degradation of biologically active compounds. Because of the above-mentioned drawbacks, new methods of extraction have been used such as ultrasound-assisted extraction, supercritical carbon dioxide extraction and microwave-assisted extraction (MAE).

Microwave-assisted processes are among several dominant trends of the 'green chemistry', applying the accelerated microwave irradiation as an alternative reaction condition in chemical processes. The presence of microwaves greatly improves the rate of many chemical processes with strong reduction of the reaction time, through which dual goals, environmental protection and economic benefit, can be achieved. Microwave-assisted extraction is a process that uses microwave energy to heat the solvent in contact with the sample in order to partition the analytes from the sample matrix into the solvent (*9*). Because of a different heating mechanism than the one in conventional extraction, MAE can result in a yield increase in shorter time at the same temperature using less solvent. The principle of heating using microwave energy is based on its direct impact on polar solvents and is governed by two mechanisms: ionic conduction and dipole rotation (*10*). Dipole rotation means realignment of the dipoles with the applied field, while ionic conduction occurs in a solution containing ions when electromagnetic field is applied (*11*). In order to heat rapidly under microwave radiation, the solvent must have high both dielectric constant and dissipation factor, *i.e.* the ability to absorb microwave energy and pass it on as heat to the surrounding molecules (*12*). Dissipation factor is given by the equation (*13*):

$$\tan \delta = \varepsilon'' / \varepsilon' \qquad /1/$$

where ε'' is the dielectric loss, indicating the efficiency with which electromagnetic radiation is converted into heat, and ε' is the dielectric constant describing the ability of molecules to be polarized by the electric field. Consequently, polar solvents and ionic solutions (acids) will strongly absorb microwave energy because of their permanent dipole moment, while non-polar solvents will not heat up when exposed to microwaves. The efficiency of the MAE depends on the used solvent, duration of the process, applied temperature and microwave power. The proper selection of these parameters is strongly dependent on the properties of the sample and target compounds.

In the past few years, there has been a growing trend to find new extraction methods based on the principles of green chemistry. Many reports have been published on the application of MAE of secondary plant metabolites (*14*–*18*). Considering the lack of published reports about the application of MAE for the isolation of sage polyphenols, the aim of this research is to study the influence of extraction solvent, time and microwave power on the composition and concentration of phenolic compounds during MAE in order to determine optimal conditions for the extraction of sage polyphenols. The optimized MAE method was compared with CE of wild sage polyphenols.

Materials and Methods

Chemicals and standards

Ethanol and acetone used for the extraction were of analytical grade, purchased from Gram-Mol (Zagreb, Croatia). Anhydrous sodium carbonate was obtained from Kemika (Zagreb, Croatia) and the Folin-Ciocalteu reagent from Merck (Darmstadt, Germany). Solvents used in high-performance liquid chromatography (ethanol, acetonitrile, formic acid) were of HPLC grade and purchased from Sigma-Aldrich (Steinheim, Germany).

All phenolic acid (rosmarinic, caffeic, syringic acids) and flavonoid (luteolin-3'-glucoside and apigenin-7-glucoside) standards were purchased from Sigma-Aldrich. All standards were prepared as stock solutions in 30 % (by volume) aqueous ethanol at concentrations (in mg/mL): rosmarinic acid 0.5, caffeic and syringic acids 2, apigenin-7-glucoside and luteolin-3'-glucoside 1. Working standard solutions were prepared by diluting stock standards in 30 % aqueous ethanol to yield concentrations ranging between 0.042 and 0.167 mg/mL for phenolic acids and apigenin-7-glucoside. Concentration range for luteolin-3'-glucoside was between 0.083 and 0.333 mg/mL.

Plant material

Wild sage (*Salvia officinalis* L.) was collected in its natural habitat, in Pučišća, on the island of Brač in Croatia during August 2008. Plant material was identified and confirmed in the Laboratory for Biology and Microbial Genetics at the Faculty of Food Technology and Biotechnology, University of Zagreb. The sage leaves were separated from the wooden stem and dried immediately after harvesting in a shady and well-aired place for two weeks. Then, the dry plant material was packed in paper bags and kept in a dark and dry place. Before analysis dry sage was homogenized in a house blender (Mixy, Zepter International, Milan, Italy).

Extraction of phenolics

Conventional extraction

The phenolic compounds in dry wild sage were extracted according to the previous laboratory research of the influence of extraction solvent (distilled water, 30, 50 and 70 % aqueous ethanol and acetone solutions), temperature (from 60 to 90 °C) and extraction time (30, 60 and 90 min) on the composition and concentration of wild sage phenolic compounds (results not shown). The highest mass fraction of total phenols was obtained under the following extraction conditions: extraction temperature of 80 °C and extraction time of 30 min. Using 30 % aqueous solution of ethanol and acetone, similar mass fractions were obtained and therefore both extraction solvents were chosen as efficient for the extraction of sage phenols. Although distilled water was not found to be effective solvent for conventional extraction, it was used in this research along with 30 % aqueous ethanol and acetone solutions, in order to investigate its influence on the microwave-assisted extraction and compare it to the

one in the conventional extraction. The extraction of polyphenols was performed as follows: (1±0.001) g of crushed dried sage leaves was mixed with 45 mL of appropriate solvent in 100-mL Erlenmeyer flask and refluxed in water bath at 80 °C for 30 min. The obtained extracts were cooled, filtered through Whatman no. 40 filter paper (Whatman International Ltd., Kent, UK), placed in volumetric flasks and made up to 50 mL with an appropriate solvent. All extracts were stored at –18 °C in inert gas atmosphere until analysis.

Microwave-assisted extraction

For MAE a single-mode focused microwave reactor (Milestone, Start S Microwave Labstation for Synthesis, Bergamo, Italy) operating at 2450 MHz with adjustable microwave power output was used. General extraction parameters were: time required to achieve extraction temperature 2 min, stirring 50 %, ventilation after extraction 1 min. Samples ((1±0.001) g) were mixed with the same solvents (45 mL) used in the conventional extraction in round-bottom flasks.

Extraction was carried out at constant temperature of 80 °C using three different solvents (distilled water, 30 % aqueous ethanol, and 30 % aqueous acetone) and various microwave power levels (500, 600 and 700 W) and extraction time (3, 5, 7, 9 and 11 min). Afterwards, the extracts were cooled, filtered through Whatman no. 40 filter paper (Whatman International Ltd.), transferred in 50-mL volumetric flasks made up with an appropriate solvent, and stored at –18 °C in inert gas atmosphere until analysis.

All extracts obtained using CE and MAE were prepared in duplicate and were used for both spectrophotometric determination of total phenols and determination of individual phenols using HPLC.

Total phenolic content determination

Total phenolic (TP) content of the extracts was determined by Folin-Ciocalteu method, according to the procedure of Ough and Amerine (*19*) with some modifications. The aliquots (250 μL) of each extract were added to 25-mL volumetric flasks followed by the addition of 15 mL of distilled water, 1.25 mL of Folin-Ciocalteu reagent and 3.75 mL of saturated sodium carbonate, and were made up to 25 mL with the appropriate solvent used for the extraction. The absorbance was measured at 765 nm after tempering in water bath at 50 °C for 30 min. All determinations were carried out in duplicate. The TP content was calculated according to the rosmarinic acid standard calibration curve (y=0.0011x, R^2=0.991) and expressed as mg of rosmarinic acid equivalents (RAE) per g of dry sage±standard deviation (S.D.).

HPLC analysis

Separation of phenolics was performed by HPLC, using a Varian ProStar System (Varian Inc., Palo Alto, CA, USA) equipped with a ProStar Solvent Delivery Module 230, Injector Rheodyne 7125, ProStar 330 UV/VIS photo diode array detector. Chromatographic separation was performed on a Zorbax ODS C18 column (250×4.6 mm i.d., 5 μm particle size) including Zorbax ODS C18 guard column (10×4.6 mm i.d., 5 μm particle size) (Agilent Technologies, Palo Alto, CA, USA). The solvent composition and used gradient conditions had been described previously by Fecka and Turek (*20*) with modification considering the amount of formic acid in mobile phases. For gradient elution, instead of 5 % formic acid, mobile phase A contained 3 % formic acid in acetonitrile (by volume) and mobile phase B contained 3 % formic acid in water (by volume). The following gradient was used: 0–25 min, from 10 % A, 90 % B to 40 % A, 60 % B; 25–30 min, from 40 % A, 60 % B to 70 % A, 30 % B; 30–35 min, from 70 % A, 30 % B to 10 % A, 90 % B. Operating conditions were: constant flow rate 0.9 mL/min, column temperature 20 °C, injection volume 20 μL, UV-photo diode array detection at 278 and 340 nm.

Identification of phenolic compounds was carried out by comparing retention times (t_R) and spectral data of separated peaks with those of authentic standards. Quantification was done using calibration curves of standards (caffeic acid: y=430358x, R^2=0.980; rosmarinic acid: y=204324x, R^2=0.981; syringic acid: y=634804x, R^2=0.978; luteolin-3'-glucoside: y=164772x, R^2=0.998; apigenin-7--glucoside: y=300345x, R^2=0.995). For those compounds lacking reference standards, identification was done according to the t_R, polarity and characteristic spectra of sage polyphenols as reported previously (*5,20,21*). Vanillic acid was quantified using syringic acid calibration curve, sagerinic acid and salvianolic acid K and I using rosmarinic acid calibration curve, luteolin glycosides were quantified as luteolin-3'-glucoside and apigenin-7-glucuronide as apigenin-7-glucoside. All determinations were done in duplicate and the results were expressed as mean values±standard deviations (S.D.).

Statistical analysis

The data were analysed using STATISTICA v. 9 (StatSoft Inc, Tulsa, OK, USA). The results of the total phenolic determination were analysed by two-way analysis of variance (ANOVA) with the extraction time and the type of solvent at the set microwave power being the two parameters and the mass fraction of total phenols being the response variable. Also, the results of the HPLC analysis were statistically analyzed by two-way ANOVA with the extraction type and solvent being the two parameters and the mass fraction of phenolic compounds (phenolic acids and flavonoids) being the response variable.

Results and Discussion

Total phenolic content

The TP content of sage extracts obtained with CE and MAE with three different solvents (distilled water, 30 % (by volume) aqueous ethanol and 30 % (by volume) aqueous acetone) was determined by the Folin--Ciocalteu method and the results are shown in Table 1.

CE of sage phenols was carried out at 80 °C for 30 min using the 30 % aqueous ethanol, 30 % aqueous acetone and distilled water as extraction solvents. The highest extraction yield was obtained using 30 % aqueous acetone as extraction solvent ((46.5±0.6) mg of RAE per g), followed by 30 % aqueous ethanol ((41.2±0.3) mg of RAE per g), while water extracts had the lowest amount of total phenols ((33.5±0.5) mg of RAE per g).

Table 1. Total phenols[a] in sage extracts obtained by conventional extraction (CE) and microwave-assisted extraction (MAE) with three different solvents

Extraction		Extraction solvents		
		30 % ethanol	30 % acetone	water
		RAE/(mg/g)±S.D.		
CE		41.2±0.3	46.5±0.6	33.5±0.5
MAE				
Power/W	Time/min			
500	3	35.2±0.3	36.6±1.3	23.5±1.0
	5	39.7±0.1	41.7±0.9	27.7±0.8
	7	38.0±1.3	39.1±1.3	28.5±0.6
	9	47.0±0.3	52.1±1.0	36.7±0.1
	11	45.9±0.2	47.6±0.6	24.1±0.7
600	3	43.1±0.3	46.6±0.8	35.1±0.1
	5	44.7±0.3	48.4±1.0	31.7±0.3
	7	42.1±0.6	43.2±0.9	34.6±0.5
	9	40.8±0.5	39.3±0.6	32.2±0.4
	11	38.1±0.8	37.1±1.1	30.8±0.5
700	3	43.8±1.0	42.0±0.9	25.1±0.1
	5	44.1±0.9	45.0±0.5	31.6±0.2
	7	39.1±0.6	38.5±0.6	33.3±0.5
	9	35.3±0.3	37.1±0.6	34.1±0.2
	11	31.7±0.3	32.5±0.8	33.5±0.2

[a]mean of duplicate analyses of each extracted duplicate (N=4) Results are given in mg of rosmarinic acid equivalent (RAE) per gram of dry sage±standard deviation (S.D.)

Constant temperature of 80 °C, used in MAE, was selected upon previous research showing an increase in total phenolic content when applying higher temperature. Generally, higher temperature increases desorption of the analyte from plant matrix, but can also lead to degradation if not selected properly. According to Huang and Zhang (*16*) the optimal temperature for MAE of quercetin from guava is 100 °C. Also, Inglett *et al.* (*22*) reported the increase in phenolic content of buckwheat when applying temperatures higher than 50 °C.

First, a lower microwave power of 500 W was applied at constant temperature of 80 °C for the extraction of sage polyphenols. The amount of total phenols increased with longer time of extraction, giving the highest yield at 9 min and decreasing at 11 min with all extraction solvents. The highest mass fraction of TP was obtained using 30 % aqueous acetone as extraction solvent ((52.1± 1.0) mg of RAE per g), followed by 30 % aqueous ethanol ((47.0±0.3) mg of RAE per g), while water extracts had the lowest mass fraction of TP ((36.7±0.1) mg of RAE per g). These results are in accordance with microwave theory that microwave-transparent solvents are more efficient than microwave-absorbing ones. Water has the highest dielectric constant of all common solvents but has lower dissipation factor, meaning that it absorbs microwave energy at higher rate than the system dissipates the heat. Acetone and ethanol have high both dielectric constant and dissipation factor and, therefore, are more efficient solvents for MAE (*9*). This can be one of the reasons why both ethanol and acetone have higher extraction capacity than water.

Higher microwave power of 600 W enhanced the extraction of polyphenols, giving high extraction yield in short time so the mass fraction of TP was the highest after 5 min when using 30 % ethanol and acetone extracts ((44.7±0.3) and (48.4±1.0) mg of RAE per g), while in water extracts the highest extraction yield of (35.1± 0.1) mg of RAE per g was obtained after 3 min. When applying longer extraction time, the extraction yield decreased with all solvents. Statistical analysis showed a significant influence ($p<0.05$) of both extraction solvent and time on the amount of TP at microwave power of 500 and 600 W (Table 2).

Table 2. Influence of extraction time (3, 5, 7, 9 and 11 min) and type of solvent (30 % aqueous ethanol, 30 % aqueous acetone and distilled water) used in MAE at different values of microwave power on the amount of total phenols

Microwave power/W	Source of variation	F_{exp}	p-value
500	time	9.03	0.00462[a]
	solvent	39.32	0.00007[a]
600	time	4.61	0.03179[a]
	solvent	30.82	0.00017[a]
700	time	1.09	0.42200
	solvent	4.27	0.05470

[a]significant differences obtained at $p\leq0.05$

At microwave power of 700 W, the amount of TP in ethanol and acetone extracts was almost similar to the one at 600 W. The highest mass fractions were obtained after 5 min of extraction, (44.1±0.9) mg of RAE per g in 30 % ethanol and (45.1±0.5) mg of RAE per g in 30 % acetone extracts. At longer extraction time, the loss of polyphenols was greater when compared to the results at 600 W and especially those at 500 W. Only in water extracts, there was an increase in TP amount with longer extraction time, so the highest mass fraction was obtained after 9 min of extraction. At this microwave power (700 W), statistical analysis showed no significant influence ($p>0.05$) of the used solvent and extraction time on the amount of TP (Table 2).

Other authors have also done research on the influence of microwave power and time on MAE, reporting the microwave power and extraction time as two factors that influence each other to a great extent. There are two approaches: low or moderate power with long exposure and high power with short exposure, but the former is considered to be a wiser choice since high microwave power can reduce purity of the extract (*12*). On the other hand, extraction time varies depending of the extracted analyte, plant material and extraction solvent. Using polarsolvents with high dielectric constant (water, ethanol, methanol and acetone) during longer extraction time can result in degradation of the analyte, as microwave radiation causes rapid temperature increase of these solvents (*14*,*15*,*23*). At lower microwave power (500 W), the highest extraction yield was obtained after 9 min of ex-

traction, while at higher power of 600 and 700 W, similar concentrations were obtained after 5 min, although slightly lower than those at 500 W after 9 min.

Generally, microwave power higher than 500 W did not increase the amount of TP. Therefore, because of the energy savings and high extraction yield, microwave power of 500 W was selected as optimal for MAE. The obtained results are in accordance with the findings of other authors. Latha (*17*) reported that the amount of extracted embelin from *Embelia ribes* did not change significantly when applying microwave power of 300 and 450 W, but higher power of 450 W reduced the purity of the extract.

At microwave power of 500 W, the highest mass fraction of TP was obtained after 9 min, so the selected optimal extraction parameters for the isolation of phenols from dry sage leaves at constant temperature of 80 °C were: microwave power 500 W and extraction time 9 min.

Under all applied extraction conditions (microwave power, time), the influence of extraction solvent was the same, *i.e.* the highest fraction of TP was obtained in 30 % aqueous acetone, followed by 30 % aqueous ethanol and then water. Using aqueous ethanol and acetone as extraction solvents resulted in almost similar extraction yield under all conditions except the ones selected as optimal, when the TP amount was (52.1±1.0) mg of RAE per g in acetone and (47.0±0.3) mg of RAE per g in ethanol extracts.

When comparing the amount of TP in the extracts obtained by MAE under optimal conditions with those obtained by CE, the CE extracts had lower amount of phenolic compounds than MAE extracts. This is in accordance with previous reports that MAE results in higher extraction yield when compared to CE. Proestos and Komaitis (*24*) reported that the amounts of TP in aromatic herbs (*Styrax officinalis*, *Origanum dictamnus*, *Rosmarinus officinalis*, *Origanum majorana*, *Teucrium polium* and *Vitex agnus-cactus*) obtained by MAE in 4 min were comparable to those obtained by CE in 2 h.

Generally, using the MAE at 80 °C and microwave power 500 W for 9 min, the amounts of extracted TP were higher than those obtained by CE at 80 °C for 30 min. Microwaves enhanced the extraction of sage polyphenols, resulting in higher extraction yield after 9 min than the one using the CE for 30 min.

Individual polyphenolic content

MAE extracts obtained under optimal conditions (80 °C, 500 W, 9 min) using three different solvents (30 % aqueous ethanol, 30 % aqueous acetone and distilled water) and CE extracts were analysed using HPLC analysis with UV/VIS PDA detection in order to determine the influence of microwaves on the content of individual phenols. HPLC chromatograms of sage extracts obtained by MAE under optimal conditions with three different solvents are shown in Fig. 1. Seven phenolic acids (vanillic, caffeic, syringic, sagerinic, rosmarinic, salvianolic K

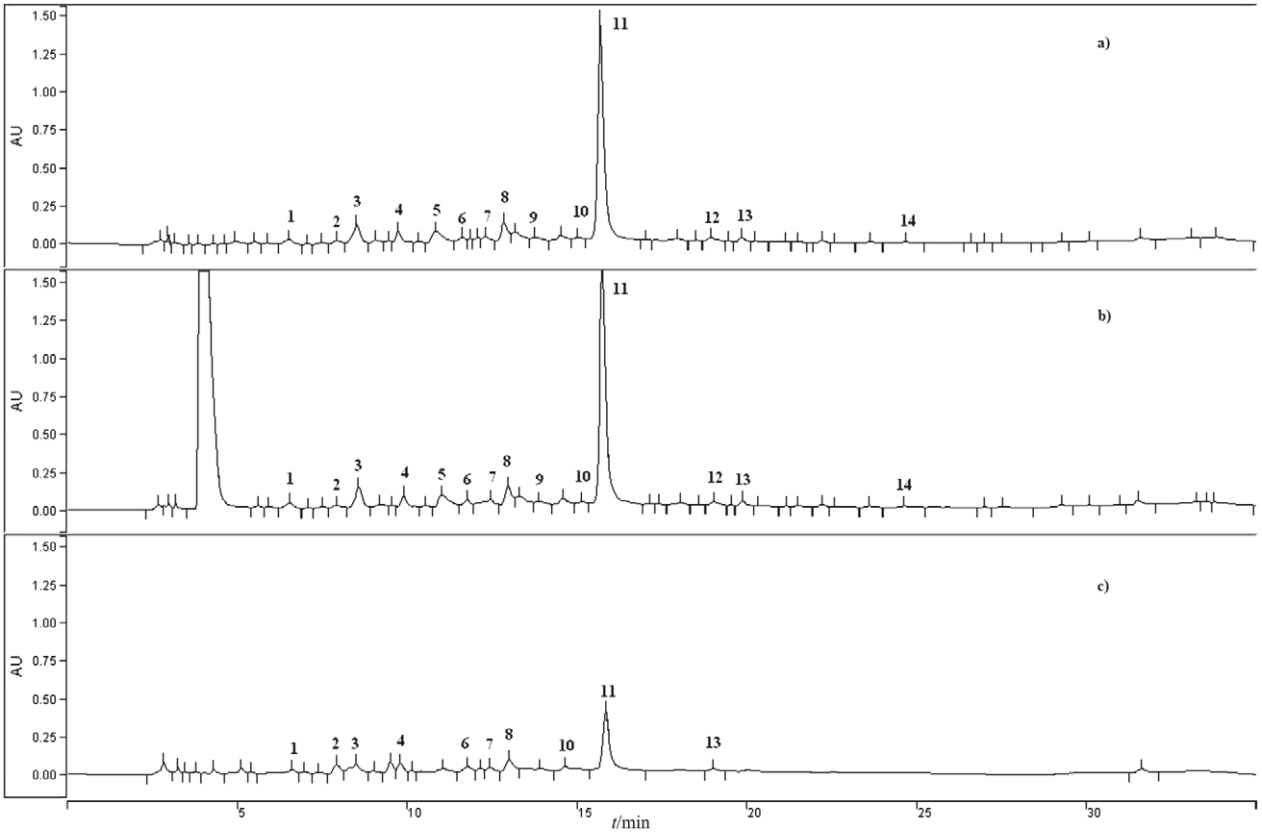

Fig. 1. HPLC chromatogram (wavelength 280 nm) of sage polyphenols isolated by MAE using three different solvents: a) 30 % aqueous ethanol, b) 30 % aqueous acetone, c) distilled water. Peaks: 1. vanillic acid, 2. caffeic acid, 3. syringic acid, 4. 6-hydroxyluteolin-7-glucoside, 5. luteolin-7-glucuronide, 6. sagerinic acid, 7. luteolin-7-glucoside, 8. luteolin-3'-glucuronide, 9. apigenin-7-glucuronide, 10. apigenin-7-glucoside, 11. rosmarinic acid, 12. salvianolic acid K, 13. salvianolic acid I, 14. methyl rosmarinate

and salvianolic I), methyl rosmarinate and six flavone glycosides (6-hydroxyluteolin-7-glucoside, luteolin-7-glucuronide, luteolin-7-glucoside, luteolin-3'-glucuronide, apigenin-7-glucuronide and apigenin-7-glucoside) were identified using retention times and spectra of authentic standards.

Quantification results of phenolic acids and flavonoids obtained by CE and MAE using three different solvents (30 % aqueous ethanol, 30 % aqueous acetone and distilled water) are shown in Table 3. The results of quantification are shown individually for rosmarinic acid as it was the most abundant compound in sage extracts, while the results for other identified phenolic acids (vanillic, caffeic, syringic, sagerinic, salvianolic K and salvianolic I) are shown as the sum because they were present in very low mass fractions. Methyl rosmarinate was present only in traces in all extracts and therefore was not quantified. Also, the results of quantification of flavonoids are expressed individually for 6-hydroxyluteolin-7--glucoside and luteolin-3'-glucuronide, while other identified flavonoids (luteolin-7-glucuronide, luteolin-7-glucoside, apigenin-7-glucuronide and apigenin-7-glucoside) are shown as a sum due to their low mass fractions.

In the extracts obtained by CE mass fraction of rosmarinic acid was the highest in 30 % aqueous acetone extract ((14.6±0.3) mg/g), followed by 30 % aqueous ethanol ((13.4±0.3) mg/g) and the lowest in the water extract ((0.6±0.0) mg/g). Mass fraction of luteolin-3'-glucuronide was almost similar in ethanol and acetone extracts ((2.4±0.1) to (2.6±0.1) mg/g), while in the water extract it was lower ((1.2±0.1) mg/g). All phenolic compounds were in significantly lower concentrations in water extracts and some of them were present only in traces (caffeic, syringic, sagerinic acid, luteolin-7-glucoside and glucuronide, apigenin-7-glucuronide and glucoside) and therefore could not be quantified.

In the extracts obtained by MAE under optimal conditions, mass fractions of all identified phenolic compounds were higher than in the extracts obtained with CE. Rosmarinic acid level was the highest in acetone extract ((35.0±0.2) mg/g), followed by ethanol ((32.3±0.6) mg/g), while it was the lowest in the water extract ((7.1± 0.2) mg/g). Luteolin glycosides were determined in almost the same amounts in both acetone and ethanol extracts, and in the lowest amount in water extracts. In water extracts, mass fractions of all phenolic compounds were low when compared to ethanol and acetone extracts, although microwaves enhanced the extraction, so the mass fractions of all identified phenols were higher in MAE than in CE extracts. Some compounds that were not identified in water extracts obtained by CE were determined in small amounts in MAE extracts (caffeic, syringic, sagerinic acid, luteolin-7-glucoside, and apigenin--7-glucuronide). Statistical analysis showed a significant influence of extraction type on the content of other phenolic acids, 6-hidroxyluteolin-7-glucoside, luteolin-3'-glucuronide and total flavonoids ($p<0.05$) (Table 4). In all acetone extracts (both CE and MAE), a high peak was determined at the beginning of the chromatogram, with spectra that do not belong to polyphenol spectra. Therefore, the high mass fraction of TP in all acetone extracts may be the result of that compound reacting with Folin--Ciocalteu reagent although it is not a polyphenol. Considering the above mentioned, using 30 % ethanol and acetone aqueous solutions as extraction solvents results in similar extraction yield, while water is not efficient solvent for the extraction of sage polyphenols. Since non-phenolic component was not present in the ethanol extract and both ethanol and acetone aqueous solutions have high extraction capacity, 30 % aqueous ethanol can be considered as more efficient solvent for the isolation of sage polyphenols. Statistical analysis showed significant influence of the extraction solvent on other phenolic acids ($p<0.05$) (Table 4).

Similar results have been reported by other authors, so Durling *et al.* (*8*) reported 55 to 70 % aqueous ethanol solutions as the most efficient solvents for the extraction of sage polyphenols. Wang *et al.* (*25*) compared extraction capacity of ethanol, methanol, acetone, acetonitrile and water for the extraction of rosmarinic and caffeic acids from sage, thyme, basil and lavender. All solvents had high extraction capacity, except for water, where the

Table 3. Phenolic acids[a] and flavonoids[a] determined in sage extracts obtained by conventional (CE) and microwave-assisted extraction (MAE) with three different solvents

	CE (80 °C, 30 min)			MAE (80 °C, 500 W, 9 min)		
	30 % ethanol	30 % acetone	water	30 % ethanol	30 % acetone	water
Phenolic acids	*w*(mg/g)					
rosmarinic acid	13.4±0.3	14.6±0.3	0.6±0.0	32.3±0.6	35.0±0.2	7.1±0.2
sum of other acids[b]	1.4±0.1	1.0±0.1	0.4±0.0	2.6±0.1	2.6±0.1	1.8±0.1
Σ	14.8	15.6	1.0	34.8	37.6	8.9
Flavonoids	*w*(mg/g)					
6-hidroxyluteolin-7-glucoside	1.1±0.1	1.1±0.1	1.0±0.0	2.5±0.1	2.5±0.1	1.8±0.1
luteolin-3'-glucuronide	2.4±0.1	2.6±0.1	1.2±0.1	7.5±0.2	7.5±0.1	3.8±0.1
sum of other flavonoids[c]	1.9±0.1	1.9±0.1	0.1±0.0	6.1±0.1	6.0±0.0	1.6±0.1
Σ	5.4	5.6	2.3	16.1	16.0	7.2

[a]mean value of duplicate analyses of each extracted duplicate (N=4)
[b]the sum of vanillic, caffeic, syringic, sagerinic, salvianolic acid K and salvianolic acid I concentrations
[c]the sum of luteolin-7-glucuronide, luteolin-7-glucoside, apigenin-7-glucuronide and apigenin-7-glucoside concentrations
Results are given in mg per g of dry sage±standard deviation

Table 4. Influence of the type of extraction (CE and MAE) and solvent (30 % aqueous ethanol, 30 % aqueous acetone and distilled water) on the mass fraction of phenolic compounds

Phenolic compounds	Source of variation	F_{exp}	p-value
rosmarinic acid	extraction	12.00	0.07415
	solvent	9.23	0.09779
other phenolic acids	extraction	170.95	0.00579
	solvent	23.28	0.04118[a]
total phenolic acids	extraction	14.23	0.06364
	solvent	9.86	0.09204
6-hidroxyluteolin-7-glucoside	extraction	42.64	0.02265[a]
	solvent	2.52	0.28401
luteolin-3'-glucuronide	extraction	27.03	0.03506[a]
	solvent	4.41	0.18492
other flavonoids	extraction	13.73	0.06573
	solvent	5.65	0.15046
total flavonoids	extraction	22.48	0.04172[a]
	solvent	5.03	0.16572

[a]significant differences obtained at $p<0.05$

extraction capacity was lower by 20 %. They also reported the optimal concentration of ethanol in aqueous ethanol solutions for the extraction, which was from 30 to 60 % (by volume).

Conclusions

The present research showed that microwave-assisted extraction results in higher extraction yield in significantly shorter time than conventional reflux method. Based on the amount of total phenols determined by Folin-Ciocalteu method, optimal conditions for MAE of sage polyphenols were chosen as follows: temperature 80 °C, extraction time 9 min and microwave power 500 W. Solvents 30 % aqueous acetone and 30 % aqueous ethanol both have high extraction capacity, but 30 % aqueous ethanol is more efficient solvent giving extracts with fewer non-phenolic compounds. Higher microwave power and longer extraction time decreased the amount of total phenols, emphasizing the importance of proper selection of these parameters according to the characteristics of the sample and targeted compounds. Using HPLC with UV/VIS PDA, it was determined that the most abundant polyphenols in sage are caffeic acid derivatives, with rosmarinic acid at the highest concentration, followed by luteolin glycosides.

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