PHARMACIA

Pharmacia 66(4): 209–215
DOI 10.3897/pharmacia.66.e38976

Research Article

Application of the Folin-Ciocalteu method to the evaluation of *Salvia sclarea* extracts

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Received 10 August 2019 ♦ **Accepted** 9 October 2019 ♦ **Published** 31 December 2019

Citation: Hudz N, Yezerska O, Shanaida M, Sedláčková VH, Wieczorek PP (2019) Application of the Folin-Ciocalteu method to the evaluation of *Salvia sclarea* extracts. Pharmacia 66(4): 209–215. https://doi.org/10.3897/pharmacia.66.e38976

Abstract

Polyphenols are valuable group of phytoconstituents due to their high antioxidant activity and healing properties. Antioxidant properties of sages are attributed mainly to a high level of phenolic compounds. The aim of the present study was to elaborate an analytical procedure for the evaluation of the content of secondary metabolites of the polyphenol nature in the herb of Clary Sage (*Salvia sclarea* L.). Four crude extracts of *Salvia sclarea* herb obtained with different technologies were used to develop an analytical procedure for the total phenolic content (TPC) assay by spectrophotometric method. The optimum conditions for the analysis (time of the reaction, wavelength, and reference substances) were chosen and experimentally justified (60–80 min, 760 nm, gallic acid and rutin, respectively). Under these conditions, the developed analytical procedure is robust in the indicated time and easy for performing in phytochemical or technological laboratories. The yield of TPC from the herb of *Salvia sclarea* was the highest in the extracts prepared by heating at a temperature of 36–46 °C and with using the ultrasonic bath. TPC was the highest in the extract in which solvent-to-herb ratio was the least (10:1) and particle size was in the range of 2–5 mm. As a result of the studies, the analytical procedure of the determination of TPC was developed and its parameters were justified. This methodology complies with the requirements for pharmaceutical analysis to ensure the reliability of results during pharmaceutical development and routine control of *Salvia sclarea* extracts.

Keywords

Salvia sclarea, herb, extract, Folin-Ciocalteu reagent, total phenolic content

Introduction

The genus *Salvia* L. belongs to the *Mentheae* tribe within the *Nepetoideae* Burnett. subfamily of the *Lamiaceae* Martinov family. *Salvia* L. is a genus with 900–1000 species in the world (Cai et al. 2006; Coisin et al. 2012; Dent et al. 2013; Kharazian 2013; Abdelkader et al. 2014; Erdoganet al. 2014; Jamzad et al. 2014; Kharazian 2014; Rajabi et al. 2014; Bo et al. 2015; Sepahvand et al. 2015). Many *Salvia* species are used in food, cosmetics, perfumery and pharmaceutical industries throughout the world (Cai et al. 2006; Coisin et al. 2012; Ibrahim 2012; Kharazian 2014; Rajabi et al. 2014; Sepahvand et al. 2015; Jafariet al. 2017). They have been used as folk medicines for a long time (Cai et al. 2006; Ibrahim 2012; Abdelkader et al. 2014; Rajabi et al. 2014; Bo et al. 2015). *Salvia* species are generally known for their multiple

pharmacological effects including antibacterial, antiviral, antioxidative, antimalarial, anti-inflammatory, antidiabetic, antitumor, anticancer cardiovascular, sedative ones, etc. (Ibrahim 2012; Abdelkader et al. 2014; Erdoganet al. 2014; Rajabi et al. 2014; Sepahvand et al. 2015). Representatives of this genus accumulate polyphenols (flavonoids, phenolic acids, etc.) and terpenoids (sesquiterpenoids, diterpenoids, sesterterpens and triterpens) which can induce above-mentioned pharmacological activities (Coisin et al. 2012; Dent et al. 2013; Abdelkader 2014; Kharazian 2014; Sepahvand et al. 2015). Antioxidant properties of *Lamiaceae* species are mainly due to their phenolic compounds and rarely terpenoids (Stanković et al. 2011; Abdelkader et al. 2014; Sepahvand et al. 2014; Dent et al. 2017; Jasicka-Misiak et al. 2018; Shanaida et al. 2018b).

Among the phenolic acids in the aerial parts of *Salvia* genera representatives were identified gallic, rosmarinic, sinapic, caffeic, ferulic, *o*-coumaric, *m*-coumaric, *p*-coumaric, *trans*-cinnamic, chlorogenic, gentisic, syringic, *p*-hydroxybenzoic, and salicylic ones (Bandoniene et al. 2005; Cai et al. 2006; Coisin et al. 2012; Dent et al. 2013; Erdogan et al. 2014; Rajabi et al. 2014; Bo et al. 2015). *Salvia* spp. are rich also in flavonoids such as flavones, flavanones, flavonols, isoflavones, dihydroflavonols, and chalcones (Valant-Vetchera et al. 2003; Coisin et al. 2012; Ibrahim 2012; Kharazian 2013). Therefore, gallic acid and rutin can be used as widespread analytical markers in the analytical procedure of the total phenolic content (TPC) determination.

Clary Sage (*Salvia sclarea* L.) is a xerophitic biennial plant which is typical for the European Mediterranean basin and Africa up to the Atlantic Ocean (Cai et al. 2006). It contains phenolic acids and flavonoids (apigenin, 5-OH-7,4′-diOMe flavone, luteolin, salvigenin (5′-OH-6,7,4′-triOMe flavone) (Valant-Vetchera et al. 2003; Coisin et al. 2012; Bo et al. 2015).

Researchers are interested in studying sage polyphenols for their high antioxidant activity and medical properties (Bandoniene et al. 2005; Khomdram and Singh 2011; Coisin et al. 2012; Dent et al. 2013; Jamzad et al. 2014; Jafari et al. 2017; Dent et al. 2017).

Measurements of TPC are an important tool for the understanding of importance of plant species from the point of view of health (Khomdram and Singh 2011). TPC is considered to be one of quality parameters of herbal preparations (Erdogan et al. 2014). Among the factors affecting the final result of TPC are: the chosen reference substance, volume of an extract and the reagent for the analytical procedure, optimal reaction time, the wavelength for measurements, temperature for the colour development, etc. (Blainski et al. 2013; Abdelkader et al. 2014; Agbor et al. 2014).

Polyphenols available in plant extracts react with specific redox complex Folin-Ciocalteu reagent (FCR) to form a blue complex that can be quantified by visible-light spectrophotometry (Blainski et al. 2013; Agbor et al. 2014; Abdelkader et al. 2014). The Folin-Ciocalteu method is described in the Europeian Pharmacopeia (European Pharmacopoeia 2016). The reaction forms a blue chromophore constituted by a phosphotungstic-phosphomolybdenum complex (Agbor et al. 2014), where the maximum absorption of the chromophores depends on the alkaline solution and the concentration of phenolic compounds (Blainski et al. 2013). This method is very sensitive and precise. The reaction generally provides accurate and specific data for several groups of phenolic compounds because many compounds change the FCR colour from yellow into blue (Blainski et al. 2013; Agbor et al. 2014).

The FCR consists of a mixture of the heteropolyacids, phosphomolybdic and phosphotungstic acids, in which the molybdenum and the tungsten are in the 6+ oxidation state. As a result of such a reaction with a reductant, the molybdenum blue and the tungsten blue are formed and the mean oxidation state of the metals is between 5 and 6 (Agbor et al. 2014). Agbor at al. provide the mechanism of the polyphenols interaction with the FCR:

$$Na_2WO_4/Na_2MO_4 \text{ yellow} \Rightarrow (\text{Phenol-}MoW_{11}O_{40})^{-4} \text{ blue}$$
$$Mo^{+6} \text{ (yellow)} + e^{-1} \Rightarrow Mo^{+5} \text{ (blue)}$$
$$Mo^{+5} + e^{-1} \Rightarrow Mo^{+4} \text{ (blue)}$$
$$ØOH \Rightarrow ØO^{\bullet} + H^{+1} + e^{-1}$$
$$ØO^{-1} \Rightarrow ØO^{\bullet} + e^{-1} \text{ (Agbor et al. 2014).}$$

As far as could be ascertained via a scientific publications survey, studies of the elaboration and justification of an ananalytical procedure of the TPC determination have not been previously reported for *Salvia sclarea* extracts developed for cosmetics or pharmaceutical industries. These extracts could be considered as herbal preparations with promising antioxidant and antimicrobial performance for the development of medicinal products for the treatment of inflammation and infection processes in the oral cavity or for formulations of curative toothpastes and elixirs with above-mentioned properties. Therefore, the aim of this study was to develop the analytical procedure for the evaluation of the content of polyphenols in four extracts of *Salvia sclarea* herb obtained with different technologies.

Materials and methods

Chemicals

The FCR, gallic acid and sodium carbonate were obtained from POCH (Polish Chemical Reagents, Poland). Rutin trihydrate was obtained from Ukrainian Scientific Pharmacopoeial Center for Quality of Medicines. All the chemicals were of analytical grade.

The aerial parts of *Salvia sclarea* were collected at a late flowering stage in August 2017 in the Sector of mobilization and saving herbal resources of the Rice Institute of the National Agrarian Academy of Sciences of Ukraine located in Plodove of Kherson region in Ukraine (latitude: 46°39'20.92"N, longitude: 32°37'4.08"E). The plant material was dried at room temperature, then crushed (to particle size of 0.5–5 mm) and subjected to extraction with ethanol of different concentrations (65% and 70%).

Four extracts of *Salvia sclarea* herb and analytical procedure of the TPC determination were the objects of these studies. The two identical voucher specimens of *Salvia sclarea* are deposited at the Department of Drug technology and biopharmaceutics (Danylo Halytsky Lviv National Medical University, Ukraine) and Sector of mobilization and saving herbal resources (Rice Institute of the National Agrarian Academy of Sciences of Ukraine, Plodove, Kherson region).

The grinded particles of *Salvia sclarea* were extracted with 65% and 70% ethanol. The characteristics of the extracts used in this study are provided in Table 1.

Correlation analysis

Achim Buyul and Peter Tsefel's classification was employed to estimate correlation coefficients (r) between absorbance and time: up to 0.2 is very weak, up to 0.5 is weak, up to 0.7 is medium, up to 0.9 is high and over 0.9 is very high (Bühl and Zöfel 2005).

Estimation of TPC

Spectrophotometric method was used in this study for the development of the analytical procedure for assessing TPC by Folin-Ciocalteu method (Shanaida et al. 2018a; Singh and Aggrawal 2018; Raipuria et al. 2018), and three parameters were analyzed in the tested extracts of *Salvia sclarea*: (1) reaction kinetics for choosing an optimum time of the reaction, (2) maximum absorption wavelength, and (3) two reference substances that characterize TPC. The used method is based on the general procedure recommended by the European Pharmacopoeia (European Pharmacopoeia 2016) for the determination of total tannins with slight modifications. We used a solution of 20% anhydrous sodium carbonate, which is corresponding to 29% of sodium carbonate decahydrate.

The stock solutions of gallic acid monohydrate (1100 mg/L) and rutin trihydrate (1200 mg/L) were prepared using purified water and 50% aqueous solution of ethanol, respectively.

The TPC in the extracts was calculated using expression C = c•10•k, where C is TPC of the tested extract, c is TPC taken from the calibration curve, 10 is coefficient of dilution of the extract for testing, k – coefficient for the calculation of rutin tryhydtate into rutin (0.9187) and 1 gallic acid. The mean of three measurements was used for each concentration of the active marker. Spectra were read in the range of 700 to 780 nm for establishing the optimum wavelength.

TPC of the extracts was determined according to the following analytical procedure. 100 μL of each extract dilution (1:10) was mixed with 100 μL of the FCR, later 1500 μl of purified water and 300 μL of 20% solution of sodium carbonate were added. The mixture was mixed by vortex and incubation was done for 150 minutes at room temperature at darkness. Such time was used for the study of reaction kinetics. The absorbance was read at 760 mm using spectrophotometer «Genesys 20». Purified water was used as the blank. The test was carried out for each extract in triplicate. The mean of three readings was used for the calculations of mean TPC. The results were expressed as gallic acid and rutin equivalents: mg eq-gallic acid and mg eq-rutin acid per 1 liter of an extract.

The kinetics of the reaction for the extracts was evaluated by comparing r ($\sqrt{R^2}$) between the absorbance at 760 nm and the reaction time. r≥0.9 was established as the acceptance criterion.

All the spectra were run using the spectrophotometer «Hitachi U-2810».

Results and discussion

Different parameters were analyzed for choosing optimal conditions for the assay of TPC in the obtained extracts from *Salvia sclarea* herb. As a rule, the interaction time of the FCR with polyphenols of an extract is in the range of 30–120 min at room or elevated temperatures (Stanković 2011; Blainski et al. 2013; Abdelkader et al. 2014; Agbor et al. 2014; Dent et al. 2017; Jasicka-Misiak et al. 2018) or even at a temperature of boiling water bath (Khomdram and Singh 2011). Usually, gallic acid (Stanković 2011; Blainski et al. 2013; Dent et al. 2013; Abdelkader et al. 2014; Erdogan et al. 2014), tannic acid (Blainski et al. 2013), catechol (Khomdram and Singh 2011), catechin (Blainski et al. 2013), pyrogallol (Blainski et al. 2013; European Pharmacopoeia 2016), rosmarinic acid (Dent et al. 2017) are used as reference substances in this method. According to Blainski et al. (2013), gallic acid, tannic acid, catechin and pyrogallol give approximately the same spectra with FCR. According to our studies, gallic acid solutions and extracts of *Salvia sclarea* give also the same spectra (Figs 1, 2). This phenomenon could be explained that the absorption maximum is induced by products of the FCR reduction, which may be the same and independent on the nature of an extract.

Researchers employ different wavelengths and times of the reaction for the absorbance measurements with FCR. For example, such wavelengths are used in the Folin-Ciocalteu method: 760 or 765 nm (European Pharmacopoeia 2016), 760 nm (Blainski et al. 2013), 750 nm (Abdelkader

Table 1. Main technological characteristics of *Salvia sclarea*extracts.

Identification number of an extract	Particle size of the herb	Ratio of raw material to solvent	Maceration dates	Maceration time and conditions	Yield of an extract, ml
E-1	0.5–5 mm	5.0 g to 110 ml of 70 % ethanol	02.03.2018–03.03.2018	200 min at ultrasound and a temperature of 40–46 °C plus 21 hour of maceration at room temperature	89.5
E-2	0.5–5 mm	5.0 g to 108 ml of 65 % ethanol	04.04.2018–10.04.2018	6 days at room temperature	82.5
E-3	0.5–5 mm	5.0 g to 108 ml of 65 % ethanol	04.04.2018–10.04.2018	100 min at 36–41 °C plus 6 days at room temperature	81.5
E-4	2–5 mm	5.05 g to 50 ml of 70 % ethanol	27.04.2018–04.05.2018	7 days at room temperature	32

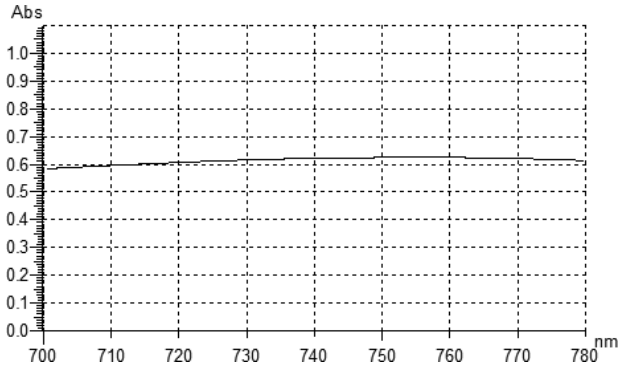

Figure 1. Dependence of absorbance on wavelength for the interaction of gallic acid (100 μg/L) with the FCR (after 60 min of reaction time).

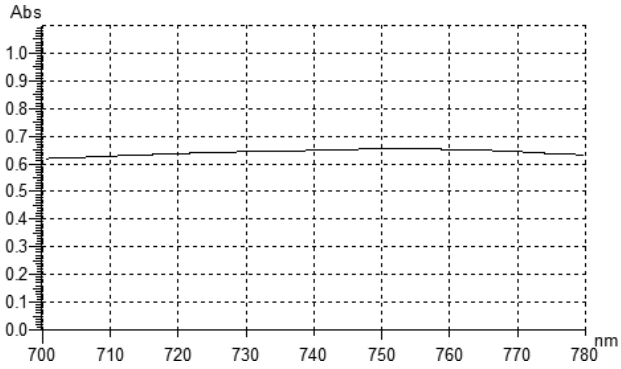

Figure 2. Dependence of absorbance on wavelength for E-4 of *Salvia sclarea* (dilution 1:10) with the FCR (after 60 min of reaction time).

et al. 2014). The European Pharmacopeia provides a wavelength of 760 nm for the measurement of absorbance, 30 min as the reaction time and pyrogallol as a reference substance (European Pharmacopoeia 2016). For polyphenols assay in different species of *Salvia* the following conditions were used: 765 nm, incubation for 30 min, gallic acid as a reference substance (Jafari et al. 2017); a wavelength of 750 nm, 120 min as the reaction time, gallic acid as a reference substance for aqueous-alcoholic extracts of *Salvia officinalis* leaves (Abdelkader et al. 2014); a wavelength of 760 nm, 120 min as the reaction time, gallic acid as a reference substance for methanolic extracts of *Salvia officinalis* leaves (Jasicka-Misiak et al. 2018). Stankovič et al. (2011) employed a wavelength of 765 nm, incubation at a temperature of 45 °C for 45 min, and gallic acid as a reference substance for the determination of TPC in methanolic extracts of *Marrubium peregrinum* (Stanković 2011). A wavelength of 650 nm, incubation in a boiling water for 1 min, catechol as a reference substance are used for TPC determination in ethanolic extracts of eight species of *Lamiaceae* (Khomdram and Singh 2011). The authors of the published papers with exception of Blainski et al. (2013) did not explain why they selected the proper marker, a wavelength or reaction time for the evaluation of TPC in the tested extracts.

The first step of the study was to choose an appropriate wavelength for measurements of TPC. A wavelength of 760 nm was chosen as a working one for measurements on the base of the experimental studies with gallic acid and the extracts presented in Table 2.

As can be seen in Table 2, and Figs 1, 2, the absorbance in a very wide range of 735–770 nm changes very insignificantly (approximately ±1%). The absorption maxima are either close to a wavelength 760 nm or the absorbance at the wavelength of maximum absorption differs very insignificantly from the absorbance at a wavelength of 760 nm. Therefore, a wavelength of 760 nm was chosen and experimentally justified. Moreover, it is recommended by the European Pharmacopeia for polyphenols and tannins determination (European Pharmacopoeia 2016).

The calibration curves of gallic acid and rutin trihydrate were plotted in the range of concentrations of 20 mg/L to 150 mg/L and 62 mg/L to 310 mg/L, respectively. 100 μl of the obtained solutions of gallic acid monohydrate and rutin trihydrate were mixed with 100 μl of FCR, later 1500 μl of purified water and 300 μl of 20% sodium carbonate were added. The mixtures were mixed by vortex. After incubation at room temperature at darkness for 60, 90 and 120 min the absorbance of the reaction mixtures was measured at 760 nm at different time points. Purified water was used as blank. The calibration equations for gallic acid and rutin trihydrate at different time points were the following:

- $y = 0.0057x + 0.0783$, $R^2 = 0.9984$ – for five concentrations of gallic acid solutions at 60 min of the reaction,
- $y = 0.0058x + 0.0728$, $R^2 = 0.9992$ – for five concentrations of gallic acid solutions at 90 min of the reaction,
- $y = 0.0058x + 0.0685$, $R^2 = 0.9995$ – for five concentrations of gallic acid solutions at 120 min of the reaction,
- $y = 0.0026x + 0.0788$, $R^2 = 0.9983$ – for five concentrations of rutin trihydtate solutions at 60 min of the reaction,
- $y = 0.0026x + 0.0801$, $R^2 = 0.9988$ – for five concentrations of rutin trihydtate solutions at 90 min of the reaction,

Table 2. Experimental data for choosing the wavelength for the measurements of TPC in the gallic acid solutions and the investigated extract of *Salvia sclarea* (E-1).

Name of object	Absorbance at the wavelength of							
	735 nm	740 nm	745 nm	750 nm	755 nm	760 nm	765 nm	770 nm
Gallic acid, 20 μg/L, 60 min	0.157	0.159	0.140	0.161	0.162	**0.162**	0.162	0.162
Gallic acid, 100 μg/L, 60 min	0.630	0.633	0.632	0.635	0.637	**0.637**	0.636	0.634
Gallic acid, 150 μg/L, 60 min	0.903	0.906	0.908	0.908	0.908	**0.908**	0.903	0.898
Salvia sclarea E-1	0.527	0.529	0.531	**0.532**	0.531	**0.530**	0.528	0.526

- $y = 0.0026x + 0.0794$, $R^2 = 0.9985$ – for five concentrations of rutin trihydtate solutions at 120 min of the reaction.

As it can been seen, calibration equations differ insignificantly at 60, 90 and 120 min that indicate the complete interaction of gallic acid and rutin with the FCR. In the calculations of this study the calibration equations for gallic acid and rutin trihydrate for 60 min of the reaction were used. Additionally, in our study square correlation coefficient (R^2) for gallic acid was higher compared with one in the papers ($R^2 = 0.901$ and $R^2 = 0.9365$) (Abdelkader et al. 2014; Singh and Aggrawal 2018) and was approximately the same as it is indicated in the references (Blainski et al. 2013; Raipuria et al. 2018).

The results of the reaction kinetics study of FCR with the *Salvia sclarea* extracts are presented in Table 3. These data indicate that 60 min is enough for the almost complete interaction of *Salvia sclarea* polyphenols with the FCR. The results provided in Table 3 demonstrate that there is a very high correlation between the absorbance and time up to 60 min ($r > 0.9$). After 60 min the absorbance increases insignificantly, namely time of 60 min is enough for the complete reaction of polyphenols with the FCR. Further measurements showed that increasing reaction time did not cause a significant increase in the absorbance. The square correlation coefficient (R^2) of absorbance dependence on time of the reaction at 60 min is more than 0.95. The further measurements at a wavelength of 760 nm at 80 min give its insignificant elevation and a significant decrease in R^2, for example, it was 0.8732 at 80 min, 0.8029 at 105 min for sample 1, etc.). In fact, there is an insignificant increase in the absorbance (approximately +1%) at 80 min compared with the absorbance at 60 min. According to the requirements of the State Pharmacopeia of Ukraine (The State Pharmacopeia of Ukraine 2015), such a deviation is considered to be insignificant at the limits of active substances in the range of 90–110% of the stated content.

Such regularity was established in the performed studies: the longer was the reaction time, the less was the R^2 between the absorbance and time of reaction of the FCR with the extracts of *Salvia sclarea*. In addition, 60 and 80 min can be chosen as the time for checking robustness of the analytical procedure of the TPC determination in the *Salvia sclarea* extracts. The deviations in the absorbances between 60 and 80–90 min were in the range of -0.6% to +1.24% that is in the limits of full uncertainty of analysis (±3%) in the concentration range of an active substance of 90 to 110% of the stated content (The State Pharmacopeia of Ukraine 2015). Therefore, on the base of experimental studies it was set up and justified that 60 min is the time of the almost complete interaction of *Salvia sclarea* polyphenols with the FCR.

The yield of extractive substances of the polyphenol nature from *Salvia sclarea* herb was the highest in E-1 and E-3 for preparation of which higher temperature (36–46 °C) had been used. Our studies are in line with studies of Dent at al. 2013 and Tzima et al. 2018 who stated that TPC depends on the type of extraction solvent, solvent-to-sample-ratio, solvent composition, extraction time and temperature. Data on optimum conditions of polyphenol extraction for *Salvia officinalis* are controversial. According to Durling et al. (2007), optimal extraction conditions giving the highest yield of all the three active compounds (rosmarinic acid, carnosic compounds and essential oil) were the following: particle diameter of 1 mm, an extraction temperature of 40 °C, solvent-to-sage ratio of 6:1 and 55–75% ethanol for up to 3 hours. However, according to Dent et al. (2013), the aqueous solutions of ethanol or acetone (30%), extraction temperature of 60 °C and extraction time of 30 min were the most efficient for the extraction of polyphenols from dry sage leaves.

The yield of TPC was the highest in E-1 for preparation of which additionally ultrasonic extraction had been used. These data are in accordance with data of Veličkovic et al. (2008) who stated that ultrasonic extraction is reported to be very promising and effective for obtaining biologically active substances from Sage ensuring higher yields of extractive substances in much shorter times compared with the classical extraction (Veličkovic et al. 2008). TPC was the highest in E-4 in which solvent-to-sage ratio was the least 10:1 and particle size was in a narrower range, but

Table 3. Statistical data for the regression equation of absorbance dependence of the *Salvia sclarea* herb extracts with the FCRon reaction time.

E-1			E-2			E-3			E-4		
Time	**Mean absorbance ±SD**	**Correlation equation, R^2**	**Time**	**Mean absorbance ±SD**	**Correlation equation, R^2**	**Time**	**Mean absorbance ±SD**	**Correlation equation, R^2**	**Time**	**Mean absorbance ±SD**	**Correlation equation, R^2**
20	0.510±0.023	-	33	0.373±0,002	–	24	0.446±0.044	–	23	0.645±0.005	–
40	0.540±0.025	y=0.0015x+0.48, $R^2 = 1$	51	0.395±0,006	y=0.0012x+0.3327, R^2=1	42	0,468±0.044	y=0.0012x+0.4168, R^2 =1	40	0.663±0.185	y=0.0011x+0.6206, $R^2 = 1$
60	**0.553**±0.026	**y=0.0011x+0.49, R^2= 0.9505**	**62**	**0.402±0.011**	**y=0,001x+0.3403, $R^2 = 0.9772$**	**60**	**0.479**±0.041	**y=0.0009x+0.4258, $R^2 = 0.9643$**	**60**	**0.674±0.009**	**y=0.0008x+0.6288, $R^2 = 0.9661$**
80	0.557±0.025	y=0.0008x+0.5015, R^2=0.8732	80	0.407±**0.011**	y=0.0007x+0.3536, R^2 = 0,8923	86	0,484±0.037	y=0.0006x+0.4379, R^2 = 0,8608	91	0.670±0.015	y=0.0003x+0.6446, R^2 = 0.6163
105	0.561±0.024	y=0.0006x+0.5102, R^2= 0.8029	102	0.409±**0.014**	y=0.0005x+0.3652, R^2 = 0.7888	119	0.478±0.028	y=0.0003x+0.4508, R^2 = 0.5683	118	0.656±0.022	y= 9E-05x+0.6557, R^2 = 0.0867
126	0,565±0.023	y=0,0005x+0,5152, R^2=0.7839	136	0.398±**0.011**	y=0.0002x+0.3815, R^2 = 0,3422	136	0.483±0.025	y=0,0002x+0.4536. R^2 = 0.5779	139	–	–
146	0,565±0.023	y=0,0004x+0.5196, R^2=0.7471	168	0.403±**0.017**	y=0.0001x+0.3861, R^2 = 0,2824	159	0.481±0.025	y=0,0002x+0.4568, R^2=0.5251	–	–	–

the yield of extractive substances of the polyphenol nature from herb of *Salvia sclarea* was the least. Therefore, it is possible to reach a desired TPC in extracts of *Salvia sclarea* changing technological factors (temperature, ultrasonic, solvent-to-sage ratio, concentration of ethanol, etc.) in order to maximally deplete the raw material.

TPC in the tested *Salvia sclarea* extracts (A) and the dry raw material of the plant (B) is presented in Table 4. Comparison between the results of TPC in the herb and liquid extracts of *Salvia sclarea* herb and the results of other reports show differences which, in first turn, are induced with different technics of obtaining extracts (different solvents, for example, methanol, 30% aqueous ethanol, petroleum ether, extraction by reflux method with the following evaporation, extraction at 60 °C in a water bath for 30 min (Khomdram and Singh 2011; Ibrahim 2012; Dent et al. 2017), different solvent-to-herb ratio, etc., places of growing *Salvia spp.* and times of harvesting (Ibrahim 2012; Abdelkader et al. 2014; Dent et al. 2017). Considerable contents of polyphenols in obtained *Salvia sclarea* extracts may be due to high amounts of phenolic acids and flavonoids in its aerial part (Altan et al. 2014; Jasicka-Misiak et al. 2018).

Several studies have been carried out with the polyphenols content in *Salvia* species. TPC has been found as 41.23 mg gallic acid equivalents (GAE) per gram of dry extract of *Salvia sclarea* and 89.88 mg GAE per gram of dry extract of *Salvia multicalius* (Jafari et al. 2017). The TPC for the methanolic extracts from the aerial part of *Salvia bicolor* was 326.76 mg ± 1.62 mg GAE per gram of dry sample of the plant extract (Ibrahim 2012). The TPC value for the ethanolic extracts from the aerial part of wild dalmatian sage (*Salvia officinalis*) growing in Croatia was in the range of 55.02–77.82 mg GAE per g dry matter of the leaves (Dent et al. 2013, 2017). However, it is impossible to compare these results as they are provided for dry extracts or fresh weight of herb (Ibrahim 2012; Abdelkader et al. 2014; Erdogan et al. 2014; Jafari et al. 2017).

Table 4. TPC in the tested *Salvia sclarea* herb extracts (A – mg eq-gallic acid/L and mg eq-rutin/L) and TPC with reference to the dry raw material (B – mg eq-gallic acid/g and mg eq-rutin/g).

Reference substance	Number of investigated extract							
	E-1		E-2		E-3		E-4	
	A	B	A	B	A	B	A	B
Gallic acid at 60 min	832.8	**14.91**	567.9	9.37	703.0	11.46	**1045.9**	6.69
Rutin at 60 min	1675.6	**30.01**	1142.01	18.84	1414.09	23.05	**2103.12**	13.46

Conclusions

It is obvious that *Salvia sclarea* growing in Ukraine is a valuable species in terms of the TPC. The optimum conditions for the TPC determination were chosen and experimentally justified (60–80 min of interaction of the extract with the FCR, a wavelength of 760 nm for measurements, and gallic acid and rutin as reference substances). Under these conditions, the developed analytical procedure was robust in the indicated time, and easy for performing in laboratories.

The yield of TPC from herb of *Salvia sclarea* was the highest in extracts for the preparation of which higher temperature (36–46 °C) and ultrasonic had been used. TPC was the highest in the extract in which the solvent-to-herb ratio was the least (10:1) and particle size was in the range of 2–5 mm, but yield of the TPC from the herb (depletion degree of the raw material) was the least.

Such an approach for the elaboration of the analytical procedure of TPC determination could be used to ensure the results of reliability during the pharmaceutical development and routine control of *Salvia sclarea* herb extracts and other herbal extracts.

Acknowledgments

These studies were supported by a scholarship from Slovak Academic Information Agency (SAIA) (the Selection committee of SAJA awarded 13.06.2017).

All the coauthors are thankful to Svydenko L.V. for providing samples of *Salvia sclarea* herb (Sector of Mobilization and Saving Herbal Resources of the Rice Institute of the National Agrarian Academy of Sciences of Ukraine, Plodove, Kherson region).

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