Journal of Medicinal Plants and By-Products (2024) 4: 1118– 1128
https://doi.org/10.22034/jmpb.2024.359883.1523

Original Article

Screening of *Salvia* L. Species from Iran for Antioxidant Activity and Evaluation of Rosmarinic Acid Content to Meet Highly Active Extract

Hossein Hashempour* and Parvin Abedi-Ghobadloo

Department of Chemistry, Faculty of Basic Sciences, Azarbaijan Shahid Madani University, Tabriz, Iran

Article History

Received: 10 January 2023
Accepted: 17 January 2024

Keywords
Antioxidant activity index
Ultrasound-assisted extraction
Response surface methodology
Salvia sclarea L.
Rosmarinic acid

***Corresponding author**
hosseinhashempour@gmail.com
hashempour@azaruniv.ac.ir
ORCID: 0000-0001-8518-6411

ABSTRACT

This study aims to screen *Salvia* L. species to select a plant species with a high level of antioxidant activity and rosmarinic acid content to consider as a potential source for possible use in food industries and human health supplements. For this purpose, eight *Salvia* species growing widely in Iran were selected to investigate their polar extract in terms of antioxidant activity and rosmarinic acid content. The polar extract of aerial parts of studied plants was obtained by methanol/water (1:1) and their DPPH radical scavenging potential was evaluated. *Salvia sclarea* L. showed the highest antioxidant activity and was selected to optimize the ultrasound-assisted extraction process. Antioxidant activity and rosmarinic acid were optimized by response surface methodology to obtain high amounts in the extract. The optimized condition for antioxidant activity was obtained as 40 min, 50% aqueous methanol, 0.1% acid concentration, and 0.07 plant-to-solvent ratio. Antioxidant activity for all studied plants resulted in the range of 1.66 and 0.16 for *S. scalarea* and *S. chloroleuca* Rech.f. & Aellen, respectively. In addition, the optimized condition for rosmarinic acid content was conducted as 30 min, 70%, 0.3%, and 0.05 plant-to-solvent ratio. Rosmarinic acid values in all plants studied were obtained at 0.30 and 23.24 mg/g plant dried weight for *Salvia sahendica* Boiss. & Buhse and *S. sclarea*, respectively. These findings indicate that optimizing extraction parameters for a distinct compound can be generalized for other plant species in the studied genus. However, optimization for some properties like antioxidant activity which originated from all components in the extract cannot be generalized for other plants. Furthermore, this study suggests that the optimized extract of *S. sclarea* promisingly can be applicable as a source of rosmarinic acid with considerable antioxidant activity in food industries and human nutrition supplements.

INTRODUCTION

Antioxidant active compounds play an important role in human health by scavenging and reducing harmful oxidants in the body [1]. Oxidants are compounds with an unpaired electron called free radicals. Reactive Oxygen Species (ROS) are oxygen-containing free radicals produced in the human body in normal metabolic processes or by external sources such as harmful chemicals in diets, air pollutants, cigarette smoking, and so on. These compounds are very reactive and can easily attack the biological molecules in cells including proteins, lipids, and DNA leading to cell damage [2]. Antioxidants target and inhibit free radicals which causes to make a balance between production and decaying of them. The decrease in antioxidants leads to an increase in oxidants which results in different diseases. Therefore, using antioxidants in a nutrition diet inhibits free radicals and controls their amounts [3]. Plant-related extracts and compounds are a big source of antioxidants [4, 5]. Accordingly, there is an effort to introduce new plant sources for antioxidants.

The plant species belonging to the *Salvia* genus from the *Lamiaceae* family are distributed worldwide. This genus comprises 900 species 58 of which are growing in different parts of flora *Iranica* with 17 native species. Many studies on the *Salvia* genus have reported the importance of species in traditional medicine applications, a variety of phytochemical components and interesting biological activities. For example, *Salvia sclarea* L. (*S. sclarea*) has been used in folk traditional medicine in Italy for treating mouth-related

disorders including gingivitis, stomatitis, and aphthae. Also, a broad range of activities have been reported for *S. sclarea* including antimicrobial, anti-inflammatory, antispasmodic, antioxidant, hypoglycemic, and digestive agent [6, 7]. In this study, eight selected *Salvia* species were investigated for their antioxidant activity. The antioxidant activity of extracts for *Salvia* genus species is produced by phenolic compounds which are the major non-volatile compounds found plentifully in the genus [8-12]. Several compounds such as rosmarinic acid, caffeic acid, p-coumaric acid, chlorogenic acid, ferulic acid, sagerinic acid, and salvianolic acid have been reported from *Salvia* species [9, 13, 14]. The reported compounds corroborated the great potential of *Salvia* species as a source of active phenolic compounds, especially as antioxidants. Rosmarinic acid has been reported as one of the important compounds related to human health and the treatment of many diseases which has attracted great attention [15-18].

Various methods such as ultrasound-assisted, microwave-assisted, supercritical fluid, and pressurized liquid extraction have been reported for natural product extraction [19-21]. Among them, ultrasound-assisted extraction (UAE) has been demonstrated as a more simple, inexpensive, rapid, and high-efficiency method [19, 22, 23]. This technique has been used to extract diverse natural compounds such as phenolic compounds, essential oils, and carotenoids from plant materials [24].

Response surface methodology (RSM) is a collection of mathematical and statistical tools that are widely used for developing, improving, and optimizing processes. RSM evaluates the relative significance of several independent parameters and optimizes experimental conditions. Several RSM methods such as central composite design (CCD) and box-behnken design have been used widely in variant fields such as natural products, chemistry, biology, and food. Amongst them, CCD has been confirmed as a powerful, more efficient, less time-use and widely used design for the RSM method [25-28]. In this work, CCD was used to optimize UAE conditions for obtaining of rosmarinic acid-rich and antioxidant-active extract.

This research aims to study selected *Salvia* species from Iran to introduce a plant source for antioxidant activity with a considerable content of rosmarinic acid. For this purpose, firstly, plant species were screened for antioxidant activity and rosmarinic acid content of their polar extracts. Next, the plant with high antioxidant activity and rosmarinic acid content was selected for ultrasound-assisted extraction optimization. Then, all studied plants were extracted in optimal conditions and evaluated for their antioxidant activity and rosmarinic acid content. Finally, the obtained results were compared to a conclusion.

MATERIALS AND METHODS

Plant Materials

The aerial parts of eight *Salvia* species were collected in the flowering stage from spring to summer of 2021. The location and herbarium number of species are shown in Table 1. The collected plants were dried at room temperature, and aerial parts were separated and powdered before extraction.

Chemicals and Solvents

Rosmarinic acid, ascorbic acid, 2,2-diphenyl-1-picrylhydrazyl (DPPH), gallic acid, methanol, HPLC grade methanol, and trifluoroacetic acid (TFA) were purchased from Merck. The Folin-Ciocalteu reagent for determining total phenol content was prepared from Sigma-Aldric.

Ultrasound-assisted Extraction

Ultrasound-assisted extraction was performed in an ultrasonic cleaner DSA100-XN2 (Fuzhou Desen, China). The plant materials (1.0 g) were placed into an Erlenmeyer with different volumes of extraction solvent and exposed to ultrasound waves with a frequency of 40 kHz and power of 100 W at room temperature. The temperature of the ultrasound bath was adjusted by adding ice. For screening of antioxidant activity, 15 ml of methanol/water (1:1) was used for extraction of 1 g of dried plant at room temperature for 20 minutes.

Selection of Effective Variables

The effect of many parameters such as extraction time, temperature, solvent type, plant-to-solvent ratio, and acid concentration has been reported as effective variables in the extraction of phenolic compounds and antioxidants [29]. In this context, based on many reports on the extraction of phenolic compounds, a mixture of water and methanol was selected as an extraction solvent [30, 31]. The effect of temperature on antioxidant activity was assayed at 30, 40, 50, 60 and 70°C. The acid concentration

effect on extract properties was estimated at 0, 0.1, and 0.3% of hydrochloric acid concentrations. The effect of extraction time and plant-to-solvent ratio are known to affect extraction; therefore, they were selected as effective variables. The selection of other effective variables was based on antioxidant activity and rosmarinic acid content.

Experimental Design

For optimization of the experimental parameters of UAE, a CCD design with four independent variables at five levels (30 experiments) was used. The variables and their levels are as follows: methanol concentration (40-80% (v/v)), HCl concentration (0.0-0.3%), plant-to-solvent ratio (0.025-0.125%), and extraction time (10-50 min). Antioxidant activity index and rosmarinic acid content were selected as the responses for the design experiments. Table 2 shows all the coded values. In this study, the predicted response was calculated by a second-order polynomial model. The second-order polynomial model for response surface analysis is shown as follows:

$$Y = b_0 + \sum_{i=1}^{n}(b_i x_i) + \sum_{i=1}^{n}(b_{ii} x_{ii}^2) + \sum_{i<j=1}^{n}(b_{ij} x_i x_j)$$

In the above equation, Y is the response value. b_0, b_i, b_{ii} and b_{ij} are constant term for intercept, linear, quadratic and interaction effects, respectively. X_i and X_j are independent variables. The experimental data were analyzed by Design Expert (10.0.6 version) program.

Table 1 The studied plants with their collecting locations and herbarium numbers

Plant species	Location	Altitude	Herbarium No.
S. sahendica	Kandovan, Tabriz	2350 m	[c]848-MPH
S. virgata	Farooj, Khorasan	1850 m	38127 (FUMH)
S. nemorosa	Ghoochan, Oghaze Kohne, Khorasan	1780 m	36605 (FUMH)
S. leriifolia	Bajestan, Ghonabad, Khorasan	1336 m	26879 (FUMH)
S. atropatana	Einali,Tabriz	1700 m	[b]2227-1A
S. sclarea	Ferdows, Abegarm, Khorasan	1500 m	22520 (FUMH) a
S. chloroleuca	Pivejan, Khorasan	2090 m	36528 (FUMH)
S. spinosa	Ferdows, Abegarm, Khorasan	1500 m	32280 (FUMH)

[a]Ferdowsi University of Mashhad Herbarium, Herbarium of Research Institute of Forests and Rangelands, Iran. [c]Medicinal Plants and Drug Research Institute Herbarium, Shahid Beheshti University.

Table 2 Range for coded and actual values for central composite design

Independent variables	Code units	Coded levels				
		-2	-1	0	1	+2
Extraction time (min)	A	10	20	30	40	50
Methanol concentration (%, v/v)	B	40	50	60	70	80
HCl concentration (% v/v)	C	0	0.1	0.2	0.3	0.4
Plant to solvent ratio (%)	D	0.025	0.05	0.075	0.1	0.125

DPPH radical Scavenging Assay and Antioxidant Activity Index

The antioxidant activity of extracts was determined by DPPH radical scavenging according to the Brand-Williams method [32] with a little modification in a microplate reader (BioTeK epoch spectrophotometer). Ascorbic acid was used as a positive control in the concentration range of 2.5-10 $\mu g.ml^{-1}$. Briefly, 187.5 µl of extract or standard was added to 62.5 µl of 500 µM of DPPH˚ in methanol. The absorbance was read at 517 nm After 30 min shake and incubation at 25ºC. The percentage of inhibition of DPPH˚ was obtained by the following equation:

$$Inhibition\ of\ DPPH^{\circ}\ \% = \left(\frac{Acontrol - Asample}{Acontrol}\right) * 100$$

Where Acontrol was the absorbance of the blank and Asample was the absorbance in the presence of assayed extract or standard at different concentrations. The antioxidant activity was expressed as IC_{50} (concentration with 50% inhibition) which was calculated by using a calibration curve plotting the concentration against the related scavenging result in the linear range.

Antioxidant activity index (AAI) [33] was calculated by the following equation that expressed the antioxidant activity of tested samples as a constant independent of its and DPPH˚ radical concentrations.

$$AAI = \frac{\text{final concentration of DPPH (µg/ml)}}{\text{IC}_{50}\ (\mu g/ml)}$$

Where the final concentration of DPPH˚ was expressed as a concentration at the start point of assay. The final concentration of DPPH˚ was calculated graphically from a calibration curve of different concentrations of DPPH˚ (50-200 µg/ml) against absorbance at 520 nm.

Determination of Total Phenolic Content

The total phenolic content (TPC) of the extracts was measured via the Folin-Ciocalteu method using gallic acid as the standard. Briefly, 100 µl of the extract or standard was mixed with 100 µl of Folin-Ciocalteu reagent. The solution was incubated at room temperature for 5 min. After incubation 1.5 ml of 20% sodium carbonate was added and again incubated at 25 ˚C for 90 min. Absorbance was measured at 760 nm. TPC was described as mg of gallic acid equivalent (GAE) per gram of plant dried weight.

HPLC Analysis

A BFRL HPLC system was used with a SY-8100 pump and SY-8100 UV detector for rosmarinic acid detection and quantification. The chromatographic separations were carried out on a Eurospher II C_{18} column (5 µm, 100 Å, 4.6×250 mm) with 1 mL min-1 of flow rate and 325 nm of detection wavelength. An isocratic elution system was used with a mobile phase of methanol/water (25:75, 0.1% TFA) based on reported study [34] with a little modification. The rosmarinic acid peak was identified by comparing it with its standard retention time and quantified by an external calibration curve.

RESULTS AND DISCUSSIONS

Screening for Antioxidant Active Plant

The obtained results for the estimation of the antioxidant capacity of eight selected *Salvia* species are shown in Table 3. The IC_{50} values of DPPH radical scavenging for extracts of studied plants were obtained between 46.83 and 305.11 µg/ml. *S. sclarea* showed the highest antioxidant activity with the lowest IC_{50} and the highest AAI about 46.83 µg/ml and 1.095, respectively. Therefore, *S. sclarea* was selected as the species which had the highest antioxidant activity.

Selecting Effective Variables on Extraction

In this work, different aqueous methanol concentrations were applied for extraction including 30-80%. The results that are shown in Figure S1 indicate that the highest values for AAI and rosmarinic acid content were obtained at 50% and 70% methanol, respectively. Therefore, aqueous methanol concentration levels as effective parameters were selected as 40, 50, 60, 70, and 80% for the optimization process. The acid concentration effect was evaluated by extracting with 50% aqueous methanolic acidic solvent (0.1-0.3% acid hydrochloric) which indicated acid contraction is an effective parameter (Fig. S2). Consequently, acid concentration levels were selected as 0.0, 0.1, and 0.3% for optimization. The results for the evaluation of temperature effect on extraction showed a negative effect on antioxidant activity and the results for AAI were presented as a curve in Figure S3. This finding followed previously reported studies that observed a negative effect of temperature on the antioxidant activity of the extract. Elevated temperatures could be effective on the degradation of phenolic compounds leading to decreasing antioxidant activity [29, 30, 35], or may help to increase the amount of non-antioxidants in the extract causing dilution of antioxidants. Therefore, the temperature was omitted from the extraction variables. In addition, the other parameters such as time and plant-to-solvent ratio in many reports were investigated as effective variables. Consequently, extraction time, methanol concentration, acid concentration, and plant-to-solvent ratio were selected as the variables for optimization.

Fitting the Model

The response of rosmarinic acid content and AAI for independent variables was listed in Table 4. Among the experiments, experiment 10 (extraction time of 40 min, methanol % of 50, acid concentration of 0.1% and plant to solvent ratio of 0.1) and experiment 24 (extraction time of 40 min, methanol % of 50, acid concentration of 0.3% and plant to solvent ratio of 0.05) provided highest and lowest antioxidant activity index (1.91 and 0.324), respectively. The highest rosmarinic acid content was obtained in experiment 8 (extraction time of 40 min, methanol % of 70, acid concentration of 0.3%, and plant-to-solvent ratio of 0.05) with the value of 27.36 mg/g of dried plant.

Table 3 Extraction Yield and Antioxidant activity of eight *Salvia* species

Plant species	Extract yield (%)	IC_{50} (µg/ml)	[a]AAI
S. sclarea	12.97	46.83	1.095
S. leriifolia	10.24	151.83	0.308
S. chloroleuca	8.66	305.11	0.166
S. virgata	15.36	58.94	0.793
S. nemorosa	14.93	74.93	0.687
S. spinosa	16.07	193.51	0.277
S. atropatana	15.66	206.74	0.250
S. sahendica	24.57	152.94	0.298
[b]Ascorbic acid	-	6.31	8.72

[a]Antioxidant activity index, [b]standard for antioxidant activity

Table 4 Antioxidant activity and Rosmarinic acid content of the extract of *S. sclarea* under different conditions of ultrasound-assisted extraction in accordance with central composite design (CCD) for response surface analysis

Run	Extraction conditions				Analytical results		
	Time (min)	Methanol %	HCl %	Plant/Solvent	Yield %	AAI	RA content (mg/g dried plant)
1	30 (0)	60 (0)	0.2 (0)	0.075 (0)	24.79	0.65	14.50
2	20 (-1)	50 (-1)	0.1 (-1)	0.1 (1)	17.08	0.74	9.58
3	30 (2)	60 (0)	0 (-2)	0.075 (0)	19.54	1.70	12.16
4	10 (-2)	60 (0)	0.2 (0)	0.075 (0)	34.02	0.48	13.10
5	20 (-1)	50 (-1)	0.1 (-1)	0.05 (-1)	23.80	0.58	14.23
6	30 (0)	60 (0)	0.4 (2)	0.075 (0)	26.02	0.54	18.12
7	30 (0)	60 (0)	0.2 (0)	0.075 (0)	22.55	0.98	13.75
8	40 (1)	70 (1)	0.3 (1)	0.05 (-1)	38.80	0.53	27.36
9	30 (0)	60 (0)	0.2 (0)	0.075 (0)	23.21	0.83	13.48
10	40 (1)	50 (-1)	0.1 (-1)	0.1 (1)	17.69	1.91	9.48
11	30 (0)	60 (0)	0.2 (0)	0.075 (0)	21.83	0.96	16.01
12	40 (1)	70 (1)	0.3 (1)	0.1 (1)	23.69	0.34	11.38
13	20 (-1)	50 (-1)	0.3 (1)	0.05 (-1)	31.80	0.39	22.25
14	40 (1)	70 (1)	0.1 (-1)	0.1 (1)	14.32	1.15	10.59
15	30 (0)	80 (2)	0.2 (0)	0.075 (0)	26.00	0.62	20.75
16	40 (1)	70 (1)	0.1 (-1)	0.05 (-1)	23.85	1.15	20.15
17	20 (-1)	70 (1)	0.3 (1)	0.05 (-1)	29.40	0.90	22.47
18	30 (0)	60 (0)	0.2 (0)	0.025 (-2)	37.90	0.63	25.46
19	20 (-1)	70 (1)	0.1 (-1)	0.1 (1)	16.48	0.55	8.53
20	40 (1)	50 (-1)	0.1 (-1)	0.05 (-1)	26.58	1.42	15.20
21	20 (-1)	70 (1)	0.1 (-1)	0.05 (-1)	22.76	1.20	16.55
22	30 (0)	60 (0)	0.2 (0)	0.075 (0)	23.56	0.64	14.52
23	50 (2)	60 (0)	0.2 (0)	0.075 (0)	21.09	0.89	10.01
24	40 (1)	50 (-1)	0.3 (1)	0.05 (-1)	29.31	0.32	18.78
25	20 (-1)	70 (1)	0.3 (1)	0.1 (1)	22.22	0.48	10.13
26	30 (0)	40 (-2)	0.2 (0)	0.075 (0)	26.98	0.56	11.46
27	40 (1)	50 (-1)	0.3 (1)	0.1 (1)	27.92	0.89	10.14
28	30 (0)	60 (0)	0.2 (0)	0.075 (0)	26.56	0.88	16.71
29	20 (-1)	50 (-1)	0.3 (1)	0.1 (1)	24.27	0.45	9.11
30	30 (0)	60 (0)	0.2 (0)	0.125 (2)	19.11	0.56	6.70

Values were obtained as the mean of three replicates (n=3)

In contrast, experiment 30 (extraction time of 30 min, methanol % of 60, acid concentration of 0.2, and plant-to-solvent ratio of 0.125) showed the lowest rosmarinic acid content with a value of 6.7 mg/g of dried plant. The significance and importance of the proposed model to predict the experimental results were evaluated by analysis of variance (ANOVA). The proposed quadratic polynomial model was highly significant with a p-value of less than 0.0001 for AAI and rosmarinic

acid content. The F-value for the AAI model was 34.95, which is higher than the critical F-value at a 95% confidence level. The F-value (54.34) for rosmarinic acid content of the quadratic polynomial model showed significance at a 90% confidence level.

Response Surface Analysis of AAI

The extraction factors such as extraction time, methanol concentration, acid concentration, and plant-to-solvent ratio were studied. F-values and P-values were determined by the significance of each coefficient shown in Table 5. The regression equation based on experimental results for AAI was:

$$AAI = 0.72 + 0.13A - 0.28C - 0.15AB - 0.17AC + 0.11AD - 0.16BD + 0.098C^2$$

The quadratic relationship between the predicted results of the AAI and extraction factors showed a good regression coefficient (R^2=0.9175). A, C, AB, AC, AD, BD, and C^2 were significant as p-values that were less than 0.05. However, B, D, BC, DC, A^2, $B^{2,}$ and D^2 were not significant because of presenting a higher p-value. The distribution of each element effect was presented by percentage in the Pareto chart (Fig. S4). Acid concentration and extraction time showed the highest linear effect on antioxidant activity (AAI) by 45.88 and 10.85 %, respectively.

The complex interaction between methanol %, plant-to-solvent ratio, extraction time and acid concentration is shown in Figure 1. The highest AAI was observed at the lowest acid concentration and highest extraction time (Fig. 1A). This finding which decreased acid concentration resulted in high antioxidant activity which was in accordance with the literature [36]. A complex interaction of methanol concentration with plant-to-solvent ratio and extraction time was observed (Fig. 1B). For achieving the high antioxidant activity in low extraction time, the high value of methanol concentration should be used. However, high antioxidant activity could be achieved in low methanol concentration and high extraction time. This result could explain that methanol is more responsible for dissolving different classes of antioxidant phenolic compounds than water [37, 38]. The high plant-to-solvent ratio (0.1) led to high antioxidant activity in mild methanol concentration (50%). In contrast, the low plant-to-solvent ratio (0.05) extracted the highest antioxidants in higher methanol concentration (70%). This finding could be explained that when the plant material is enough, the solvent in a low proportion of methanol can extract more antioxidants. On the other hand, the increasing of methanol proportion can extract more non-antioxidants. The model capability to predict AAI Unit was conducted from the closeness of adjusted R-squared (0.89) to R-squared (0.91) which indicated the high degree of correlation between predicted and observed values.

Response Surface Analysis of Rosmarinic Acid Content

The relationship between the Rosmarinic acid and extraction factors with a good regression value for rosmarinic acid content (R^2=0.9452) is shown in Table 5. The quadratic polynomial equation for RA of *S. sclarea* with significant parameters is given below:

$$y = 15.47 + 1.54B + 1.63C - 4.82D + 0.83AB - 0.86BD - 1.38CD - 0.90A^2$$

The parameters of the B, C, D, AB, BD, CD, and A^2 were the most significant. A, AD, AC, BC, D^2, $B^{2,}$ and C^2 were not significant due to a higher p-value (0.1). The distribution of each element effect was presented by percentage in the Pareto chart (Fig. S5). Plant-to-solvent ratio, acid, and methanol concentration showed the high effect on RA content by 73.47, 8.47, and 7.5%, respectively.

The relationship between rosmarinic acid content and extraction factors such as extraction time, methanol %, acid concentration, and plant-to-solvent ratio is shown in Figure 2. The increase in methanol and acid concentration caused a rise in the rosmarinic acid content. However, the increase of plant to solvent ratio showed a negative effect on rosmarinic acid content. The factor of extraction time had no linear relationship with rosmarinic acid content while having a quadratic effect on the extraction of rosmarinic acid content. Therefore, the rosmarinic acid content increased a little around 30 min.

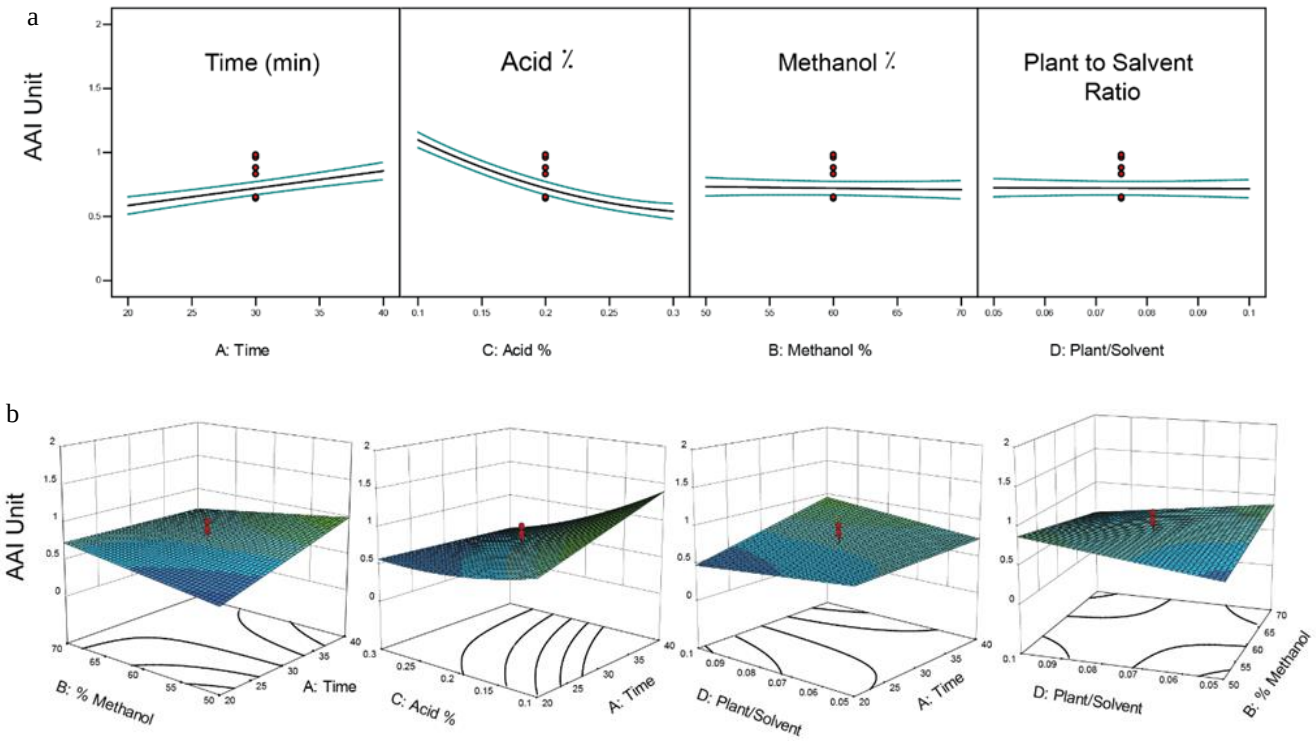

Fig. 1 Antioxidant activity index fluctuation, (a) One-factor effects, (b) three-dimensional response surface graphs

Table 5 Analysis of variance (ANOVA) for the fitted model for the optimization of process parameters

Source	Antioxidant Activity Index (R^2 = 0.9175)					Rosmarinic Acid Content (R^2 = 0.9452)				
	SS	DF	MS	F-Value	P-Value	SS	DF	MS	F-Value	P-Value
Model	3.95	7	0.56	34.95	< 0.0001	754.45	7	107.78	54.34	<0.0001
Lack of fit	0.25	17	0.014	0.66	0.7657	35.54	17	2.09	1.29	0.4188
Pure error	0.11	5	0.022	-	-	8.10	5	1.64	-	-
Residual	0.36	22	0.016	-	-	-	-	-	-	-

SS, sum of squares; DF, degree of freedom; MS, mean square

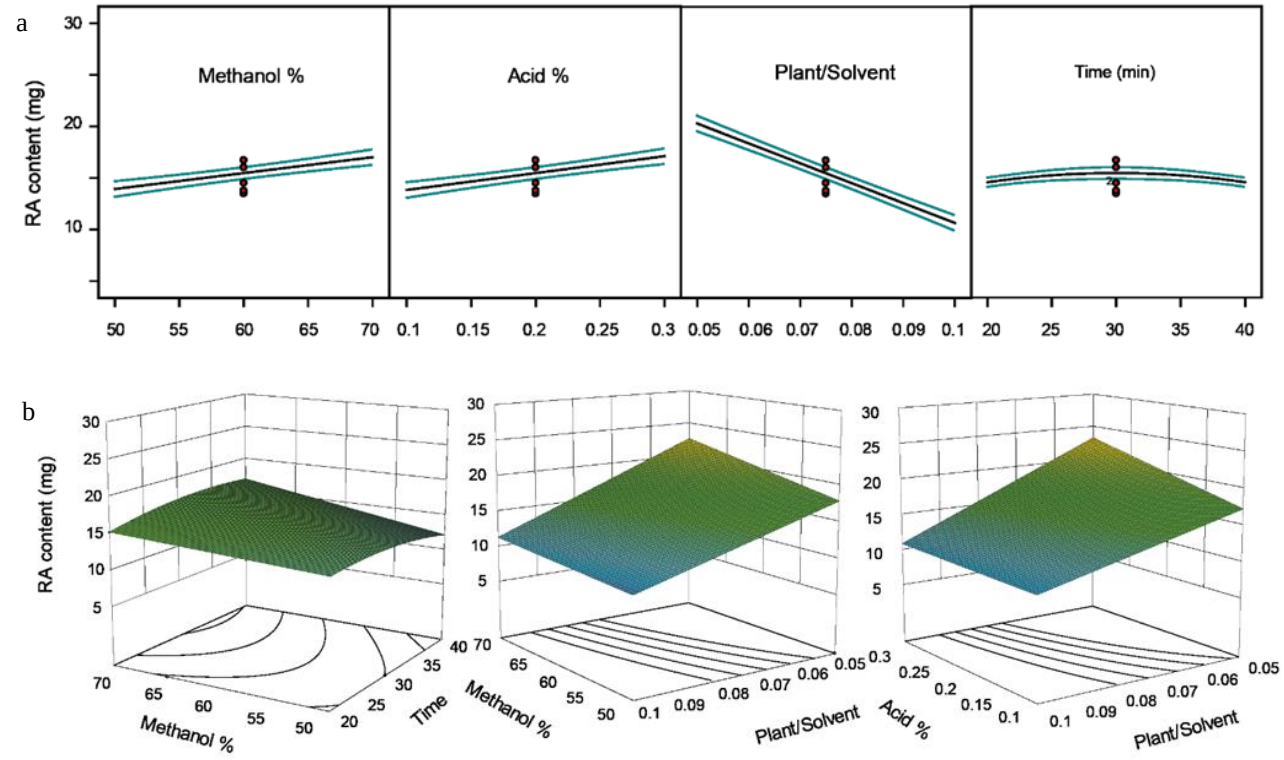

Fig. 2 Rosmarinic acid variation, (a) One-factor effects, (b) three-dimensional response surface graphs

Figure 2A Shows the interaction between acid concentration and plant-to-solvent ratio which the highest rosmarinic acid content was observed at higher acid concentration (0.3%) and lower plant-to-solvent ratio (0.05). The rosmarinic acid content increased with decreasing plant-to-solvent ratio and increasing methanol % which was shown in Figure 2B. The interaction between extraction time and methanol % is shown in Figure 2C. Increasing methanol % and extraction time caused to increase in rosmarinic acid content. This finding was following other reported studies [36, 39, 40]. A high degree of correlation was observed between predicted and actual values which were confirmed by closeness of R-adjusted (0.92) and R-squared (0.94).

Optimization and Validation of the Model

The optimal UAE conditions for the extract with a high AAI value based on the RSM model were: extraction time of 40 min, methanol % of 50, acid concentration of 0.1% and the plant-to-solvent ratio of 0.07. The predicted AAI value for optimal conditions was obtained at 1.56 from the model. The model was validated by experiments (n=3) under the mentioned optimal condition. The experimental value was 1.66 for AAI, which was close to the predicted value. The finding showed a close correlation between predicted and experimental values stating the adequacy of the models to predict UAE conditions. There was an improvement in AAI from 1.09 (none optimized) to 1.66 (optimized) which increased antioxidant activity by 34.04%. That was the state of the art for optimization of the extraction process of natural products.

The optimal UAE conditions for rosmarinic acid extraction based on the RSM predictive model were estimated: extraction time of 30 min, methanol % of 70, the acid concentration of 0.3% and the plant-to-solvent ratio of 0.05. The model validation was performed by experiments (n=3) at optimal conditions. The predicted and experimental values at optimal conditions were 24.88 and 23.24 mg/g dried plants. The nearness of these results showed the close correlation between predicted and experimental values indicating the model's adequacy to predict UAE conditions for rosmarinic acid extractions. None optimized extraction process showed 17.96 mg/g of the dried plant which in optimized extraction it improved to reach 23.24 with an increase of 22.71%. The obtained results showed the optimization was considerably useful to increase rosmarinic acid content in extract.

HPLC Analysis of Rosmarinic Acid

The rosmarinic acid content of extracts for experimental design and non-optimized UAE was identified and determined by HPLC (Fig. 3). Rosmarinic acid was detected at a wavelength of 320 nm and identified by the retention time of its standard. Rosmarinic acid standard was used for spiking on extracts for identification. An external standard calibration curve was used for rosmarinic acid quantification.

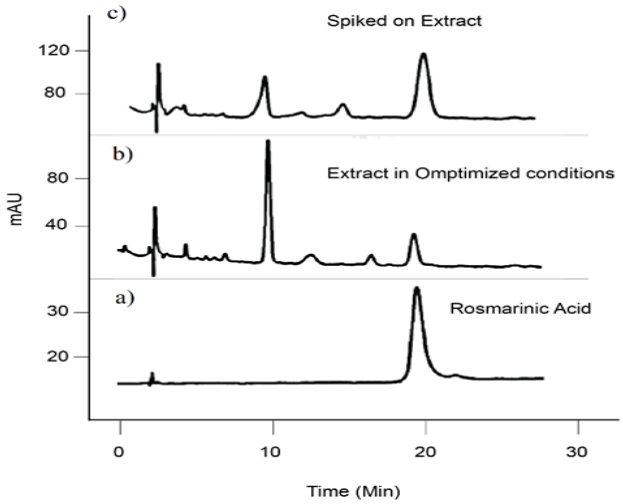

Fig. 3 HPLC chromatogram of (a) rosmarinic acid standard, (b) extract in optimized conditions, (c) standard spiked on optimized extract

Evaluation of Studied Plants in Optimal Conditions

In this part, the polar extracts of studied plants were obtained based on optimal conditions. All extracts were evaluated for their antioxidant activity and rosmarinic acid content. A comparison of obtained results in optimal and non-optimal conditions for antioxidant activity index and rosmarinic acid content was shown in Figure 4 and 5, respectively. For the antioxidant activity index, an obvious increasing pattern was not observed (Fig. 4).

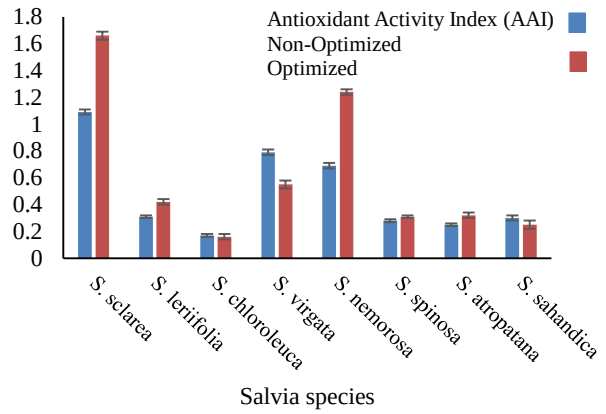

Fig. 4 Comparison of antioxidant activity for studied plant extracts in optimized with non-optimized conditions

In this case, a considerable increase for *S. sclarea* and *S. nemorosa* and also a considerable decrease for *S. virgata* and *S. sahendica* were observed. This finding indicated that generally, optimization of antioxidant activity in a distinct plant cannot apply to other plants to get high values even in the same genus.

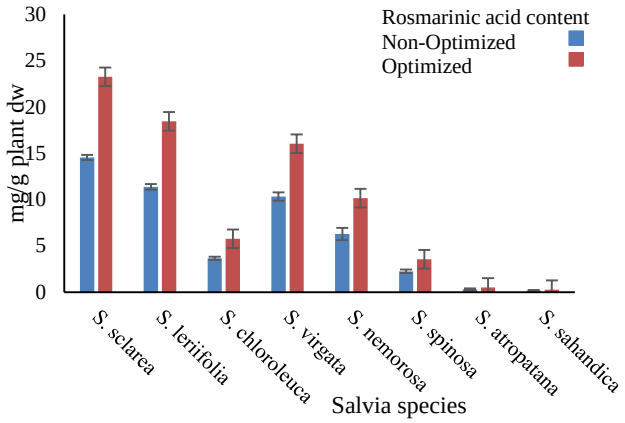

Fig. 5 Rosmarinic acid content comparison of studied plant extracts in optimized and non-optimized conditions

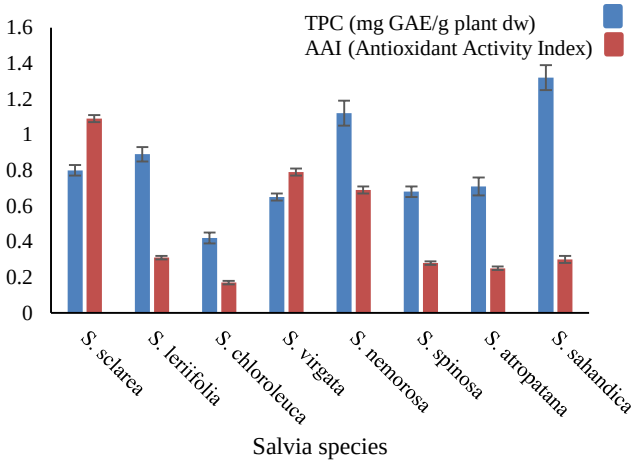

Fig. 6 Obtained results for total phenol content of studied plant extracts in optimized conditions for antioxidant activity,

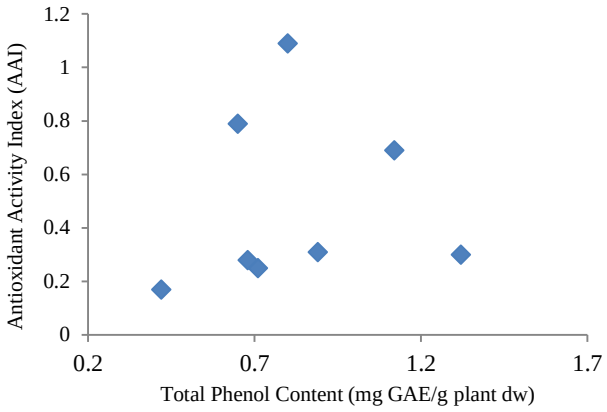

Fig. 7 Correlation of antioxidant activity and total phenol content of studied plants extracts in optimized conditions for antioxidant activity, (1; S. cholorolucea, 2; S. virgata, 3; S. spinosa, 4; S. atropatana, 5; S. sclarea, 6; S. lerrifolia, 7; S. nemorosa and 8; *S. sahendica*)

This finding can be explained by the origin of antioxidant activity which is drawn from all compounds in the extract.

Rosmarinic acid content results for optimized and none-optimized extracts in Figure 5 showed an obvious increasing pattern in optimized conditions. This result can be explained by the fact that extraction of a compound in many cases is independent from other compounds in the plant materials. So, optimized conditions for extraction of a distinct compound in a plant can apply to other plant species from a plant genus.

In addition, the total phenol content of extracts in optimum conditions for antioxidant activity was evaluated and compared with AAI (Fig. 6). Subsequently, the comparison of obtained results for eight extracts showed no linear correlation between Antioxidant activity and total phenol content (Fig. 7).

CONCLUSIONS

In the present study, ultrasound-assisted extraction was used to extract antioxidants and rosmarinic acid from *S. sclarea* which was selected for its high antioxidant capacity among eight *Salvia* species. The RSM using the CCD method was successfully used to optimize UAE conditions on *S. sclarea* for extraction of distinct antioxidants. The optimization process caused make considerable increase in both distinct content and antioxidant activity (AAI) by 22.71 and 34.04%, respectively. The rest of the *Salvia* species were extracted by UAE and evaluated their antioxidant activity and rosmarinic acid

content. The obtained results confirmed that optimized conditions for extraction of a distinct compound from a plant species can apply to another species in the genus. Whereas, the optimization of extraction based on an activity like antioxidant activity in a plant species cannot be generalized for other plant species. The obtained results showed that *Salvia sclarea* has higher rosmarinic acid content and antioxidant activity in its polar extract. Based on a literature survey, *Salvia sclarea* has been cultivated industrially for its essential oil which is used widely in perfumes and as a muscatel flavoring for vermouths, wines, and liqueurs. This study suggests that the parallel use of plant materials for hydro-distillation on essential oil and extraction process might be applicable for the extraction of polar extract to use its valuable properties in food industries and human health nutrition supplements.

ACKNOWLEDGEMENTS

The authors thank the research deputy of Azarbaijan Shahid Madani University for financial support.

REFERENCES

1. Bagchi K., Puri S. Free radicals and antioxidants in health and disease: A review. Eastern Mediterranean Health Journal. 1998;4(2):350-60.
2. Cheeseman K., Slater T. An introduction to free radical biochemistry. British Med Bulletin. 1993;49(3):481-93.
3. Sies H. Oxidative stress: oxidants and antioxidants. Experimental Physiology: Translation and Integration. 1997;82(2):291-95.
4. Gupta V.K., Sharma S.K. Plants as natural antioxidants. 2006.
5. Miguel M.G. Antioxidant activity of medicinal and aromatic plants. A review. Flavour and Fragrance J. 2010;25(5):291-312.
6. Kostić M., Kitić D., Petrović M.B., Jevtović-Stoimenov T., Jović M., Petrović A., Živanović S. Anti-inflammatory effect of the Salvia sclarea L. ethanolic extract on lipopolysaccharide-induced periodontitis in rats. Journal of ethnopharmacology. 2017; 199: 52-59.
7. Leporatti M.L., Pavesi A., Posocco E. Phytotherapy in the Valnerina marche (central Italy). J. Ethnopharmacology. 1985;14(1):53-63.
8. Al-Qudah M.A., Al-Jaber H.I., Abu Zarga M.H., Abu Orabi S.T. Flavonoid and phenolic compounds from Salvia palaestina L. growing wild in Jordan and their antioxidant activities. Phytochemistry. 2014; 99: 115-20.
9. Wang M., Li J., Rangarajan M., Shao Y., LaVoie E.J., Huang T.-C., Ho C.-T. Antioxidative Phenolic Compounds from Sage (Salvia officinalis). J. Agric. Food Chem. 1998; 46(12): 4869-4873.
10. Generalić I., Skroza D., Šurjak J., Možina S.S., Ljubenkov I., Katalinić A., Šimat V., Katalinić V. Seasonal variations of phenolic compounds and biological properties in sage (Salvia officinalis L.). Chem Biodiversity. 2012; 9(2): 441-457.
11. Lu Y., Foo L.Y. Antioxidant activities of polyphenols from sage (Salvia officinalis). Food Chem. 2001; 75(2): 197-202.
12. Martins N., Barros L., Santos-Buelga C., Henriques M., Silva S., Ferreira I.C.F.R. Evaluation of bioactive properties and phenolic compounds in different extracts prepared from Salvia officinalis L. Food Chem. 2015; 170: 378-85.
13. Llurba-Montesino N., Schmidt T.J. Salvia Species as Sources of Natural Products with Antiprotozoal Activity. Int J. Molecular Sci. 2018; 19(1): 264.
14. Bahadori M.B., Asghari B., Dinparast L., Zengin G., Sarikurkcu C., Abbas-Mohammadi M., Bahadori S. Salvia nemorosa L.: A novel source of bioactive agents with functional connections. LWT-Food Sci. Technol. 2017; 75: 42-50.
15. Huang S.-s., Zheng R.-l. Rosmarinic acid inhibits angiogenesis and its mechanism of action in vitro. Cancer letters. 2006; 239(2): 271-80.
16. Ngo Y.L., Lau C.H., Chua L.S. Review on rosmarinic acid extraction, fractionation and its anti-diabetic potential. Food and Chemical Toxicology. 2018; 121: 687-700.
17. Petersen M., Simmonds M.S. Rosmarinic acid. Phytochemistry. 2003; 62(2): 121-125.
18. Scognamiglio M., Buommino E., Coretti L., Graziani V., Russo R., Caputo P., Donnarumma G., D'Abrosca B., Fiorentino A. Phytochemical investigation and antimicrobial assessment of Bellis sylvestris leaves. Phytochemistry Letters. 2016; 17: 6-13.
19. J. Mason T., Chemat F., Vinatoru M. The Extraction of Natural Products using Ultrasound or Microwaves. Current Organic Chem. 2011; 15(2): 237-47.
20. Lang Q., Wai C.M. Supercritical fluid extraction in herbal and natural product studies—a practical review. Talanta. 2001; 53(4): 771-782.
21. Mustafa A., Turner C. Pressurized liquid extraction as a green approach in food and herbal plants extraction: A review. Analytica Chimica Acta. 2011; 703(1): 8-18.
22. Shirsath S.R., Sonawane S.H., Gogate P.R. Intensification of extraction of natural products using ultrasonic irradiations—A review of current status. Chemical Engineering and Processing: Process Intensification. 2012; 53: 10-23.
23. Esclapez M.D., García-Pérez J.V., Mulet A., Cárcel J.A. Ultrasound-Assisted Extraction of Natural Products. [journal article]. Food Engin. Reviews. 2011; 3(2): 108.
24. Chemat F., Rombaut N., Sicaire A.-G., Meullemiestre A., Fabiano-Tixier A.-S., Abert-Vian M. Ultrasound assisted extraction of food and natural products. Mechanisms,

techniques, combinations, protocols and applications. A review. Ultrasonics Sonochemistry. 2017; 34: 540-60.
25. Zodi S., Potier O., Lapicque F., Leclerc J.-P. Treatment of the industrial wastewaters by electrocoagulation: Optimization of coupled electrochemical and sedimentation processes. Desalination. 2010; 261(1-2): 186-90.
26. Khayet M., Zahrim A., Hilal N. Modelling and optimization of coagulation of highly concentrated industrial grade leather dye by response surface methodology. Chem. Engin. J. 2011; 167(1): 77-83.
27. Poroch-Seritan M., Gutt S., Gutt G., Cretescu I., Cojocaru C., Severin T. Design of experiments for statistical modeling and multi-response optimization of nickel electroplating process. Chem. Engin. Res. Design. 2011; 89(2): 136-47.
28. Baş D., Boyacı I.H. Modeling and optimization I: Usability of response surface methodology. J. Food Engin. 2007; 78(3): 836-845.
29. Dorta E., Lobo M.G., Gonzalez M. Reutilization of mango byproducts: study of the effect of extraction solvent and temperature on their antioxidant properties. J. Food Sci. 2012; 77(1): C80-C88.
30. Ling L.T., Yap S.-A., Radhakrishnan A.K., Subramaniam T., Cheng H.M., Palanisamy U.D. Standardised Mangifera indica extract is an ideal antioxidant. Food Chem. 2009; 113(4): 1154-59.
31. Sariburun E., Şahin S., Demir C., Türkben C., Uylaşer V. Phenolic content and antioxidant activity of raspberry and blackberry cultivars. J. Food Sci. 2010; 75(4): C328-C35.
32. Brand-Williams W., Cuvelier M.-E., Berset C. Use of a free radical method to evaluate antioxidant activity. LWT-Food Sci. Technol. 1995; 28(1): 25-30.
33. Scherer R., Godoy H.T. Antioxidant activity index (AAI) by the 2, 2-diphenyl-1-picrylhydrazyl method. Food Chem. 2009; 112(3): 654-658.
34. Haghi G., Hatami A. Simultaneous Quantification of Flavonoids and Phenolic Acids in Plant Materials by a Newly Developed Isocratic High-Performance Liquid Chromatography Approach. J. Agric and Food Chem. 2010; 58(20): 10812-10816.
35. Şahin S., Aybastıer Ö., Işık E. Optimisation of ultrasonic-assisted extraction of antioxidant compounds from Artemisia absinthium using response surface methodology. Food Chem. 2013; 141(2): 1361-68.
36. Aybastıer Ö., Işık E., Şahin S., Demir C. Optimization of ultrasonic-assisted extraction of antioxidant compounds from blackberry leaves using response surface methodology. Industrial Crops Prod. 2013;44:558-65.
37. Luthria D.L., Mukhopadhyay S., Kwansa A.L. A systematic approach for extraction of phenolic compounds using parsley (Petroselinum crispum) flakes as a model substrate. J. Sci. of Food and Agric. 2006;86(9):1350-58.
38. Ma Y.-Q., Chen J.-C., Liu D.-H., Ye X.-Q. Simultaneous extraction of phenolic compounds of citrus peel extracts: effect of ultrasound. Ultrasonics Sonochemistry. 2009;16(1):57-62.
39. Escribano-Bailon M. Polyphenol extraction from foods. Methods in polyphenol analysis. 2003.
40. Escribano-Bailon M., Santos-Buelga C. Methods in polyphenol analysis. Polyphenol Extraction from Foods. RSC, Cambridge, UK. 2003:2-16.