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ORIGINAL PAPER

Total polyphenols from *Solanum retroflexum* Dun. fruit: extraction and optimization by response surface methodology

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Abstract

Total phenolic (TPC), flavonoid (TFC) content and antioxidant activity (AOA) of *Solanum retroflexum* fruit extracts obtained by conventional extraction with different aqueous ethanol solution (25, 50, 75% v/v), temperatures (20, 40, 60 °C) and extraction time (15, 30 and 45 min) were investigated. The extract obtained by 75% aqueous ethanol at 60 °C after 45 min shows the highest AOA determined by DPPH (EC_{50} = 60.67 mg/ml) and FRAP (1.55 µmol Fe^{2+}/mg) methods. TPC and TFC were in ranges from 63.50 ± 0.43 to 92.07 ± 1.07 mg of gallic acid/g of dry extract and from 29.17 ± 0.35 to 48.22 ± 0.43 mg of rutin/g of dry extract, respectively. In order to commercialize the extraction of polyphenolic compounds, as the most important group of the plant bioactive compounds, the extraction conditions were optimized by response surface methodology. Under proposed optimum extraction conditions (60 °C, 40.3 min and ethanol concentration of 74.7%) extract with the maximal TPC (92.29 mg gallic acid/g of dry extract) could be obtained. The set of the extraction conditions which gave the maximum TPC while simultaneously minimizing the time, energy and ethanol consumption were also proposed (economic extraction conditions). The results confirm that this insufficiently tested fruit represent a good source of polyphenolic compounds and are worthy of further investigation.

Keywords *Solanum retroflexum* · Antioxidant activity · Total phenolic · Optimization · Response surface methodology

Introduction

Solanum retroflexum (sunberry, nightshade, wonderberry) is a wild leafy annual plant indigenous to South Africa [1]. The plant typically attains a height of 1.5 m, blooming from June to September, while purple–black berries ripen gradually starting from September [2–4]. The fruits are rich with anthocyanins, 100 g of fresh fruit could accumulate from 450 to 700 mg anthocyanins expressed as cyanidin-3-glucoside chloride equivalent, while the petunidin-3-(*p*-coumaroylrutinoside)-5-glucoside is identified as the main anthocyanin [4].

This species can be found throughout temperate and tropical regions, up to the altitude of 2300 m and more [5]. It is considered to be an inedible plant and persistent weed in Europe and America, whereas its status completely differs in Africa, India, Indonesia and China where this fast-growing annual vegetable crop is used for human consumption, as a source of colored pigments and for various medical purposes [6, 7]. This crop, known for their nutritional and economic importance in most parts of South Africa [7], grows wildly in Serbia.

Considering that extraction conditions significantly affect the extraction process, the estimation of individual and combined effects of process parameters, as well as the process optimization is a crucial step in the extraction of bioactive compounds from plant materials [8]. Therefore, use of the response surface methodology (RSM) for development, improvement and optimization of extraction processes is becoming more common and indispensable [9, 10].

However, to the best of this authors' knowledge, the polyphenolic content and antioxidant activity (AOA) of *S. retroflexum* fruit extracts have not been reported yet. Therefore, the main goals of this work were to optimize extraction conditions, to evaluate the effect of extraction conditions on the

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antioxidant capacity, total phenolic (TPC) and flavonoids (TFC) content of *S. retroflexum* fruit extracts, as well as to assess the importance of this insufficiently tested plant species as a potential source of bioactive compounds and natural antioxidants.

Experimental

Plant material

Fresh fruits of *S. retroflexum* were collected in South Serbia (Toplica district) in September 2013. The plant material was harvested at the stage of full maturity and stored at + 4 °C until use (no longer than 4 days). Botanical identification of plant material was done by Prof. dr Novica Randjelović (Botany Department, Faculty of Science, University of Nis, Serbia).

Extraction of plant material

Freshly ground fruit (5 g) was extracted with aqueous ethanol solution (25, 50, 75% v/v), with plant material to solvent ratio of 1:10 g/ml, at three temperatures (20, 40 and 60 °C) for 15, 30 and 45 min. After the extraction, the liquid extract was separated, evaporated to dryness at 40 °C, and stored at 4 °C until further analysis.

Determination of AOA, TPC and TFC

AOA of extracts was estimated using DPPH and FRAP methods. The TPC and TFC contents of the extracts were determined by the Folin–Ciocalteu and aluminum chloride colorimetric method, respectively [11].

Experimental design

The RSM with three-level full factorial experiment design was used to optimize the conditions for the maximal recovery of TPC from *S. retroflexum* fruit. The influence and combined effects of temperature (20, 40 and 60 °C, X_1), ethanol concentration (25, 50 and 75%, X_2) and extraction time (15, 30 and 45 min, X_3) on TPC (dependent parameter, Y) were investigated (Design Expert software, trial version 7.0.0, STAT-EASE Inc., Minneapolis, USA).

The analysis of variance (ANOVA) was used to investigate the adequacy of the proposed polynomial model and to assess a statistical significance of the analyzed factors (individual and combined).

Results and discussion

AOA

AOA of aqueous ethanol extracts of the fruit *S. retroflexum* obtained under different extraction conditions was shown in Table 1.

The results indicate that the fruits of this plant species are rich in compounds with antioxidant capacity. The efficiency of neutralization of DPPH radicals is expressed by the value of EC_{50}, i.e. an effective concentration of the extract required to neutralize 50% of DPPH radicals [11]. Lower EC_{50} value is being an indicator of a greater AOA. A good correlation ($R^2 = 0.925$) between the values of AOA of DPPH and FRAP methods was calculated in the cases of analyzed extracts obtained under different operating conditions.

Ethanol represents one of the most commonly used solvents for the extraction of polyphenol components from plant material. Aqueous solutions of ethanol were selected for extraction due to lower toxicity and cost, higher yield, as well as due to the possible further application of the obtained extracts in food industries [12]. The increase in the proportion of ethanol in the extraction system from 25 to 75% increased the AOA of obtained extracts by an average of about 9%. An extract of *S. retroflexum* obtained by conventional extraction with a 75% ethanol solution at 60 °C for 45 min exhibited the highest AOA ($EC_{50} = 60.67$ µg/ml, 1.55 µmol Fe^{2+}/mg; determined by DPPH and FRAP methods, respectively). Better AOA, determined by DPPH test, with an increase in ethanol concentration is in accordance with the previously published results in the case of the conventional extraction of three types of black currant "Record", "Blackdown" and "Ronix" [13]. In the case of potato peel extract, the maximum AOA was observed at a concentration of ethanol of 66.4% with the extension of time [14]. The analysis of the extracts of the bark *Azadirachta indica, Acacia nilotica, Eugenia jambolana*, leaves and roots of *Moringa oleifera*, fruit of *Ficus religiosa*, and the leaves of *Aloe barbadensis*, showed that the extracts obtained with 80% ethanol had a much better AOA than those obtained with pure ethanol [15].

On the basis of the results, it was concluded that an extract with greater AOA is obtained with the increase in temperature and time of extraction, regardless of the solvent used. It was also noticed that the AOA of the obtained extracts increases by about 25% with the increase in extraction time from 15 to 45 min, regardless of the temperature and ethanol concentration.

In accordance with the results obtained in this work, the positive effects of temperature and extraction time were

Table 1 Antioxidant activity of *S. retroflexum* fruit extracts obtained under different operating conditions determined by DPPH and FRAP method

		20 °C			40 °C			60 °C		
		15 min	30 min	45 min	15 min	30 min	45 min	15 min	30 min	45 min
25% EtOH										
DPPH	EC_{50}, μg/ml*	91.99 ± 1.32	86.50 ± 1.19	82.57 ± 1.38	89.44 ± 1.18	83.05 ± 1.13	73.74 ± 0.91	78.98 ± 0.94	70.97 ± 1.02	66.88 ± 1.03
FRAP	μmol Fe^{2+}/mg	1.12 ± 0.07	1.19 ± 0.13	1.22 ± 0.23	1.15 ± 0.08	1.24 ± 0.05	1.29 ± 0.11	1.23 ± 0.09	1.32 ± 0.05	1.41 ± 0.02
50% EtOH										
DPPH	EC_{50}, μg/ml*	86.65 ± 1.11	80.51 ± 0.53	76.45 ± 0.96	80.38 ± 1.05	73.26 ± 1.07	71.17 ± 0.89	73.74 ± 0.31	68.07 ± 0.52	65.10 ± 0.41
FRAP	μmol Fe^{2+}/mg	1.08 ± 0.09	1.19 ± 0.02	1. 31 ± 0.06	1.25 ± 0.04	1.32 ± 0.01	1.39 ± 0.07	1.36 ± 0.036	1.39 ± 0.039	1.49 ± 0.028
75% EtOH										
DPPH	EC_{50}, μg/ml*	82.44 ± 1.23	75.47 ± 1.17	71.22 ± 1.07	74.40 ± 0.62	71.27 ± 0.84	66.40 ± 1.06	68.82 ± 1.12	65.14 ± 1.08	60.67 ± 0.96
FRAP	μmol Fe^{2+}/mg	1.18 ± 0.08	1.29 ± 0.07	1.33 ± 0.07	1.31 ± 0.50	1.37 ± 0.36	1.48 ± 0.58	1.42 ± 0.74	1.54 ± 0.65	1.55 ± 0.35

*EC_{50}—an effective concentration of the extract required to neutralize 50% of DPPH radical

confirmed in the following cases: conventional extraction of artichoke leaves [16], red grapes *Barbera* [17], kinema, a *Bacillus*-fermented soybean food [18]. This is mainly attributed to the solubility increase in polyphenolic components responsible for the AOA, as well as to the extraction speed increase and solvent viscosity reduction.

The total content of phenolic and flavonoids compounds

The total content of phenolic and flavonoid compounds in aqueous ethanolic extracts of *S. retroflexum* fruit obtained under different operating conditions (Table 2) indicate that the fruits of this plant species have a high content of these compounds and, as such, represent a good source of natural antioxidants.

On the basis of the obtained results, it has been established that the temperature of the extraction system has the greatest positive effect on the total content of phenolic and flavonoid compounds, which means that the content of these components in extracts increases with the increase in temperature. These results are consistent with the available literature data which show similar dependence of polyphenolic component content on temperature in the extracts of green tea [19], grape pomace [20], peanut skins [21] and olive pits [22]. The positive impact of the increase in temperature (from 20 to 100 °C) on the content of TPC and TFC in aqueous extracts was found in 16 different types of fruit tea [23]. However, it was found that the content of polyphenolic compounds in aqueous extracts of papaya leaves increases with the increase in temperature from 50 to 70 °C, after which it decreases as the temperature further increases up to 100 °C [19]. Such effect of the temperature rise can be explained by the improved extractive matter solubility at higher temperatures and reduced solvent viscosity which facilitate the penetration of a solvent into the particles of plant material and increase the diffusion rate of extractive matter.

Increasing the concentration of ethanol is positively affected the content of TPC and TFC in the obtained aqueous ethanolic extracts of *S. retroflexum* fruits. It has been shown that the increase in the temperature and ethanol concentration in the extraction system encourage severing the bonds between phenols on one hand and polysaccharides and proteins on the other, whereby a greater amount of extraction of phenolic compounds occurs [24].

With the prolongation of the extraction time, the content of bioactive components increases in the analyzed extracts of *S. retroflexum* fruits which is consistent with the previous studies [22, 25].

Using the comparative analysis of the total content of phenolic compounds and flavonoids in aqueous ethanolic extracts of plant species *S. retroflexum* and their AOA determined by DPPH and FRAP methods, it was found that there

Table 2 Total content of phenolic and flavonoid compounds in *S. retroflexum* fruit extracts

Ethanolic concentration, %	20 °C			40 °C			60 °C		
	15 min	30 min	45 min	15 min	30 min	45 min	15 min	30 min	45 min
TPC, mg gallic acid/g dry extract									
25	63.5 ± 0.43	69.8 ± 0.33	73.3 ± 1.31	64.2 ± 1.65	66.6 ± 125	69.8 ± 1.14	70.2 ± 0.51	79.4 ± 1.08	85.1 ± 0.69
50	66.3 ± 0.68	72.4 ± 0.99	75.1 ± 1.05	67.0 ± 0.81	73.5 ± 0.84	78.3 ± 0.53	72.9 ± 1.22	84.6 ± 0.77	87.7 ± 0.39
75	70.1 ± 0.23	74.5 ± 1.02	76.1 ± 0.97	73.5 ± 1.21	78.6 ± 0.44	79.9 ± 0.98	84.3 ± 0.71	89.6 ± 0.99	92.1 ± 1.07
TFC, mg rutin/g dry extract									
25	29.2 ± 0.35	31.9 ± 0.72	35.9 ± 0.46	31.0 ± 1.29	32.8 ± 0.50	37.4 ± 0.59	32.5 ± 0.08	37.9 ± 1.16	43.5 ± 0.16
50	30.9 ± 0.53	33.6 ± 0.24	37.2 ± 0.34	33.4 ± 0.28	37.2 ± 0.56	42.5 ± 0.43	34.0 ± 0.91	41.1 ± 0.16	46.8 ± 0.28
75	32.6 ± 0.48	34.3 ± 2.31	38.5 ± 0.88	39.1 ± 0.34	41.8 ± 0.75	43.5 ± 0.78	40.5 ± 0.73	43.6 ± 1.02	48.2 ± 0.43

Table 3 Experimental design

Run	Design matrix						Response
	Coded factors			Uncoded factors			Total phenolic compounds, mg gallic acid/g dry extract
	Factor X_1	Factor X_2	Factor X_3	Temperature, °C (X_1)	Ethanol concentration, % (X_2)	Time, min (X_3)	
1	0	−1	−1	60	50	45	87.69
2	−1	0	0	60	75	30	89.59
3	0	−1	0	40	25	45	69.84
4	+1	0	+1	40	50	30	73.59
5	+1	−1	0	40	50	45	78.23
6	−1	+1	0	60	25	30	79.44
7	+1	+1	0	20	25	30	69.84
8	0	+1	+1	60	75	15	84.28
9	−1	−1	0	20	25	45	73.34
10	0	0	0	40	75	15	73.55
11	0	+1	0	40	25	15	64.21
12	+1	−1	−1	40	50	15	67.04
13	0	0	−1	40	50	30	73.59
14	+1	−1	+1	40	50	30	73.59
15	0	+1	−1	60	50	15	72.99
16	0	0	0	60	75	45	92.07
17	−1	+1	−1	20	75	15	70.06
18	0	0	0	40	25	30	66.56
19	−1	0	−1	40	50	30	73.59
20	0	0	0	60	25	45	85.07
21	−1	−1	+1	60	50	30	84.60
22	0	0	0	20	50	45	75.08
23	+1	+1	+1	20	75	30	74.52
24	−1	+1	+1	20	75	45	76.01
25	+1	+1	−1	20	25	15	63.50
26	0	−1	+1	20	50	30	72.43
27	−1	−1	−1	40	50	30	73.59
28	0	0	+1	60	25	15	70.23
29	+1	0	0	40	50	30	73.59
30	0	0	0	20	50	15	66.28
31	−1	0	+1	40	75	30	78.60
32	+1	0	−1	40	75	45	79.87

are good linear correlations ($R^2 > 0.8723$). Such good correlations point to the fact that polyphenol compounds are also responsible for the AOA of the analyzed extracts.

RSM modeling and optimization

Experimental design, all experimental runs with different combinations of the three extraction parameters (temperature, ethanol concentration and extraction time), experimental and predicted values for TPC as a response were shown in Table 3, while the ANOVA results is shown in Table 4.

A second order polynomial model which predicts the yield of TPC from *S. retroflexum* fruit was generated by multiple nonlinear regression analysis of the experimental data (X_1, X_2 and X_3 are the coded values for variables).

$$Y_{TPC} = 73.46 + 5.83X_1 + 4.25X_2 + 4.73X_3 + 1.44X_1X_2 + 1.06X_1X_3 - 0.85X_2X_3 + 4.60X_1^2 + 0.19X_2^2 - 1.68X_3^2$$

High F value (73.98) and the p values lower than 0.05 ($p < 0.0001$) indicated statistical significance of suggested model, while low value of the variation coefficient (2.03%) and non-significant lack of fit value further validates the model. The coefficient of determination (R^2) and adjusted coefficient of determination (Adj R^2) values were close to one indicating a good fit of the suggested model to the experimental data.

According to the ANOVA results all analyzed extraction parameters (temperature, ethanol concentration and extraction time) had a very significant effect ($p < 0.0001$) on the yield of TPC, as well as the quadratic effects of temperature and extraction time, and the interaction between temperature and the other two analyzed factors (X_1X_2 and X_1X_3). Temperature and extraction time are the most important factors influencing the response.

All statistically significant factors and interactions had a positive effect on the yield of TPC except quadratic effects of an extraction time. A positive effect of the independent factors means that their positive changes lead to an increase in the response values [26]. Similar results, that increase in an extraction temperature, ethanol concentration or extraction time positively affect the extraction efficiency of TPC were also reported earlier for the different berries fruit: black chokeberry [26], cherry laurel [27] grape [28], black mulberry [29] and mulberry pulp [30].

The model response surface 3D plots (Fig. 1) clearly presents positive effects of all analyzed variables on yield of TPC. The yield of TPC increased with the increase of the extraction time, but the effect of extraction time on the yield of TPC is more pronounced at lower temperatures.

The optimum conditions for maximizing the yield of TPC proposed by the model was: temperature of 60 °C, ethanol concentration of 74.7%, and extraction time of 40.3 min. Under this conditions the predicted yield of TPC was 92.29 mg gallic acid per g of dry extract, while the yield of TPC obtained under the same conditions in the laboratory (three experiments) was 90.69 ± 0.39 mg gallic acid per g of dry extract.

Finally, in order to found more cost-effective conditions for obtaining biologically valuable compounds, numerical optimization was done on the basis of new criteria, maximizing the yield of TPC with simultaneous minimizing the time, energy and ethanol consumption. The newly proposed extraction conditions, accepted as the economic condition, were: temperature of 20 °C, ethanol concentration of 25%, and extraction time of 28.16 min, while the predicted yield of TPC was 69.03 mg gallic acid per g of dry extract.

Table 4 ANOVA results

Source of variation	Sum of squares	Degrees of freedom (df)	Mean squares	F-value	p-value
Model	1547.75	9	171.97	73.98	**< 0.0001**
X_1—temperature	611.33	1	611.33	262.97	**< 0.0001**
X_2—ethanol concentration	325.30	1	325.30	139.93	**< 0.0001**
X_3—time	401.96	1	401.96	172.90	**< 0.0001**
X_1X_2	24.91	1	24.91	10.72	**0.0035**
X_1X_3	13.53	1	13.53	5.82	**0.0246**
X_2X_3	8.76	1	8.76	3.77	0.0652
X_1^2	151.09	1	151.09	64.99	**< 0.0001**
X_2^2	0.26	1	0.26	0.11	0.7435
X_3^2	20.30	1	20.30	8.73	**0.0073**
Residual	51.14	22	2.32		
Lack of fit	51.14	17	0.027	1.82	0.2450
Cor total	1598.00	31			
CV = 2.03%	$R^2 = 0.9680$	Adj $R^2 = 0.9549$	Predicted $R^2 = 0.9250$		

Bold values are significantly different at $p < 0.05$

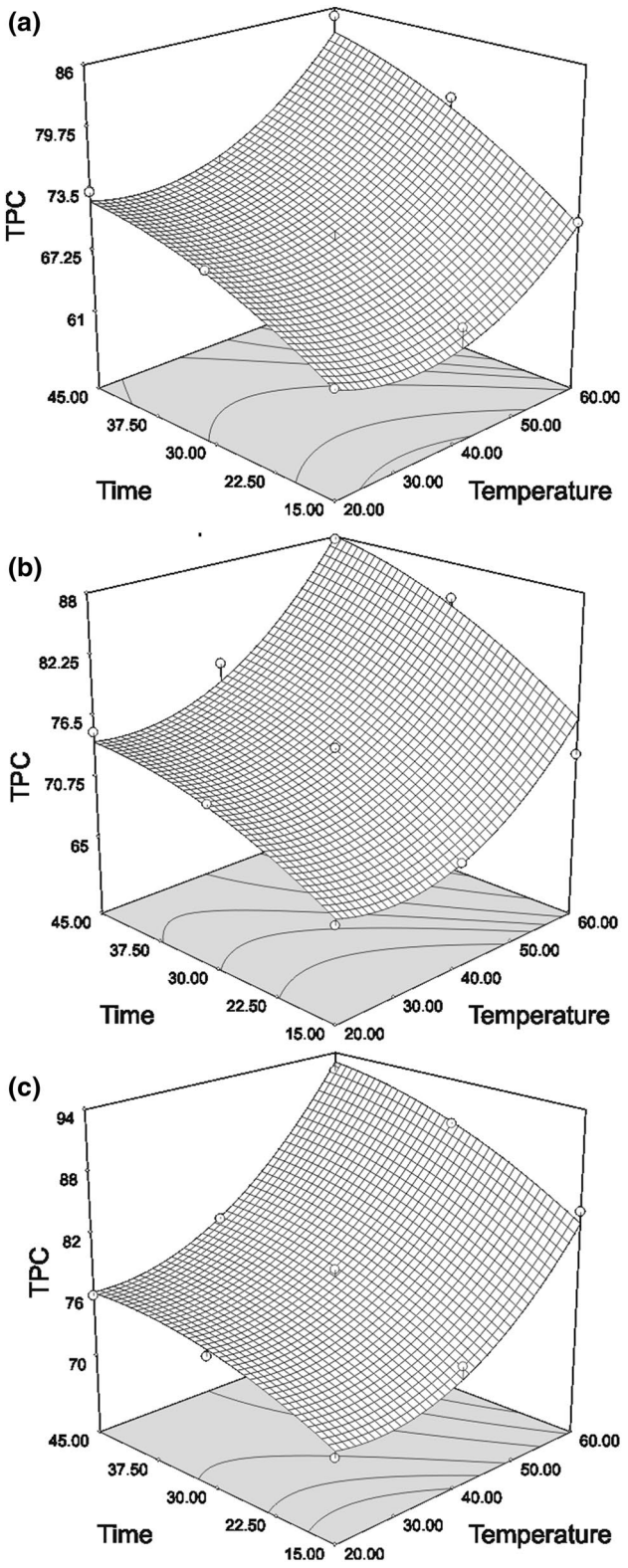

Fig. 1 Response surface 3D plots of temperature, time and ethanol concentration of 25% (**a**), 50% (**b**) and 75% (**c**) effects on the yield of total polyphenol content from *S. retroflexum* fruit

The yield of TPC in the extract of *S. retroflexum* fruit obtained under the economic condition (three experiments) was 68.72 mg gallic acid per g of dry extract. A good agreement between experimental and the predicted yields of TPC for both set of conditions (optimal and economic) also confirmed the adequacy of the suggested model.

By choosing the proposed economic extraction conditions it could be possible to obtain an extract with 24% lower yield of TPC, three times lower ethanol and energy consumption and significantly shorter time (70%). Therefore, this extraction conditions could be recommended for the commercial extraction of *S. retroflexum* fruit polyphenol compounds.

Conclusion

The extract of *S. retroflexum* fruit obtained by the conventional extraction with a 75% aqueous ethanol at 60 °C for 45 min has the highest AOA and the content of TPC and TFC in relation to other extracts. The proposed optimal and economic conditions for the polyphenolic compounds extraction by the RSM were temperature of 60 °C, ethanol concentration of 74.7%, extraction time of 40.3 min and temperature of 20 °C, ethanol concentration of 25%, extraction time of 28.16 min, respectively. Under the optimum level for all three extraction parameters, the maximum predicted yield of TPC was 92.29 mg gallic acid per g of dry extract, while under the suggested economic condition it is possible to obtain 76% of the maximum predicted yield of TPC but with significantly reduced the price per unit of the final product.

In addition, presented results should also contribute to the popularization of *S. retroflexum* fruit as a promising source of natural antioxidants, while complete polyphenolic characterization of this plant needs to be the object of further studies.

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