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Original Research Article

Effect of solvent, time and method of extraction on the amounts of phenolic, flavonoid and antioxidant activity of *Ixiolirion tataricum* (Pall.) Schult. & Schult.f. extract

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ABSTRACT

Solvent type, extraction time and method are among important parameters in the evaluation of extraction efficiency. The selection of appropriate conditions to increase the extraction efficiency is of great importance. The response surface method (RSM) is a statistical and mathematical technique which can be used to examine the dependence between the responses with variables and also to determine the optimal conditions. In this study, the experimental design of general full factorial (GFF) was used using Minitab 17 software. The variables were time (60, 90 and 120 min), solvent (water, methanol and *n*-hexane) and method (soxhlet and ultrasound-assisted extraction). The main effects and also interaction terms were investigated on amounts of total phenolic content (TPC), total flavonoid content (TFC) and the antioxidant activity (according to IC_{50}, FRAP and BCB) of the extract of *Ixiolirion tataricum* (Pall.) Schult. & Schult.f.. Under optimal conditions, the best extraction method for the TFC was the ultrasound-based approach, whereas for other responses the soxhlet-based was better. Water was the optimal solvent for TPC, FRAP and BCB, but methanol was found as the best solvent for the determination of TFC and IC_{50}. Also, the extraction time for all of the responses was 90 min. The amounts of TPC, TFC, IC_{50}, FRAP and BCB in the optimum conditions obtained were 22.45 ± 0.60 (mg GAE/g extract), 114.57 ± 1.59 (mg QC/g extract), 0.36 ± 0.08 (mg/mL), 1014.7 ± 12.4 (mmol Fe^{2+}/g extract) and 53.02 ± 0.05, respectively. The R^2 values for responses were close to unity, which indicates the compatibility between the experimental and the real data. A linear correlation was observed between phenolic compounds and antioxidant activity. Also, the effect of solvent was more important than time and extraction method on the amounts of TPC, TFC, IC_{50}, FRAP and BCB.

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1. Introduction

The Amaryllidaceae family with 1000 species in 79 genera has been distributed in Andean South America, the Mediterranean Sea, Asia and South Africa (Nair and Staden, 2013). It is a source of certain alkaloids that are effective in the treatment of patients who are suffering from Alzheimer's disease (Osorio et al., 2010). The genus *Ixiolirion* from Amaryllis family has 3 species and based on the Iranian flora, the only observed species in Iran is *Ixiolirion tataricum* (Pall.) Schult. & Schult.f.. The antioxidant activity of phenolic compounds in plants is mainly due to their oxidation-reduction properties and chemical structures, which play an important role in neutralizing free radicals.

Alkaloids, phenol and total flavonoid in plants have the potential to eliminate free radicals (Aeschbach et al., 1994; Katalinic et al., 2006; Theriault et al., 2006).

In recent years, it has been proven that free radicals, regardless of their undesired organoleptic effects in food products, eliminating vitamins, destroying of essential fatty acids and creating toxic compounds, can cause side effects such as inflammatory diseases, diabetes and decreased immune system (Benzie, 1996; Robards et al., 1988; Antolovich et al., 2002; Tamaino et al., 2005; Estevez and Cava, 2006). Therefore, the use of antioxidants is necessary to reduce the rate of oxidation in food materials. There is a great deal

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of evidence of toxicity and undesirable effects of synthetic antioxidants such as butylatedhydroxytoluene (BHT), butylatedhydroxyanisole (BHA) and tert-butylhydroquinone (TBHQ) (Frankel, 1991). Due to this fact, the attention is focused on the use of natural antioxidants that are mainly extracted from medicinal plants (Camilo et al., 2017; Mohammadhosseini et al., 2017; Nunes and Miguel, 2017; Pavunraj et al., 2017).

Solvent type, physicochemical properties of the solvent, extraction time, agitation speed, the solvent to sample ratio and temperature are effective parameters on the extraction efficiency. Previous studies have shown that solvent type is more effective than the other parameters in the determination of phenolic and flavonoid compounds when using different extraction methods (Cheok et al., 2012).

Also, the standard extraction methods such as soxhlet (SOE), microwave-assisted (MAE), ultrasound-assisted (UAE), supercritical fluid (SFE) and accelerated solvent (ASE) extraction have a great role in optimizing the quantity and quality of effective compounds of plants. On the other hand, the selection of appropriate conditions for increasing the extraction efficiency is of prime importance. The response surface method (RSM) is a statistical and mathematical technique which can be used to examine the dependence between the responses and variables (dependent and interaction terms) in a process and also to determine the optimal conditions (Chen and Chen, 2009).

In literature, there are few reports concerning chemical composition of the essential oil of *I. tataricum* (Pall.) Schult. & Schult.f (Ghalandarnejad et al., 2014). As far as we know, the antioxidant activity of this plant has been investigated in the present attempt for the first time. In this study, the experimental design of general full factorial (GFF) was used. The variables were time (60, 90 and 120 min), solvent (water, methanol and *n*-hexane) and method (soxleht and ultrasound-assisted extraction). The main effects and interaction terms were investigated on amounts of total phenolic content (TPC), total flavonoid content (TFC) and the antioxidant activity (according to IC_{50}, FRAP, and BCB) of extract of *I. tataricum* (Pall.) Schult. & Schult.f.. The values for TPC, TFC, IC_{50}, FRAP, and BCB of extract of *I. tataricum* (Pall.) Schult. & Schult.f were determined under the optimum conditions.

2. Experimental

2.1. Reagents and materials

Acetic acid, butylated hydroxytoluene, methanol, 2,4,6-tripyridyl-s-triazine, sodium sulphate, *n*-hexane, iron(II) ammonium sulfate, acetate potassium, iron(III) chloride hexahydrate, iron(II) sulfate, gallic acid, linoleic acid, tween 80, aluminium chloride and quercetin were all purchased from Merck (Darmstadt, Germany) with high purity. β-Carotene was prepared from Sigma-Aldrich (St. Louis, MO, USA).

Fig. 1. The geographical map of the sampling area of *I. tataricum* (Pall.) Schult. & Schult.f.

Fig. 2. The photograph of the plant of *I. tataricum* (Pall.) Schult. & Schult.f.

2.2. Preparation of plant

The plant material was collected in April 2017 from the Baba Amman Mountains of North Khorasan province in Iran. The plant was identified and confirmed by Natural Products and Medicinal Plants Research Center of North Khorasan University of Medical Sciences with a voucher number of NPM4/2-1.The geographical map of the sampling area and the photograph of *I. tataricum* (Pall.) Schult. & Schult.f are shown in Fig. 1 and Fig. 2, respectively.

In this study, the aerial plants were dried at 25 ºC in the shade. After 4 days, the dried aerial parts were finely ground. The dried samples were kept in a cold and dry

place prior to their analysis.

2.3. Extraction procedure

Extraction was performed by soxhlet (SOE) and ultrasound-assisted (UAE) extraction techniques. In this regard, 5.0 g of the powered plant was mixed with 100 mL of solvent (H_2O, CH_3OH and *n*-hexane). In the soxhlet method, the mixture became homogenous and after 60, 90 and 120 min, the contents were filtered with Whatman 42 filter paper. In UAE, the homogenous contents were transferred to the ultrasonic water bath (BandelinSonorex, Germany, 480 W, 20 kHz) and the extraction process was conducted at different extraction times (60, 90 and 120 min). In both techniques, the obtained extracts were concentrated using a rotary evaporator (RV 05 BASIC 1-B 115V IKA 8017901). The temperature of the bath of the rotary evaporator was selected according to the boiling point of the solvent extraction. Then, the concentrated extracts were dried at the ambient temperature and stored at 4 ºC.

2.4. Total phenolic content assay

The total phenolic content (TPC) of *I. tataricum* (Pall.) Schult. & Schult.f extract was determined using the Folin-Ciocalteu method (Singleton and Rossi, 1965). For this purpose, 1.0 g of the obtained extract was dissolved in 1.0 mL of solvent and shaken after the addition of 1.5 mL of sodium carbonate (20% w/w) and 500 µL of Folin-Ciocalteu reagent. Then, the mixture was kept under the darkness for 2 h. The absorbance of each solution was measured at 750 nm. The standard calibration curve was plotted using gallic acid solution (0.03-0.22 mg/mL). The results were expressed as mg GAE/g extract.

2.5. Total flavonoid content assay

The total flavonoid content (TFC) of crude extract was determined by the aluminium chloride colorimetric method (Woisky and Salation, 1998). Accordingly, to 0.01 g of the prepared extract, 1.5 mL of methanol, 0.1 mL of $AlCl_3$ (10% v/v in ethanol), 0.1 mL of acetate potassium (1.0 M) and 2.8 mL of distilled water were respectively added. After 30 min at ambient temperature, the absorbance was measured at 415 nm. Quercetin solutions were used for the construction of the calibration curve within the range of 10-100 mg/L. The antioxidant capacity was expressed in mg QC/g extract.

2.6. DPPH free radical scavenging assay

3.9 mL of DPPH solution (6×10-6 mmol/L) was mixed with 100 µL of the prepared extract. This mixture was shaken and incubated for 30 min at room temperature; the absorbance of solutions was measured using a UV-Vis spectrophotometer (UVO-2960-Shimadzu) at 517 nm. Inhibition percent (I %) was calculated using the following equation (Saha et al., 2004):

$$I\% = \frac{(A_{blank} - A_{sample})}{A_{blank}} \times 100 \qquad \text{(Eqn.1)}$$

A_{blank} and A_{sample} are the absorbance of the blank and sample, respectively. IC_{50} was calculated from the plot of inhibition percentage against the extract concentration using Graph pad prism 7 software.

2.7. Ferric reducing antioxidant potential (FRAP) assay

FRAP reagent (3.0 mL) (Benzie and Strain, 1999) was mixed with 50 µL of the extract. This mixture was incubated at 37 ºC for 20 min and then its absorbance was measured at 593 nm using a UV-Vis spectrophotometer. Aqueous solutions of $FeSO_4$ were used for the plot of the calibration curve over the range of 2-10 mg/L. The antioxidant capacity was expressed in mmol Fe^{2+}/g extract.

2.8. β-Carotene bleaching(BCB) assay

In this study, 5.0 mg of β-carotene was first dissolved in 10 mL of chloroform. In the next step, 1.0 mL of β-carotene solution, 25 µL of linoleic acid and 200 mg of tween 40 were mixed (Tuntachote and Berghofer, 2005). Then, the solvent was completely removed using a rotary evaporator. Then, 100 mL of distilled water was added and the resulting mixture was emulsified. 2.5 mL of this emulsion was mixed with 350 µL of extract and incubated at 50 ºC for 48 h. The absorbance was measured at 490 nm. The antioxidant activity (AA) was calculated by the following equation:

$$AA\% = 1 - \frac{(A_0 - A_t)\text{sample}}{(A_0 - A_t)\text{blank}} \times 100 \qquad \text{(Eqn.2)}$$

A_0 and A_t are the absorbance of the blank and sample solutions at t=0 min and t=48 h; respectively.

2.9. Experimental design

Response surface methodology (RSM) was applied to determine the optimized conditions using the Minitab 17 (Minitab Inc., State College, PA, USA) software. General full factorial design (GFFD) was used to investigate the effect of independent variables (time, solvent and method) and interaction terms on the TPC, TFC, IC_{50}, FRAP and BCB. The independent variables with their corresponding levels (i, j and k) for the experimental design are shown in Table 1.

A multiple regression, first degree model was used to express the responses (Bachcecitapar et al., 2016):

$$Y_{ijkn} = \beta_0 + \beta_i X_1 + \beta_j X_2 + \beta_k X_3 + \beta_{ij} X_1 X_2 + \beta_{ik} X_1 X_3 + \beta_{jk} X_2 X_3 + \beta_{ijk} X_1 X_2 X_3$$

$$i = 1, \ldots, a;\ j = 1, \ldots, b;\ k = 1, \ldots, c \qquad \text{(Eqn.3)}$$

Table 1
The independent variables for the experimental design.

Independent variables		
Time(min) (X_1)	**Solvent (X_2)**	**Method (X_3)**
60	H_2O	Soxhlet extraction
90	CH_3OH	Ultrasound-assisted extraction
120	*n*-hexane	

Where Y_{ijkn} is the response in n'th, replicate, β_0 is regression coefficients for intercept; β_i, β_j and β_k are linear regression coefficients for main variables (X_1, X_2 and X_3); β_{ij}, β_{ik} and β_{jk} are regression coefficients for 2-way interactions and finally β_{ijk} is regression coefficient for 3-way interactions.

An analysis of variance (ANOVA) with 95% confidence level was carried out for each response (TPC, TFC, IC_{50}, FRAP and BCB) in order to test the model significance and suitability.

The significance of independent variables in the form of main effect and interaction terms were analyzed by computing the F-value at probability (p) of 0.001 and 0.05.

3. Results and Discussion

3.1. The variance analysis of variables

The experimental data of TPC, TFC, IC_{50}, FRAP and BCB for the extract of *I. tataricum* (Pall.) Schult. & Schult.f under different treatment conditions are shown in Table 2. An example of the residual plots for IC_{50} response is presented in Fig. 3. In addition, the results of ANOVA for extraction variables and regression coefficients on responses are presented in Table 3.

Based on the coefficients in Table 3, all the independent variables and interaction terms were significant ($p<0.001$) on TPC response with the exception of the time of 60 min in soxhlet method (β_{ij} (60) (SO)) ($p>0.05$). In addition, the effect of water solvent ($\beta_i(H_2O)=5.28$) and the interaction between water solvent with the soxhlet method ($\beta_{ij}(H_2O)$ (SO)=6.167) has been more effective than the other variables on the TPC value.

The results of variance analysis showed that all of variables (independent and interaction terms) were significant ($p<0.001$ and $p\leq0.05$) on the amount of TFC, IC_{50}, FRAP and BCB (see Table 3).

3.2. Effect of solvent extraction

In most cases, the highest amount for responses was found in H_2O ($p'=10.2$) and CH_3OH ($p'=5.1$) solvents, while the lowest value was observed in *n*-hexane ($p'=0.1$) solvent (Table 2). This can be related to increasing the efficiency of extraction for polar and semi-polar compounds such as alkaloids, flavonoids, terpenes and proteins (Zieliński and Kozłowska, 2000) in polar solvents compared with non-polar ones.

The optimum extraction conditions were obtained by maximizing the response of model for TPC, TFC, FRAP and BCB and minimizing of it for IC_{50} response. The amount of TFC obtained using methanol was higher than those of water and *n*-hexane solvents, which can be related to the better extraction of flavonoid aglycones in CH_3OH in addition to flavonoid glycones. Less polar solvents were used for the extraction of flavonoid aglycones, while more polar ones were used to extract flavonoid glycosides (Dordoevic et al., 2000). Velickovic et al. (2007) showed that the petroleum ether extracts of *S. officinalis* contained apolar compounds (flavonoid aglycones), the aqueous ethanolic (70% V/V) extracts contained polar and nonpolar compounds, while the water extracts did not show visible spots of flavonoid aglycones.

Water was the optimal solvent for TPC, FRAP, IC_{50}

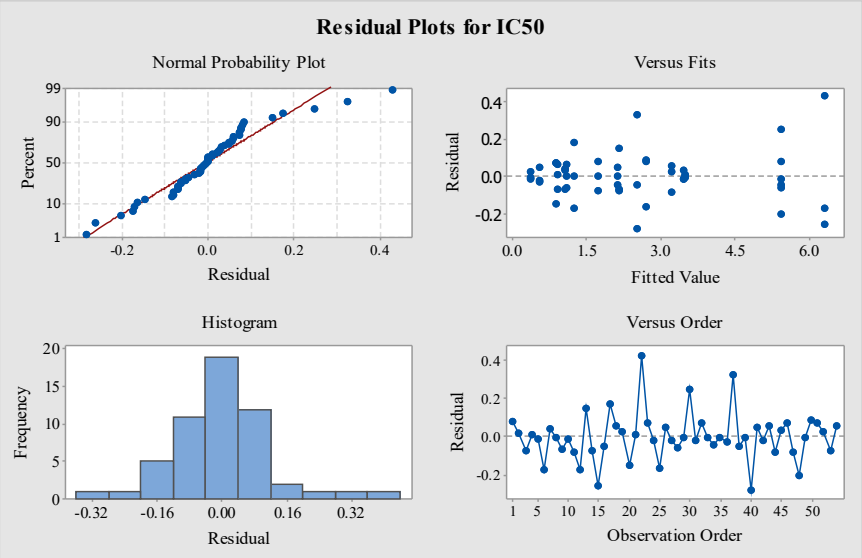

Fig. 3. The residual plots for IC_{50} responsein extract of *I. tataricum* (Pall.) Schult. & Schult.f.

Table 2

Experimental design and observed experimental data in General Full Factorial Design for extract of *I. tataricum* (Pall.) Schult. & Schult.f.

Run Order	Time	Solvent	Method	[c]TPC	[d]TFC	[e]IC_{50}	[f]FRAP	BCB	Run Order	Time	Solvent	Method	TPC	TFC	IC_{50}	FRAP	BCB
1	90	[a]UA	CH_3OH	12.77	114.57	1.83	280.38	33.33	28	60	SO	H_2O	17.18	66.64	1.05	917.04	34.28
2	90	[b]SO	H_2O	22.57	45.50	0.38	1022.05	53.20	29	60	SO	*n*-hexane	0.22	9.57	3.50	130.48	7.04
3	120	SO	*n*-hexane	0.26	17.16	2.08	207.70	6.19	30	60	SO	CH_3OH	5.52	50.87	5.70	164.87	39.04
4	120	SO	CH_3OH	4.30	63.46	0.94	632.18	46.66	31	90	SO	*n*-hexane	0.28	17.65	3.45	164.40	7.47
5	60	SO	*n*-hexane	0.21	9.98	3.49	133.03	7.14	32	90	UA	*n*-hexane	0.18	15.46	5.54	1.20	6.42
6	60	UA	H_2O	8.47	43.35	1.07	45.48	25.19	33	60	SO	H_2O	17.34	66.46	1.11	1010.24	32.85
7	120	SO	H_2O	18.92	37.35	1.12	889.66	39.05	34	60	UA	CH_3OH	6.28	63.27	2.48	133.68	28.89
8	60	UA	H_2O	8.24	43.65	1.24	45.61	24.73	35	90	UA	H_2O	4.27	48.83	2.13	279.07	31.48
9	90	UA	*n*-hexane	0.29	15.50	5.40	1.95	6.46	36	120	UA	H_2O	3.54	32.54	0.52	46.73	48.17
10	90	UA	*n*-hexane	0.21	15.35	5.45	1.25	7.35	37	60	UA	CH_3OH	6.05	63.27	2.85	135.87	27.39
11	90	UA	CH_3OH	12.54	116.98	1.67	290.70	33.23	38	90	UA	H_2O	4.22	48.09	2.08	273.97	31.03
12	120	UA	*n*-hexane	7.90	17.31	6.17	1.43	17.31	39	90	UA	CH_3OH	12.64	112.17	1.75	290.90	33.72
13	120	SO	*n*-hexane	0.26	18.69	2.30	200.05	7.62	40	60	UA	CH_3OH	6.02	63.27	2.24	133.36	29.19
14	120	SO	H_2O	17.75	39.02	1.01	872.41	38.42	41	90	UA	H_2O	4.33	48.46	2.18	279.07	30.47
15	120	UA	*n*-hexane	7.99	17.17	6.08	1.51	17.16	42	90	SO	H_2O	22.37	47.35	0.34	1003.64	53.00
16	60	SO	CH_3OH	4.72	48.27	5.40	164.82	38.09	43	60	SO	H_2O	22.41	66.05	1.17	1010.93	34.28
17	60	UA	H_2O	8.34	43.05	1.42	45.54	25.39	44	120	SO	*n*-hexane	0.27	18.93	2.07	217.12	6.00
18	120	SO	CH_3OH	4.33	63.87	0.99	654.50	44.76	45	90	SO	*n*-hexane	5.45	17.24	3.50	164.23	6.20
19	120	SO	H_2O	17.42	40.68	1.11	889.71	38.10	46	90	SO	CH_3OH	5.27	46.05	0.96	919.66	39.04
20	90	SO	CH_3OH	5.57	64.76	0.74	881.60	39.66	47	60	UA	*n*-hexane	1.54	12.13	3.16	22.10	13.93
21	60	SO	*n*-hexane	0.22	9.20	3.51	122.65	7.76	48	60	SO	CH_3OH	4.60	53.64	5.25	51.50	37.01
22	120	UA	*n*-hexane	7.32	17.16	6.77	1.69	17.17	49	90	SO	H_2O	22.42	46.42	0.35	1018.35	52.85
23	120	UA	CH_3OH	13.32	72.53	2.78	461.54	25.58	50	120	UA	CH_3OH	13.47	73.65	2.79	465.50	23.12
24	90	SO	*n*-hexane	0.20	17.35	3.45	168.62	7.57	51	90	SO	CH_3OH	5.57	64.76	0.96	919.66	39.04
25	120	UA	CH_3OH	13.16	74.76	2.54	459.00	23.04	52	60	UA	*n*-hexane	1.70	11.61	3.27	21.45	13.12
26	120	UA	H_2O	3.34	33.09	0.60	44.41	48.09	53	120	SO	CH_3OH	4.56	63.05	0.86	661.06	42.38
27	120	UA	H_2O	3.75	32.72	0.53	45.05	46.00	54	60	UA	*n*-hexane	1.67	12.91	3.30	20.80	13.42

[a]Ultrasound-assisted; [b]Soxhelt; [c]Total phenolic Content (mg GAE/g extract); [d]Total Flovanoid Content (mg QC/g extract); [e]IC_{50} (mg/mL); [f](mmol Fe^{2+}/g extract).

Table 3

Experimental design and observed experimental data in General Full Factorial Design for extract of *I. tataricum* (Pall.) Schult. & Schult.f.

Coefficient	Responses				
	TPC	TFC	IC_{50}	FRAP	BCB
β_0	7.44**	43.939**	2.4931**	352.25**	27.501**
β_i(60)	-0.769**	-2.984**	0.3519**	-112.83**	-3.127**
β_i(90)	0.365**	6.199**	-0.1509**	90.01**	0.916**
β_i(H_2O)	5.28**	2.30**	-1.4148**	188.81**	10.642**
β_i(CH_3OH)	0.339**	26.794**	-0.1193**	75.57**	7.119**
β_i(SO)	1.049**	-2.833**	-0.3898*	210.42**	2.302*
β_{ij}(60)(H_2O)	1.828**	11.781	-2535*	84.25**	-5.563**
β_{ij}(60)(CH_3OH)	-1. 515**	-10.651**	1.2609**	-184.30**	1.775**
β_{ij}(90)(H_2O)	0.394**	-4.828**	0.3159	14.96**	2.945*
β_{ij}(90)(CH_3OH)	0.879**	9.616**	-0.9045**	79.32**	0.800*
β_{ij}(60)(SO)	ns	4.176*	0.8981	-38.11**	-0.289**
β_{ij}(90)(SO)	1.076**	-6.519**	-0.3824**	43.12*	2.394
β_{ij}(H_2O)(SO)	6.167**	7.371**	0.1604*	207.86**	1.336**
β_{ij}(CH_3OH)(SO)	-3.927**	-10.264**	0.4381*	-77.15**	3.708**
β_{ijk}(60)(H_2O)(SO)	2.193**	2.803**	-0.7354**	86.76*	1.001**
β_{ijk}(60)(CH_3OH)(SO)	2.003**	2.749**	0.5169**	-98.78**	-0.943**
β_{ijk}(60)(CH_3OH)(UA)	nd	32.21	nd	nd	nd
β_{ijk}(90)(H_2O)(SO)	0.798**	nd	-0.2748*	-92.74**	4.979**
β_{ijk}(90)(CH_3OH)(SO)	-1.788**	-8.410**	-0.0976*	133.43**	-5.495**
model	**	**	**	**	**
linear	**	**	**	**	**
2-way Interactions	**	**	**	**	**
3-way Interactions	**	**	*	**	**
R-sq(adj)	0.9769	0.9895	0.9927	0.9764	0.9965
R-sq(pred)	0.9647	0.9839	0.9889	0.9746	0.9946

*Significant at p≤0.05; **Significant at p≤0.001; [ns]Not significant (p≥0.05); [nd]Not detected; β_0, β_{ij} and β_{ijk} are regression coefficients for intercept, linear and interaction terms; respectively.

and BCB, but methanol was the best solvent for TFC. Also, the extraction time for TPC, TFC, IC_{50}, FRAP and BCB was found to be 90 min.

3.3. Effect of method extraction

Sound waves create mechanical oscillations in a distinct material. Unlike electromagnetic waves, sound waves propagate in a material and produce cycles of expansion and contraction due to effect of cavitation. In the expansion mode, the negative pressure in the solvent creates bubbles. As bubbles break down at the material level, their cell wall is degraded and the process of mass transfer is made easier and faster into the solvent. The intensity of the ultrasonic cavitation effect depends on the surface tension, viscosity and vapor

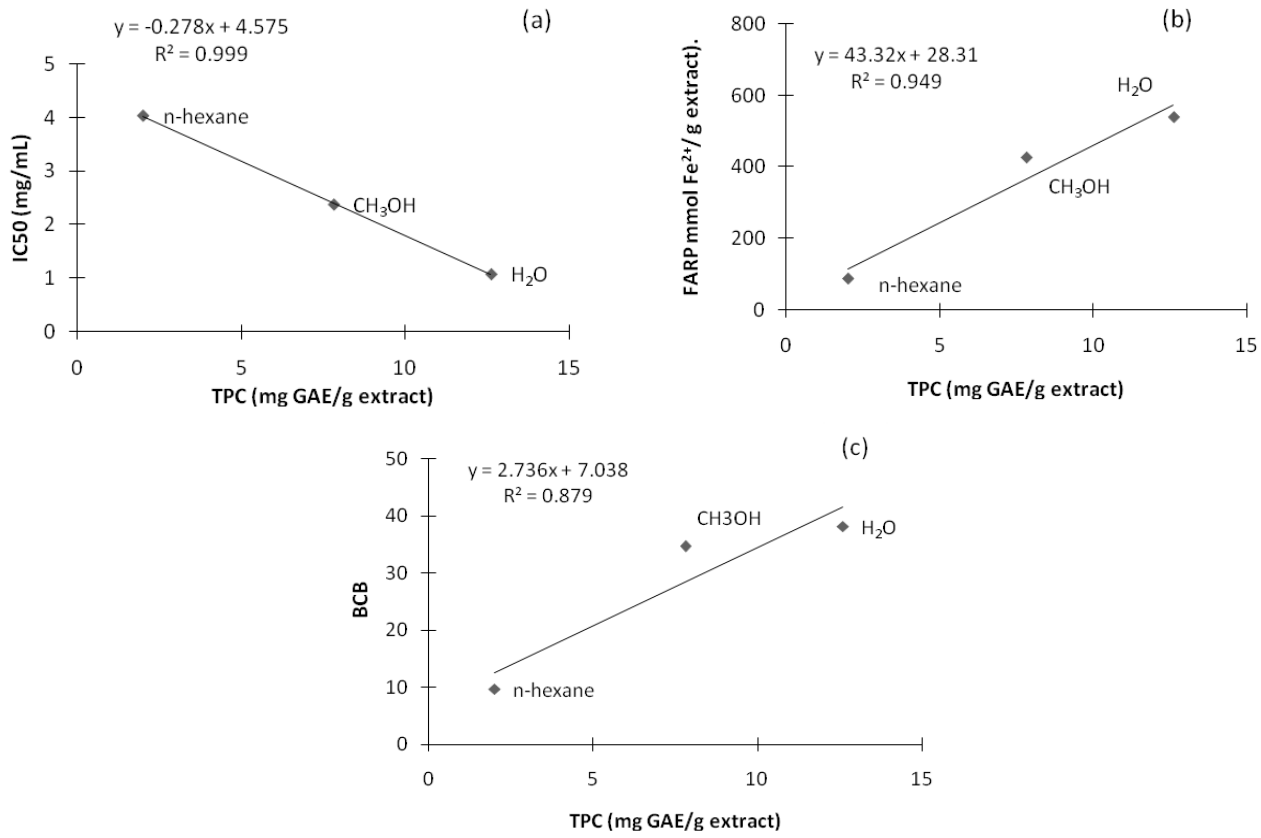

Fig. 4. Correlation between the mean of total phenol content with antioxidant activity (a) IC_{50} (b) FRAP and (c) BCB in the solvent different.

pressure of the medium (Chen et al., 2007). By reducing viscosity and surface tension, the effect of cavitation is higher due to decreased density and increased penetration coefficient. The values of viscosity and surface tension for the used solvent were found to be 0.59 cP, 22.55 dyn/cm for methanol and 1cP, 72.8 dyn/cm for water. As seen, viscosity and surface tension of water is higher than methanol; therefore the cavitation phenomenon occurs more in methanol than water. On the other hand, according to the predicted model and the obtained regression coefficient for methanol solvent ($\beta_j(CH_3OH)$=26.794) (Table 3), the effect of CH_3OH solvent is higher than the other variables in the amount of TFC as a result of increase in the efficiency of total flavonoid content of *I. tataricum* (Pall.) Schult. & Schult.f methanol extract using ultrasonic method as is expected. Falleh et al. (2012) showed that solvent of ethanol is more effective than extraction time on the amount of total phenolic content, total flavonoid content and antioxidant activity of *Mesembryanthemum edule* L.

In optimum conditions, the extraction method for TPC, IC_{50}, FRAP and BCB was soxhlet and for TFC the ultrasound-assisted method was found as the best strategy. Azwanida showed that the selection of extraction method depends on the type of plant, material contents, the suitability and economic feasibility of the method (Azwanida, 2015).

3.4. Quantitative evaluation

The amounts of TPC, TFC, IC_{50}, FRAP and BCB in the optimum conditions obtained were found to be 22.45 ± 0.60 (mg GAE/g extract), 114.57 ± 1.59 (mg QC/g extract), 0.36 ± 0.08 (mg/mL), 1014.7 ± 12.4 (mmol Fe^{2+}/g exctract) and 53.02 ± 0.05; respectively. Also, the R-sq (adj) and R-sq (pred) values (for responses (TPC, TFC, IC_{50}, FRAP and BCB) were close to unity indicating the compatibility between the experimental and the real data (Table 3).

4. Concluding remarks

The results obtained in this work showed that the antioxidant activity of extract of *I. tataricum* (Pall.) Schult. & Schult.f can be related to the phenolic and flavonoid compounds of this plant. As seen in Fig. 4, a linear correlation observed between the mean of total phenolic content with antioxidant activity in the different solvents, indicating the significant contribution of phenolics to antioxidant activity of the extract of *I. tataricum* (Pall.) Schult. & Schult.f.

Also, the effect of solvent and interaction between solvent with extraction method were more important than extraction time (as main effect) and other interaction terms on TPC, TFC, IC_{50}, FRAP and BCB responses.

Only one method cannot provide a comprehensive prediction of the effect of the involved parameters on the antioxidant capacity of the plant (Kulisic et al., 2004).

In other words, the type of the measurement method is the main criterion in the evaluation of diverse antioxidant abilities of a wide variety of plant materials. According to Table 2, the BCB value is lower than FRAP, the low presence of low-polarity antioxidant components could be also possible in the extract of *I. tataricum* (Pall.) Schult. & Schult.f..

In this study, despite the reduction in solvent consumption using the UAE approach, in the most cases, soxhlet was selected as the optimum method, similar results have been reported by Motallebi Riekandeh et al. (2016). The results of this work along with previous studies showed that the choice of extraction method depends on different factors, such as plant type and target compounds.

Conflict of interest

The authors declare that there is no conflict of interest.

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