Bioactive properties of hydroalcoholic extract from *Origanum onites* L. as affected by glycerol incorporation

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In the current study, the effect of glycerol—as a green solvent-addition into solvent mixture (50:50 ethanoldistilled water), on some biofunctional properties of *Origanum onites* L. was investigated. Response surface methodology (RSM) was used to detect the optimum conditions for the extraction process. Three variables namely glycerol concentration (X₁: 1–9 g), extraction temperature (X₂: 25–75 °C) and time (X₃: 10–30 min) were selected and also total phenolic content, total flavonoid content, antioxidant capacity and antiradical activity of *O. onites* extracts were determined. Analysis of variance (ANOVA) showed that glycerol incorporation significantly increased the total phenolic content and antioxidant activity (p < 0.05) of the samples. Maximum levels to obtain the highest bioactive properties (highest total phenolic content and antioxidant activity) were determined as to be 9 g of glycerol addition for the extraction conditions as 45.4 °C and 75 min. This study reports the effect of glycerol on bioactive properties of *O. onites* and suggests that glycerol can be used to produce hydroalcoholic extracts having higher bioactivity from *Origanum* genus.

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1. Introduction

The *Origanum* genus consists of 10 sections including 49 species and 17 hybrids native to the Mediterranean, Euro-Siberian and Irano-Siberian regions, and they are locally endemic (Jedrzejczyk, 2018). The majority of *Origanum* species (ca. 75%) is found in the eastern Mediterranean subregion (Ozel and Kaymaz 2004).

The genus (*Oregano, Lamiaceae*) consists of annual, biennial or perennial medicinal and aromatic plants which are commonly utilized by the food, cosmetic, aromatherapy and pharmaceutical industries (Jedrzejczyk, 2018). *Origanum onites* is a perennial species having woody stems and differentiated from other *Origanum* species by its one-lipped calyces (Ozel and Kaymaz 2004). Several studies were conducted to investigate the antioxidant (Ozkan and Erdogan 2011; Pizzarelli et al. 2002; Ozkan et al. 2010), anticancer (Ozkan and Erdogan 2011; Bostancıoğlu et al. 2012), antiradical (Coskun et al. 2008), analgesic (Aydin et al., 1996), antibacterial (Baydar et al. 2004; Sarac and Ugur 2008), insecticidal (Cetin and Yanıkoğlu, 2006) and disinfectant properties (Copur et al. 2010). In these studies, essential oil or plant extracts were used as bioactive substance.

Extraction is the first and crucial step of any medicinal plant and the success of qualitative and quantitative studies of bioactive compounds from plant materials depends on the selection of proper extraction method. Among conventional methods to obtain bioactive substances, hydro-distillation (HD), steam distillation, Soxhlet extraction, and simultaneous distillation–extraction can be performed (Lucchesi et al. 2004). Besides, non-conventional methods known as environmentally friendly have been developed during the last 50 years regarding to decreased use of synthetic and organic chemicals, reduced operational time, and better yield and quality of extract (Azmir et al. 2013). Glycerol is manufactured from renewable sources and is a non-toxic, biodegradable, and recyclable liquid (Wolfson et al. 2006). Additionally, glycerol has advantages with its high boiling point, no flammability and low cost (Paleologou et al. 2016) and matches the green solvent definition (Philippi et al. 2016). Recent studies reported that...
water/glycerol mixtures may be very effective in extracting polyphenols (Apostolakis et al. 2014; Karakashov et al. 2015a, b).

In addition, for high yielded extraction results, types of extraction methods used are greatly important as well as the factor consideration and their linear, quadratic and interactive effects for understanding their effect on responses (Belwal et al. 2018). Response surface methodology is a combination of mathematical and statistical techniques based on the fit of a polynomial equation to the experimental data, which must describe the behavior of a data set with the objective of making statistical provisions. This methodology is one of the most commonly used technique among multivariate statistical methods for the aim of optimization of analytical procedures and also to understand linear, quadratic and interactive effects (Bezerra et al. 2008).

Within this scope, this study was designed to study the effect of glycerol incorporation into the extraction solvent on the bioactive properties of O. onites L. Additionally, the processing variables having effects on the extraction of O. onites were optimized by response surface methodology approach. The present study seems to be the first report on gaining the bioactive properties of Oregano anum genus by using glycerol as a green solvent.

2. Material and methods

2.1. Materials

In the current study, O. onites was purchased from Boralife Medicinal Plant Company (Ankara, Turkey) as dried material. Glycerol was purchased from Tekkim Co. (Turkey).

2.2. Extraction of O. onites

For the extraction of the samples, 1 g of ground O. onites sample (aerial parts) was weighed and 30 ml of extraction solvent (50:50 ethanol:distilled water) was incorporated into the bottle. After 1 min mixing of the sample, different amounts of glycerol (1–9 g) were added to the sample and the bottles were placed into the water bath for the extraction. After the extraction of the samples at different temperatures (25–75°C) for different periods (10–50 min), the samples were cooled down and subjected to filtration by 0.45 μm syringe filter after centrifugation at 7500 rpm for 5 min at 4°C and the filtrate was used for further analysis. To model the processing variables and their levels, a response surface methodology was used. The glycerol concentration (X1), extraction temperature (X2) and extraction time (X3) were selected as processing variables and Box-Behnken experimental design was constructed. Table 1 shows the coded and uncoded levels of the studied variables.

### Table 1

| Runs | Coded values | Actual values |
|------|--------------|---------------|
|      | X1           | X2            | X3            | Glycerol (g) | Temperature (°C) | Time (min) |
| 1    | –1.00        | –1.00         | 0.00          | 1            | 25             | 30         |
| 2    | 0.00         | 1.00          | –1.00         | 5            | 75             | 10         |
| 3    | 0.00         | 0.00          | 0.00          | 5            | 50             | 30         |
| 4    | 0.00         | –1.00         | –1.00         | 5            | 25             | 10         |
| 5    | 0.00         | –1.00         | 1.00          | 5            | 25             | 50         |
| 6    | 1.00         | 1.00          | 0.00          | 9            | 75             | 30         |
| 7    | 0.00         | 1.00          | 1.00          | 5            | 75             | 50         |
| 8    | –1.00        | 0.00          | 1.00          | 1            | 50             | 50         |
| 9    | –1.00        | 0.00          | –1.00         | 1            | 50             | 10         |
| 10   | 0.00         | 0.00          | 0.00          | 5            | 50             | 30         |
| 11   | –1.00        | 1.00          | 0.00          | 5            | 75             | 30         |
| 12   | 1.00         | –1.00         | 0.00          | 9            | 25             | 30         |
| 13   | 0.00         | 0.00          | 0.00          | 5            | 50             | 30         |
| 14   | 1.00         | 0.00          | 1.00          | 9            | 50             | 50         |
| 15   | 1.00         | 0.00          | –1.00         | 9            | 50             | 10         |

2.3. Determination of total phytochemical constituent concentrations

2.3.1. Analysis of total phenolic content (TPC)

Total phenolic content of the samples was determined according to the methods of Singleton and Rossi (1965). For this aim, 200 μl of extract was placed in a tube and then 1800 μl of distilled water was added. After that, 1 ml of Folin Ciocalteu reagent (1/10 diluted) was incorporated and waited for 1 min. At the end, 2 ml of sodium carbonate (2% w/v) was added and the tubes were covered, vortexed and incubated for 2 h in a dark place at room conditions (24–25°C). The absorbance values of the samples were measured at 765 nm by a UV–vis spectrophotometer (UV–1800, Shimadzu, Japan) at the end of the incubation, and the total phenolic content of the samples was calculated as mg gallic acid equivalent (mg GAE)/g.

2.3.2. Analysis of total flavonoid content (TFC)

Total flavonoid analysis of the samples was performed according to the method suggested by Zhishen et al. (1999). Two ml of distilled water was added into tube including 0.5 ml of sample extract. Then 150 μl of sodium nitrite (5% w/v) was placed into the tubes and after 5 min waiting, 150 μl of AlCl3 (5% w/v) was incorporated. After waiting for 6 min, 1 ml of NaOH (1 M) and 1.2 ml of distilled water were added and the final mixture was vortexed and the absorbance of these mixtures was recorded at 510 nm by a UV–vis spectrophotometer (UV–1800, Shimadzu, Japan) and the total flavonoid content of the samples was calculated as mg catechin equivalent (mg CE)/g.

2.4. Determination of antioxidant activity of samples

2.4.1. Ferrous ions chelating activity (ICA)

Ferrous ions (iron) chelating activity (ICA) of the samples was determined according to methods of Rival et al. (2001) and Duh et al. (2001) after some minor modifications. For this aim, 1 ml of diluted samples as 1:30 was mixed with 3.7 ml of ethanol (96% v/v) and 100 μl of FeCl2 (2 mM in distilled water). The tubes were vortexed and covered tightly and incubated for 10 min at room conditions in a dark place. After the incubation, 200 μl of ferrozine (5 mM in distilled water) was incorporated and all samples were subjected to incubation for 10 min again. After that, the absorbance of the samples was recorded at 562 nm by a UV–vis

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Table 2

Bioactive properties of *O. onites* as affected by processing variables.

| Runs | TPC (mg GAE/g) | TFC (mg CE/g) | ICA (% Inh.) | FRAA (mg AAЕ/g) | ABTS⁺ (μg TE/g) |
|------|----------------|---------------|--------------|-----------------|-----------------|
| 1    | 32.03          | 34.17         | 58.22        | 51.88           | 75.00           |
| 2    | 40.71          | 46.65         | 51.96        | 78.58           | 96.00           |
| 3    | 39.43          | 50.50         | 51.34        | 81.00           | 95.17           |
| 4    | 30.08          | 31.02         | 56.00        | 70.81           | 65.88           |
| 5    | 40.23          | 35.95         | 48.41        | 70.13           | 90.86           |
| 6    | 53.75          | 52.54         | 64.17        | 97.76           | 123.00          |
| 7    | 45.89          | 50.73         | 49.60        | 86.33           | 101.71          |
| 8    | 41.72          | 44.89         | 49.15        | 74.64           | 77.28           |
| 9    | 39.62          | 42.35         | 50.09        | 76.02           | 98.29           |
| 10   | 43.76          | 51.02         | 48.52        | 79.56           | 104.17          |
| 11   | 39.32          | 46.79         | 47.29        | 70.81           | 95.83           |
| 12   | 38.60          | 37.03         | 57.14        | 72.13           | 83.43           |
| 13   | 42.62          | 52.33         | 50.29        | 82.85           | 99.48           |
| 14   | 59.11          | 54.70         | 54.66        | 92.99           | 114.44          |
| 15   | 38.88          | 48.17         | 57.71        | 88.25           | 90.00           |

TPC: Total phenolic content, TFC: Total flavonoid content, ICA: Iron chelating activity, FRAA: Ferric reducing antioxidant activity, ABTS⁺: 2,2-Azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) radical scavenging activity

Fig. 1. 3D surface plots for the effect of processing variables on total phenolic content (TPC) of *O. onites*. 
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Table 3
Effect of processing variables on studied parameters by ANOVA.

| Parameters¹ | TPC    | TFC    | ICA    | FRAA   | ABTS² |
|-------------|--------|--------|--------|--------|-------|
| Model       |        |        |        |        |       |
| Linear      | 18.54*** | 69.13*** | 17.69** | 22.63 | 10.6¹ |
| X₁       | 24.52*** | 1.87    | 24.08** | 0.06  | 8.93** |
| X₂       | 13.50*** | 22.03*** | 8.59*   | –     | 9.44** |
| X₃       | 25.92*** | 230.9*** | 27.46** | 34.3*** | 22.09** |
| Cross product | X₁X₂    | 11.36*** | –      | –     | 8.88** |
| X₁X₃    | –      | –      | 28.51** | –     | –     |
| X₂X₃    | –      | –      | –      | –     | –     |
| Quadratic | X₁²     | –      | 2.43   | 22.33** | 0.19 |
| X₂²     | –      | 13.98*** | –      | –     | –     |
| X₃²     | 6.37** | 111.4*** | 9.67*  | 12.1*** | –     |
| Lack of fit | 1.57    | 2.45   | 1.52   | 7.63  | 3.34  |
| R²       | 0.910  | 0.981  | 0.930  | 0.900 | 0.809 |

¹ X₁: Glycerol (g); X₂: Temperature (%); X₃: Time (min); TPC: Total phenolic content; TFC: Total flavonoid content; ICA: Iron chelating activity; FRAA: Ferric reducing antioxidant activity, ABTS²: 2,2-Azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) radical scavenging activity.

spectrophotometer (UV-1800, Shimadzu, Japan) and the chelating activity was calculated using the following Eq. 1:

\[
\text{Chelating ability} = \left( \frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}} \right) \times 100
\]  

(1)

where \( A_{\text{sample}} \) is the absorbance of the sample; \( A_{\text{control}} \) is the absorbance of control without sample.

2.4.2. Ferric reducing antioxidant activity (FRAA)

The ferric reducing antioxidant activity (FRAA) of the samples was characterized according to the methodology of Oyaizu (1986). In this regard, 1 ml of diluted extract sample with phosphate buffer (pH 6.6) was mixed with 2.5 ml of phosphate buffer (pH 6.6) again. After that, 2.5 ml of potassium ferricyanide (1% w/v) was placed into the tubes and then all samples were incubated for 6 min and finally the absorbance values of the mixture were registered at 734 nm by UV-Vis spectrophotometer (UV-1800, Shimadzu, Japan) and the results were expressed as mg ascorbic acid equivalent (mg AAE)/g.

2.5. Determination of antiradical activity

The ABTS⁺ radical scavenging activity of the samples was determined according to the methodology proposed by Wettasinghe et al. (2002) and Mathew & Abraham (2006). For this purpose, ABTS⁺ radical was prepared by dissolving the 96 mg of ABTS⁺ radical cation in 5 ml of potassium persulfate (0.1% w/v) and 20 ml of distilled water. The final solution was adjusted as 0.7 ± 0.05 at 734 nm by the spectrophotometer. After that, four different concentrations (25, 50, 75 and 100 μl) of diluted extracts (1:30) were placed into the spectrophotometer cuvette and 2 ml of ABTS⁺ radical cation was incorporated into the extracts. The final mixture was incubated for 6 min and finally the absorbance values of the samples were measured at 734 nm using a spectrophotometer (UV-1800, Shimadzu, Japan). The radical scavenging activity of the samples as % inhibition was calculated using the following equation (Eq. 2).

\[
\%\text{Inhibition} = \left( \frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}} \right) \times 100
\]  

(2)

where \( A_{\text{sample}} \) is the absorbance of ABTS⁺ with sample; \( A_{\text{control}} \) refers to the absorbance of ABTS⁺ without sample. All results were expressed as Trolox equivalent antiradical capacity (μg TE/g sample).

2.6. Data analysis, modeling and optimization

In this study, a 3-factor-3-level Box-Behnken experimental design (Box and Behnken, 1960) with three replicates at the center point was used to develop predictive models for studied parameters. Table 1 showed the factors (processing variables), levels and among the processing variables and to determine the regression coefficients of linear, quadratic and interaction term. Also, the postulated polynomial model as shown in Eq. (3).

\[
Y - \bar{Y} = \beta_0 + \sum_{i=1}^{N} \beta_i X_i + \sum_{i=1}^{N} \beta_{ij} X_i^2 + \sum_{i=1}^{N} \sum_{j=i+1}^{N} \beta_{ij} X_i X_j,
\]  

(3)

where \( Y \) is the corresponding predicted response value, \( \beta_0 \) is the intercept term, \( \beta_i \) is the linear term, \( \beta_{ij} \) is the quadratic term, \( \beta_{ij} \) is the interaction term, and \( X_i \) and \( X_j \) are the coded levels of the independent variables. The regression coefficients of linear, quadratic and interaction terms were determined by using Design Expert package software (Design-Expert® Software Version 7.0 (Stat-Ease Inc., Minneapolis, USA)) for each output parameter.

In this regard, glycerol concentration (1, 5 and 9 g), extraction temperature (25, 50 and 75 °C) and extraction time (10, 30 and 50 min) were selected as the processing variables. Design-Expert® Software Version 7.0 (Stat-Ease Inc., Minneapolis, USA) was used for the all computational work including designation of experimental points, analysis of variance, fitting of the second-order polynomial models and graphical representations and optimization. Optimization procedure was performed by using desirability function of the Design-Expert® Software Version 7.0 (Stat-Ease Inc., Minneapolis, USA) and maximum and minimum response values and their corresponding extraction conditions were calculated. Analysis of variance (ANOVA) was performed to see the differences among the processing variables and to determine the regression coefficients of linear, quadratic and interaction term.
determination of coefficients ($R^2$) were calculated and the F values were computed to show the significance of the dependent variables ($p < 0.05$). Backward elimination process was also followed to discard the non-significant parameters in the predicted regression models at the significant level of 0.05. The regression models were created as final equation in terms of coded or actual factors (Table 1).

3. Results and discussion

3.1. Total phenolic content

Table 2 shows the averaged total phenolic and flavonoid contents, antioxidant capacity and antiradical activity values of O. onites L extracts. The highest TPC value (59.11 mg GAE/g) was observed in 14th run (9 g glycerol, 50 °C and 50 min) while the lowest one (30.08 mg GAE/g) was obtained from 4th run (5 g of glycerol, 25 °C and 10 min). Linear effects of glycerol, temperature and time were determined to be significant on the total phenolic content of the samples ($p < 0.05$). Additionally, interaction effects of glycerol and temperature showed also significant increase on the TPC of the O. onites while the quadratic effect of extraction time caused a decrease for the TPC ($p < 0.05$). The fitted second order polynomial equation for total phenolic content showing the increase or decrease effect of the processing variables is as follows:

$$Y_{TPC} = 96.5 + 47.8X_1 + 55.7X_2 + 4.84X_3 + 45.3X_1X_2 - 3.51X_3$$

The effect of these processing variables on TPC could also be seen from Fig. 1. Besides, the interaction effects of glycerol and temperature resulted a significant increase for the TPC value ($p < 0.05$) and $R^2$ (0.910) and the non-significant lack of fit (F = 1.57) showed that the model was fit a good prediction (Table 3). Eyiz et al. (2020) performed an investigation for the bioactivity of red grape pomace extracted by aqueous glycerol and they reported that the use of glycerol provided a significant increase in total phenolic content of the samples. Similar result for the increased bioactivity by glycerol incorporation was also reported by Bidi et al. (2015) and Philippi et al. 2016. Semiz

![3D surface plots for the effect of processing variables on total flavonoid content (TFC) of O. onites.](image)
et al. (2018) informed that chloroform extracts of *O. onites* had the highest total phenolic content as to be 45.17 mg GAE/g while Ozkan et al. (2010) measured the total phenolic content of methanol extract from *O. onites* as in the range of 106.13 and 149.40 mg GAE/g extract. These variations could be explained by the differences of used solvent and also the used plant material. In a different study performed by Shehata et al. (2015), it was proved that glycerol incorporation up to 90% (w/v) provided an increase in the yields of total polyphenols from two Artemisia species as similar to our results. Supporting effects were also observed in the study of Karakashov et al. (2015a) showing that 10% (w/v) of aqueous glycerol at 70°C provided the higher extraction yield in total polyphenols of *Hypericum perforatum*.

### 3.2. Total flavonoid content

Total flavonoid contents of the samples were in the range of 31.02–54.70 mg CE/g. The highest TFC was for the sample showing the highest TPC (run14) and vice versa (run 4). It was observed that the temperature and time variables effected the total flavonoid content significantly (p < 0.05) while no significant effect of glycerol was monitored. The predicted second order polynomial equation is as follows:

\[
Y_{TFC} = -54.53 - 218.4X_1 + 2.26X_2 + 7.32X_3 - 110.4X_1^2 - 2.65X_2^2 - 7.55X_3^2 \quad (R^2 = 0.981)
\]

As could be seen from the equation, time and temperature variables increased the total flavonoid content while glycerol showed a non-significant decrement for the TFC. Also, quadratic effect of time and temperature showed significant decrease for the TFC of the samples (p < 0.05). Fig. 2 also shows the effects of processing variables on total flavonoid content of *O. onites*. As is seen, increase in time and temperature increased the TFC while there was a slightly change by the glycerol increment (p > 0.05). Nizioł-Łukaszewska et al. (2019) investigated the biological properties of *Plantago lanceolata* extracts and stated that the use of glycol and glycerin allowed isolation of caffeic, synaptic, and salicylic acid derivatives that were not present in the ethanolic and aqueous extracts. Based on this information, it could be said that because
of these compounds are phenolic acids, the glycerol effected the total phenolic content of extracts while it did not affect the flavonoid content of Origanum onites extracts.

3.3. Antioxidant capacity and antiradical activity

Antioxidant capacity values were measured with two methods as iron chelating (ICA) and ferric reducing antioxidant activity (FRAA). The ICA and FRAA values ranged between 47.29 and 64.17% and 51.88–97.76 mg AAE/g, respectively. The highest antioxidant capacity values determined for 6th run (9 g of glycerol, 75 °C and 30 min) for both two methods. The lowest ICA value (47.29%) was observed for 11th run (1 g of glycerol, 75 °C and 30 min) while 1st run (1 g of glycerol, 25 °C and 30 min) was for the FRAA test. In accordance to antioxidant capacity values, the highest antiradical activity value (123 l g TE/g) characterized by ABTS+ radical cation was detected in 6th run (9 g of glycerol, 75 °C and 30 min). Besides the lowest value (65.9 µg TE/g) was observed in 4th run (5 g of glycerol, 25 °C and 10 min). ICA values changed significantly with the increase of glycerol incorporation and extraction time (p < 0.05, Table 3) while temperature did not alter the ICA significantly (p > 0.05, Table 3). In addition to these results, the interaction effect of glycerol and extraction time affected the ICA values of the samples significantly (p < 0.05). Effect of processing variables on ICA and FRAA values of O. onites was also shown in Fig. 3. The fitted second order polynomial equation created by using actual factors for ICA is as follows:

$$Y_{ICA} = 77.6 - 3.91X_1 - 0.09X_2 - 0.69X_3 + 0.05X_1X_3 + 0.26X_1^2 + 0.04X_3^2 \left( R^2 = 0.930 \right)$$

Only extraction time variable effected the FRAA value significantly (p < 0.05, Table 3) while the other two variables namely glycerol and extraction temperature showed no significant effect on FRAA values of the sample (p > 0.05). The fitted second order polynomial equation for FRAA is as follows:

$$Y_{FRAA} = 73.3 - 113.9X_1 + 8.57X_3 - 93.9X_1^2 - 7.45X_3^2 \left( R^2 = 0.901 \right)$$

As could be seen from the equation, the increment of extraction time caused an increment of FRAA value. It was reported that the ferric reducing antioxidant activity of the extracts depends on the phenolic concentration and a significant correlation (r = 0.911) between phenolic concentration and ferric reducing.

Fig. 4. 3D surface plots for the effect of processing variables on ABTS+ radical scavenging activity of O. onites.
In the current research, a high and significant correlation between temperature also provided an increase of ABTS scavenging performance (p < 0.05). Similarly, extraction time and that the increase in the glycerol resulted a sharp increase in the activity. Additionally, a significant effect was also observed for the interaction of glycerol and extraction temperature. The fitted second order polynomial equation is as follows:

$$Y_{ABTS^-} = 184.7 + 80.6X_1 + 132.1X_2 + 12.7X_3 + 113.6X_1X_2 \left( R^2 = 0.810 \right)$$

Philippi et al. (2016) conducted an investigation for the ultrasound assisted extraction of eggplant peel with ethanol:water and glycerol:water and they indicated that the water/glycerol might be a more effective solvent system for total chlorogenerates, total flavonoids and total pigments, in contrast for antiradical activity and reducing power. These findings are consistent with our results and the mentioned authors also informed that correlation between the polyphenolic content and the antioxidant activity is not always statistically significant.

### 3.4. Optimization of the processing variables

The multiple response optimization values for the production of glycerol added hydroalcoholic extracts from *O. onites* could be seen from Table 4. Minimization and maximization process were performed to detect the optimum conditions for the processing variables. Minimization process results showed that minimum levels would be obtained when glycerol content was 4.27 g and extraction conditions were 25 °C and 10 min. At these conditions, TPC level was 28.83 mg GAE/g while the other values were predicted as to be 31.02 mg CE/g, 54.44%, 66.59 mg AAE/g and 73.75 μg TE/g for TFC, ICA, FRAA and ABTS values, respectively. Desirability function showing the prediction performance was calculated as 0.851 for the minimization process. For the maximization process, it was calculated that the maximum levels for the studied responses by 9 g of glycerol incorporation and extraction conditions at 45.4 °C and 75 min. At these conditions TPC value was predicted as to be 58.99 mg GAE/g sample while they were estimated as to be 54.47 mg CE/g, 63.79%, 97.55 mg AAE/g, 127.8 μg TE/g for TFC, ICA, FRAA and ABTS values, respectively. Desirability function showing the prediction performance was calculated as 0.951 for the maximization process.

### 4. Conclusion

The present study focused on the glycerol incorporation into the hydroalcoholic extraction solvent to increase the bioactive properties of extracts from *O. onites*. In most of studies related to bioactive properties of *O. onites*, different solvents were used such as methanol, ethanol, chloroform and acetone. In this study, to decrease the usage of synthetic chemicals, glycerol was incorporated into hydroalcoholic mixture and the change in bioactive properties was monitored. Besides, the best extraction conditions were determined using response surface methodology approach. Glycerol addition increased the total phenolic content, antioxidant capacity and antiradical scavenging performance of *Origanum onites*. The results of the study could be useful for different industries which process the aromatic and medicinal plants.

### Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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| Table 4 | Multiple response optimization values of *O. onites* extract. |
|---------|-------------------------------------------------|
| Response parameters | Minimization process | Maximization process |
| $X_1$ (g) | $X_2$ ($^\circ$C) | $X_3$ (min) | Desirability | $X_1$ (g) | $X_2$ ($^\circ$C) | $X_3$ (min) | Desirability |
| 4.27 | 25 | 10 | 0.851 | 9.00 | 45.4 | 75 | 0.951 |
| TPC (mg GAE/g) | 28.83 | | | 58.99 | | | |
| TFC (mg CE/g) | 31.02 | | | 54.47 | | | |
| ICA (% Inh.) | 54.44 | | | 63.79 | | | |
| FRAA (mg AAE/g) | 66.59 | | | 97.55 | | | |
| ABTS$^+$ (μg TE/g) | 73.75 | | | 127.8 | | | |
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