

## HASS AVOCADO LEAVES: OPTIMIZATION OF MICROWAVE-ASSISTED EXTRACTION PARAMETERS, PHENOLIC COMPOUNDS, ANTIOXIDANT, AND ANTIDIABETIC ACTIVITIES

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### ABSTRACT

Avocado leaves, typically considered as pruning residues, possess a significant amount of bioactive compounds. This research aimed to optimize the extraction of phenolic compounds from Hass avocado leaves using microwave-assisted extraction (MAE) and response surface method (RSM). The extraction yield and total phenolic content (TPC) were maximized by determining the optimal process conditions, which were found to be 47°C for 5 minutes and a solid/solvent ratio of 1.13 g dry leaf/100 mL, respectively. The predicted values of all models were found to be statistically significant ( $p < 0.001$ ). The aqueous extracts' antidiabetic and antioxidant activities were 64.59% and 235.6 mg TE/100 g, respectively. The amount of TPC was 591.76 µg GAE/g extract, and chlorogenic acid was the main phenolic component. These results indicated that MAE proved efficient with low energy consumption, yielding phenolic-rich avocado leaf extracts, which possess high antioxidant and antidiabetic activities.

**Keywords:** Avocado (*Persea americana* Mill.), microwave-assisted extraction response surface method (RSM), antidiabetic, pruning wastes

## HASS AVOKADO YAPRAĞI: MİKRODALGA DESTEKLİ EKSTRAKSİYON PARAMETRELERİ, FENOLİK BİLEŞİKLER, ANTIOKSİDAN VE ANTİDİYABETİK AKTİVİTELERİN OPTİMİZASYONU

### ÖZ

Budama atığı olan avokadonun yaprakları biyoaktif bileşenler bakımından zengindir. Bu çalışmanın amacı, Hass çeşidi avokado yapraklarından fenolik bileşiklerin mikrodalga destekli ekstraksiyon (MAE) ile ekstraksiyon parametrelerini yanıt yüzey yöntemi (RSM) ile optimize etmektir. Ekstraksiyon verimi ve toplam fenolik madde miktarı (TPC) için optimum proses koşulları sırasıyla 47°C'de 5 dakika ve 1.13 g kuru yaprak/100 mL olarak belirlenmiştir. Tüm modellerin tahmin edilen değerleri istatistiksel olarak anlamlı bulunmuştur ( $p < 0.001$ ). Sulu ekstraktların antidiyabetik ve

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antioksidan aktiviteleri sırasıyla % 64.59 ve 235.6 mg TE/100g olarak belirlenmiştir. TPC miktarı 591.76 µg GAE/g ekstrakt olup ana fenolik bileşen klorojenik asittir. Bu sonuçlar, MAE'nin düşük enerji tüketimi ile yüksek antioksidan ve antidiyabetik aktivitelere sahip fenolik bakımından zengin avokado yaprağı ekstreleri vererek etkili olduğunu kanıtladı.

**Anahtar kelimeler:** Avokado (*Persea americana* Mill.), mikrodalga destekli ekstraksiyon, yanıt yüzey yöntemi (RSM), antidiyabetik, budama atığı

## INTRODUCTION

*Persea americana* Mill., commonly known as avocado, is a plant in the family Lauraceae. The main avocado-growing countries are Mexico, Chile, USA, Australia, South Africa, Peru, Indonesia, and Israel (FAOSTAT, 2021). Fuerte, Bacon, Zuatona, and Hass varieties are widely grown in our country. Antalya (69.5%) and Mersin (29.2%) are among the most important provinces in the cultivation area. About 80% of Antalya's total production comes from the districts of Alanya and Gazipaşa (TUIK, 2015).

In addition to the consumption of avocado fruits as a food, it has been historically employed for their antioxidant, anticancer, antibacterial, and cardiovascular protective effects (Hughes et al., 2009; Noorul et al., 2016; Owolabi et al., 2010; Rodriguez-Mateos et al., 2014; Unsal Pinar et al., 2019). Avocado leaves, which are pruned waste, have high phenolic content such as p-hydroxybenzoic acid, gallic acid, catechin, p-coumaric acid, chlorogenic acid, rutin, caffeic acid, epicatechin, syringic acid, and quercetin (Castro-López et al., 2019; Gümüştepe et al., 2022) and *in vitro* antioxidant, antidiabetic, and anticancer activities as well as their effects on cardiovascular diseases (Castro-López et al., 2019; Gümüştepe et al., 2022).

Extraction technology of bioactive constituents is a growing field day by day and today the demand to use new extraction techniques is increasing due to some disadvantages of traditional extraction methods (Sahena et al., 2009). The main advantages of the new advanced extraction technology are compatible with automation, high extraction efficiency, minimizing energy and solvent consumption, reduce extraction time, and eco-friendly technology (Sparr Eskilsson and Björklund, 2000; Xing et al., 2017).

MAE is an innovative extraction technique that combines microwave extraction and conventional solvents (Delazar et al., 2012). By rapidly heating the mixture of sample and solvent, MAE enables extraction to be performed at the ideal temperature efficiently. In most cases, the reproducibility and recovery of the compound are high compared to conventional techniques (Sparr Eskilsson and Björklund, 2000). It has several advantages such as shorter extraction time, less solvent, high extraction rate, and low cost. Due to technological advancements, MAE has emerged as a widely adopted and economically viable technique for extraction purposes (Delazar et al., 2012).

RSM is a statistical method extensively used in food processes and mainly in extraction, process variables, and their optimization. The central composite design (CCD) is an effective tool for process optimization (Guaracho et al., 2009) and is one of the most widely used experimental designs in engineering-related studies (Radojković et al., 2013).

The extraction of phenolic compounds is carried out using different solvents and methods. In the literature, there have been a number of studies using the ultrasonic extraction method of avocado leaves and using alcohol, ethanol: water, and aqueous solvents (Che-Galicia et al., 2020; Hefzallahman et al., 2022; Monzón et al., 2021; Salar Bashi et al., 2011). However, there has been no study of microwave-assisted extraction (MAE) in avocado leaves.

Globally, the number of individuals with diabetes was 451 million in 2017, and it is expected to increase to 693 million by 2045 (Nam Han Cho., 2017). The impact of diabetes is significant, with 1.5 million adult deaths annually attributed to the disease, along with 2.2 million deaths related to hyperglycemia-related conditions (WHO, 2016).

Given these statistics, there is a growing interest in alternative and natural treatment approaches for diabetes management, driven by concerns over the rising costs of conventional treatments and their associated side effects.

Based on the above information, the objective of the study was to optimize the process conditions for microwave-assisted water extraction of phenolic compounds from Hass avocado leaves by RSM. In addition, it aimed to determine the bifunctional properties (antioxidant and antidiabetic activity) and some bioactive components of the extract.

## MATERIAL AND METHODS

### Material

Avocado Hass variety leaves were collected from the Gazipaşa district of Antalya at the time of pruning of the tree. They were brought to the laboratory on the day of collection, cleaned, and dried. Before and after drying, 5 grams of the leaves were placed in a rapid moisture analyzer (Radwag, Poland) and their moisture (%) content was calculated. Washed and dried avocado leaves were dried at 70°C by adjusting the temperature in the Mikrotest brand thermostat (TT107) drying oven (Turkey). The moisture content of the samples was reduced from 48.22% to 6.60% after drying. Before extraction, dried avocado leaves were shredded with a laboratory grinder (Waring Commercial Laboratory Blender, USA). The

ground samples were passed through a 0.5 mm sieve (Kocintok, Turkey) and were vacuum packed in polyethylene/polyamide bags and stored at -18°C until extraction.

### Methods

#### Extraction of avocado leaves

MAE, which is a modern extraction technique and the principle of MAE is based on the polarization of polar water molecules in food by the electric field whereas this vibration of molecules occurs heat which then increases extraction efficiency and decreases extraction time (Gumustepe et al., 2023). In this study, water, a solvent that is environmentally friendly and safe for human health was utilized to extract phenolic compounds.

#### Optimization of microwave-assisted water extraction process conditions

RSM was chosen for the optimization of phenolic-rich MAE aqueous extract (Millipore, Italy) from dried and ground leaves, and CCD was preferred for the experimental design. The minimum and maximum factor levels of temperature (°C) and solid/solvent ratio (g/100 mL) from the independent variables are in Table 1 and the results of the central composite design of 13 different applications including 2 central points for microwave-assisted water extraction of Hass avocado leaf are given in Table 2.

Table 1. Coded and uncoded factor levels of independent variables

Independent variables	Factor levels		
	-1	0	1
X <sub>1</sub> : Temperature (°C)	30	45	60
X <sub>2</sub> : Solid/Solvent ratio (g/100mL)	1	4.25	7.5

Experimental data were analyzed using the Minitab statistical program (Minitab 20.0). Model fit, regression tests, R<sup>2</sup>, and adjusted R<sup>2</sup> values were considered. The response surfaces and designs of the model were determined according to the second-order quadratic equation. As a response, the yield of the extracts and the total phenolic content were used.

#### Extraction yield and TPC

Extracts of avocado leaves obtained by microwave-assisted water extraction using CCD were filtered and centrifuged to remove supernatant (2-6, Sigma, Germany). A sample of 10 mL was taken from the filtrate and dried in an oven at 105 °C until constant weight. After drying, they were weighed, and the extraction

yield was calculated as % (g extract/100 g sample). Analyzes were performed in two replications.

Table 2. Central Composite Design used for RSM

Run <sup>a</sup>	Temperature(°C)	Solid/Solvent ratio (g/100mL)
1	45	7.5
2	45	4.25
3	45	4.25
4	34	1.95
5	45	1.95
6	56	1.95
7	30	4.25
8	60	4.25
9	45	4.25
10	34	6.55
11	45	4.25
12	45	4.25
13	56	6.55

<sup>a</sup>Randomized

After centrifuging the samples obtained by microwave-assisted water extraction from avocado leaves using CCD, TPC amounts of the samples were determined using the Folin & Ciocalteu method (Ainsworth and Gillespie, 2007). 0.4 mL of phenolic extracts were taken and pipetted into a tube. Then, 2 mL of 10-fold diluted Folin & Ciocalteu reagent was added and mixed by vortex (Darmstadt, Germany). Then, 1.6 mL of 7.5% Na<sub>2</sub>CO<sub>3</sub> (sodium carbonate) solution was added and mixed with vortex again. After the mixture was left in the dark for one hour, the absorbance values of the solution were measured against the blank solution with a UV/VIS spectrophotometer (T70, PG Instruments, UK) at a wavelength of 765 nm. Results were calculated using the gallic acid calibration curve. Analyzes were performed in triplicate.

#### DPPH free radical scavenging activity

After centrifuging the samples obtained by microwave-assisted water extraction from avocado leaves using CCD, the antioxidant effect of the samples was determined using the DPPH free radical scavenging activity assay developed by Dorman and colleagues (2003). 50 µL of the phenolic extract was taken, and pipetted into a

tube, 450 µL of Tris-HCl buffer (50 mM, pH: 7.4) was added and mixed by vortex. Then, 1.00 mL of DPPH (0.10 mM, in methanol) solution was added to the mixtures, vortexed, and left in a dark environment at room temperature for 30 minutes. At the end of the reaction time, the absorbance of the solution was read in the spectrophotometer at 517 nm. Pure water was used instead of phenolic extract as a control. The DPPH activities of the extracts prepared at different concentrations were calculated using the formula given below (Equation 1):

$$\% \text{ Inhibition (DPPH)} = \left[ \frac{\text{Abs}_{\text{Control}} - \text{Abs}_{\text{Sample}}}{\text{Abs}_{\text{Control}}} \right] * 100 \quad (1)$$

Here Abs<sub>Control</sub> and Abs<sub>Sample</sub> represent absorbance readings for the control and sample, respectively. The results were calculated by using the calibration curve (R<sup>2</sup>=0.9973) of the absorbance values obtained at 517 nm in the spectrophotometer of the solutions at different concentrations prepared with the Trolox<sup>®</sup> (99.87%) standard (Sigma, Germany) and given as mg TE/100 g extract.

#### Phenolic composition

The samples with the highest yield and TPC of avocado leaf extracts obtained by microwave-assisted water extraction were filtered, centrifuged, and the filtrates were placed in 2 mL Eppendorf. Then, these extracts were analyzed in the high-performance liquid chromatography (HPLC) device and diode array detector (DAD) (Shimadzu, Japan). Reversed-phase Agilent Eclipse XDB-C18 (250x4.60 mm) 5-micron analytical column was used in the study. The wavelength was set at 278 nm, the flow rate was 0.8 mL/min, the injection volume was 20 µL, and the column oven temperature was 30 °C. A: 3.0% acetic acid: methanol was used as the mobile phase. The profile of phenolic substances was determined using the gradient program of Delil and colleagues (2022). The quantification of phenolic compounds in the extract was determined by measuring the concentration in micrograms per gram of extract (µg/g) using calibration curves specific to phenolic

compounds. Analyzes were performed in triplicate.

### Antidiabetic activity

The measurement of antidiabetic activity is based on the principle of detecting the activities of  $\alpha$ -glucosidase enzymes (maltase, isomaltase, and sucrase) in rat intestinal powder (Nyambe-Silavwe et al., 2015). 200  $\mu$ L of the substrate was mixed with 200  $\mu$ L of enzyme and 100  $\mu$ L of buffer solution and incubated for 10 min at 37°C in a water bath (Selecta, Spain). Then, to stop the enzyme activity, 750  $\mu$ L of acetone was added and mixed by vortex for 10 seconds and then centrifuged for 5 minutes. Finally, the acetone in the obtained samples was evaporated with nitrogen gas. High-performance liquid chromatography (HPLC) device and refractive index detector (RID) (Shimadzu, Japan) were used to measure the amounts of fructose and glucose formed by decomposition of sucrose. Lactose was used as the internal standard. In addition, for the validation of the experiment and the antidiabetic activity of the extracts, metformin used in the treatment of diabetic patients was added to the analysis as a positive control.

For optimization of the experiment, Michaelis Constant ( $K_m/V_{max}$ ) was measured and hydrolysis of sucrose was performed at 37 °C using different enzyme concentrations and incubation times according to  $K_m$  (mM),  $V_{max}$  (0.09  $\mu$ mol sucrose hydrolysis/minute). From the data obtained, the highest value of enzyme-specific activity and rat intestinal powder concentrations were determined as optimum incubation time and enzyme concentration.

### Statistical Analysis

Extraction applications were carried out in 2 replications and analyzes in 3 parallels according to the trial plan. Minitab package program RSM was used to evaluate the responses obtained and to find the optimum points for avocado leaf extraction. Obtained experimental data were analyzed using Minitab statistical analysis software program (Minitab 20.0) and the performance of the model was evaluated by considering  $R^2$  values. CCD was used in model design. The model was

evaluated according to bivariate quadratic equality. In the equation, X was determined as an independent variable, Z dependent variable, constant coefficient, first-order (linear) equation coefficient, second-order equation coefficient, and two-factor cross-interaction coefficient.

## RESULTS AND DISCUSSION

Avocado, with its low sugar content, rich bioactive components, nutrients, and antioxidant properties has positive effects on human health. The antioxidant, antidiabetic, anticancer, and cardiovascular properties of the leaf, which is the waste of avocado, have been reported in scientific studies (Castro-López et al., 2019; Gümüštepe et al., 2022). In this study, MAE processing conditions of phenolic substances, which are the bioactive component of the avocado leaf of the Hass variety, were optimized with RSM and CCD. In addition, physicochemical properties, bioactive components, and biofunctional properties (antioxidant and antidiabetic activity) of Hass leaf water extracts extracted under optimum conditions were determined.

### Extraction Modeling of the Yield and TPC of Water Extracts of Hass Avocado Leaves

The extraction yield (%) and TPC ( $\mu$ g GAE/g extract) of Hass avocado leaves under different extraction conditions are shown in Table 3. The evaluation results of the model coefficients are given in Table 4.

In Table 3, the yields of microwave-assisted water extracts of Hass avocado leaf were found to be between 15.07% and 19.67%. In microwave-assisted water extraction of avocado leaf, the lowest extraction efficiency was determined in the samples where 7.5 g of dry leaves were applied for 45°C and 5 minutes, and the highest yield was determined in the samples where 45°C and 5 minutes were applied to 1 g of dry leaves. In a previous study conducted by Kamagate and colleagues (2016), it was found that the utilization of water, ethanol, and methanol solvents in the traditional extraction method yielded varying percentages of extracts. The extract yields obtained were 20.9%, 16.8%, and 18.6% for water, ethanol, and methanol, respectively.

When the TPC results of the extracts were examined, it was determined that the values varied between 368.20 and 709.82  $\mu\text{g GAE/g}$  extract (Table 3). The lowest TPC amount was obtained at 30°C temperature, 5 min time, and 4.25 g solid/solvent ratio, while the highest TPC amount was obtained under extraction conditions using 45°C temperature, 5 min time, and 7.5 g solid/solvent ratio. Murathan and Kaya, (2020) found that the TPC of the Hass leaf extract obtained using 80% methanol was 261.1 mg GAE/100 g extract, and this value was determined to be a higher amount than that

determined for the water extract used in the current study. Another study, in which the cultivar was not specified, found that TPC in samples extracted with 95% ethanol and methanol from avocado leaves was 82.48 mg GAE/g extract and 91.72 mg GAE/g extract, respectively Kavaz and Ogbonna, (2019). This can be explained by the fact that organic solvents or organic solvent/water mixtures are better extraction solvents than water and can extract more bioactive compounds (Dailey and Vuong, 2015).

Table 3. Experimental design and corresponding responses (Extraction time: 5 minutes)

Run <sup>a</sup>	Solid/Solvent (g/100mL)	Temperature (°C)	Extract Yield (%)	TPC ( $\mu\text{g GAE/g}$ extract)
1	7.5	45	15.07	709.82
2	4.25	45	17.51	657.53
3	4.25	45	17.45	657.42
4	1.95	34	18.45	456.17
5	1.95	45	19.67	585.14
6	1.95	56	19.34	516.47
7	4.25	30	16.63	368.20
8	4.25	60	17.67	452.35
9	4.25	45	17.49	658.27
10	6.55	34	15.36	546.48
11	4.25	45	17.49	660.61
12	4.25	45	17.49	660.60
13	6.55	56	15.98	608.18

<sup>a</sup>Randomized

Table 4. Model coefficients and fit values of the responses

Model coefficients <sup>a</sup>	Extract Yield (%)	TPC ( $\mu\text{g GAE/g}$ dw)
$\beta_0$	15.278*	-1793.0*
$\beta_1$	0.17468*	101.696*
$\beta_2$	-0.4964*	27.49*
$\beta_{11}$	-0.001442*	-1.09840*
$\beta_{22}$	-0.01007*	-0.940*
$\beta_{12}$	-0.002727*	ns
Model	***	***
R <sup>2</sup>	99.98	99.97
Adj- R <sup>2</sup>	99.97	99.96
Pred- R <sup>2</sup>	99.93	99.90
Lack of fit	0.372	0.227

<sup>a</sup> $\beta_0$ , constant coefficient;  $\beta_i$ , linear coefficient;  $\beta_{ii}$ , quadratic coefficient;  $\beta_{ij}$ , two factors' interaction coefficient; ns, not significant ( $p > 0.05$ ); \*, significant at  $p \leq 0.001$ . Subscripts 1, and 2 represent temperature and solid/solvent.

When the microwave-assisted water extraction efficiency of Hass avocado leaf is examined in Table 4, the effect of temperature and solid/solvent ratio, which is the independent variable, on the extraction efficiency in the model obtained according to RSM demonstrated a significant level of significance ( $p \leq 0.001$ ). Temperature, solid/solvent ratio, and the interaction between temperature and solid/solvent ratio among the second-order variables in the model were found to be significant at the 99.9% level. The lack of fit value is insignificant ( $p > 0.05$ ), which indicates that the model is error-free. The model explains 99% of the extract yield (%) changes depending on the temperature and solid/solvent ratio extraction parameters in the experimental design. It is seen that the predictive power of the model is high in terms of extract yield.

The variation of the extract yield depending on the effect of temperature and solid/solvent ratio is shown in Figure 1a. The figure shows that the extraction efficiency decreases as the solid/solvent ratio increases at low temperatures, and the extraction efficiency decreases as the solid/solvent ratio increases at high temperatures. It was found that the highest yields were found at high temperatures and low solids/solvent ratios. In the literature, there were no microwave-assisted water extraction modeling studies using RSM examining the effects of temperature and

solid/solvent ratio on the extraction efficiency of Hass avocado leaves.

When the variation of TPC amounts of the extracts depending on the extraction parameters was examined, first-order terms of temperature and solid/solvent ratio, solid/solvent ratio, and temperature variables exhibited a high level of significance at a 99.9% confidence level (Table 4). The lack of fit value being insignificant ( $p > 0.05$ ) indicates that the model is error-free. The model explains 99% of the extract TPC changes depending on the temperature and solid/solvent ratio extraction parameters in the experimental design. The variation of TPC in microwave-assisted water extraction of Hass avocado leaf, depending on the effect of temperature and solid/solvent ratio, is given in Figure 1b. When the figure is examined, the amount of TPC first increased and then decreased with the increase of the solid/solvent ratio at low temperatures, and the amount of TPC first increased and then decreased with the increase of the solid/solvent ratio at high temperatures. It was determined that the TPC amount was the lowest at low temperatures and low solid/solvent ratio.

In the literature review, no microwave-assisted water extraction modeling study with RSM, which investigated the effect of temperature and solid/solvent ratio on the amount of TPC in extraction, was found for Hass avocado leaf.

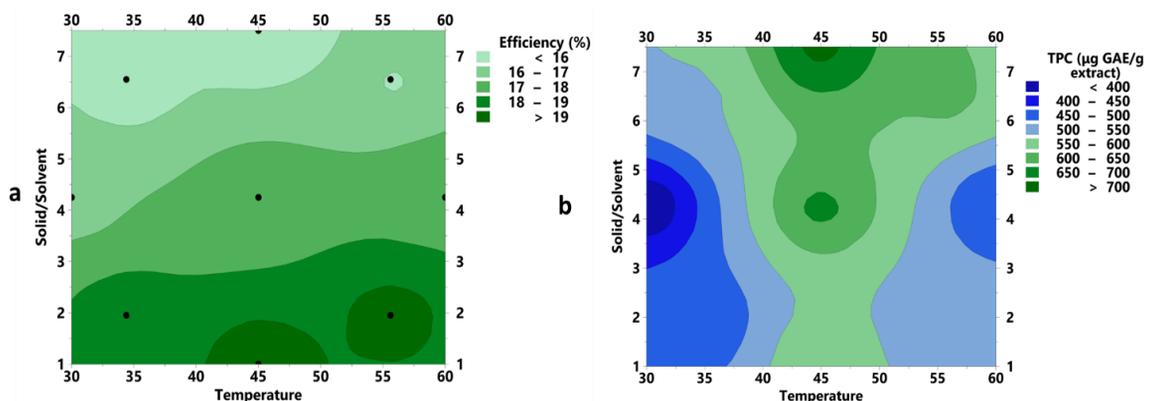


Figure 1. a) Influence of temperature (°C) and solid/solvent ratio (g/100mL) on extract yield (%), b) Influence of temperature (°C) and solid/solvent ratio (g/100mL) on extract TPC (µg GAE/g extract)

### **Optimization of Yield and TPC of Water Extracts of Hass Avocado Leaves and Validation of Models**

Optimum extraction conditions obtained by maximizing the extraction yield (%) and TPC amounts ( $\mu\text{g}$  GAE/g extract) of microwave-assisted water extracts of Hass avocado leaves using the response surface method were obtained at temperature, time, and solid/solvent ratio at  $47^\circ\text{C}$ , 5 min and 1.13 g dry leaves/100 mL water, respectively. The extraction efficiency of Hass avocado leaf was estimated as 19.62-19.70% and the TPC amount of the extract was estimated in the range of 586.83-593.81  $\mu\text{g}$  GAE/g extract under optimum microwave-assisted water extraction conditions by the model.

To experimentally verify the predictive value of the model, yield and TPC amount were determined in the extracts obtained by microwave-assisted extraction from leaf samples of the Hass variety under optimum conditions. The amount of TPC was determined as 591.76  $\mu\text{g}$  GAE/g dw, and the extraction efficiency was 19.67%. These values are within the model prediction ranges given above. The theoretical data obtained according to these results have been experimentally verified.

### **Physicochemical Properties of Water Extracts of Hass Avocado Leaves Extracted under Optimum Process Conditions**

TPC, free radical scavenging activity (DPPH), and antidiabetic activities (ADA) of the functional properties of the water extracts obtained from the leaves of Hass variety avocados under optimum conditions were determined.

TPC, DPPH and ADA as functional properties of extracts of the leaves of the Hass avocado variety were  $591.7 \pm 7.0$   $\mu\text{g}$  GAE/g extract,  $235.6 \pm 6.74$  mg TE/100 g extract, and  $64.59 \pm 0.12$  %, respectively. Murathan and Kaya (2020) determined the TPC amount of the extracts from Hass leaves with 80% methanol as 261.1 mg GAE/100 g extract and the DPPH ratio as 50.9%. In the samples extracted from avocado leaves with 95% ethanol and methanol, TFC was determined as 82.48 and 91.72 mg GAE/g

extract, respectively (Kavaz and Ogbonna, 2019). Gopalan and Tyug, (2021) reported that avocado leaf phytochemicals have a significant antioxidant capacity and also reported that the TEAC value and TPC of avocado leaves (dw) were 332.30  $\mu\text{g}$  Trolox/g and  $1199.08 \pm 6.00$   $\mu\text{g}$  GAE/g, respectively. Although these studies showed higher TPCs and antioxidant activities than the water extracts determined in our study, organic solvents or organic solvent/water mixtures were consistently reported to be better extraction solvents than water for extracting more bioactive compounds (Dailey and Vuong, 2015).

Chlorogenic acid, quercetin, p-hydroxybenzoic acid, rosmarinic acid, cinnamic acid, eriodictiol, protocatechuic acid, and gallic acid as phenolic components (mg/100g extract) of the extracts were found to be 12.309, 0.854, 0.809, 0.539, 0.270, 0.270, 0.180, and 0.135, respectively. It was determined that most of the components of optimum water extract in Hass avocado leaf consisted of chlorogenic acid. In the research findings of the literature, the values were given for caffeic acid (10.83-32.74), chlorogenic acid (28.83-852.81), and rutin (26.05-68.41) content of phenolic compound (mg/kg) in lyophilized water extracts and ethanol extracts of dried avocado (*Folium perseae*) leaves, respectively. They were detected with the HPLC-MS system (Polat Kose et al., 2020). In another study, it was determined that the phenolic compounds content (mg/100g) of avocado leaf extract were as syringic acid 31.65, ferulic acid 7.09, epicatechin 2.84, and p-hydroxybenzoic acid 2.51 (Adelusi et al., 2014).

### **CONCLUSION**

Within the scope of the findings obtained, the microwave-assisted extraction method can be used for high phenolic extraction in a short time from avocado leaves. However, in the study where we used water as the solvent, the phenolic content and functional properties of our extracts were found to be lower than in the studies where ethanol, methanol, or their aqueous mixtures were used as organic solvents. In future studies, extraction with aqueous mixtures of different organic solvents may be better. In future studies, *in vivo* studies (animal and clinical) can be done. In

addition, antioxidant and antidiabetic herbal food supplement, tea, sauce, etc. Functional foods can be produced that will attract the attention of consumers.

#### DECLARATIONS OF INTEREST STATEMENT

We declare that no conflicts of interest are associated with this manuscript.

#### AUTHOR CONTRIBUTIONS

The following statements should be used Conceptualization, EA, G.O.; methodology, EA, G.O.; formal analysis, N.K, E.A.; writing original draft, G.O.; writing and editing, E.A.; supervision, E.A.; co-supervision, G.O.

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