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A comparative assessment for efficient oleuropein extraction from olive leaf (Olea europaea L. folium)

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Abstract

Since oleuropein has long been known in the health sector and is abundant directly in our country as the fourth largest olive producer, oleuropein, the predominant phenolic ingredient in olive leaves, was recovered in this study using Soxhlet extraction. The effects of different solvent types (acetonitrile, ethanol, methanol, and water), extraction period (4 cycles, 4 h, and 8 h), particle size (250-500 µm and 900-2000 µm), and pretreatment of olive leaves on the yield of oleuropein were examined to determine the maximum yield. A greater oleuropein yield was obtained when the particle size of olive leaves utilized for extraction was lowered. Furthermore, aqueous solvents revealed a higher yield of oleuropein than pure solvents and prolonging the extraction duration resulted in a significant increase in the amount of oleuropein extracted. On the other hand, pretreatment of olive leaves resulted in a reduction in oleuropein output. As a result, with 36% extraction efficiency in terms of olive leaf conversion, the highest oleuropein extraction yield was obtained as 13.35 mg g^{-1} dry leaf for 8 h of extraction time using olive leaves with a particle size of 250-500 µm and an 80% methanol solution as solvent.

1. Introduction

Phenolic substances are prevalent in plants and serve a crucial role in plant growth, development, and reproduction by combating diseases and harmful bacteria. They are also responsible for plants and fruits' sensory qualities like color, bitterness, taste, and odor [1]. There is a wide variety of polyphenols in nature because phenolic compounds may take on a variety of structural shapes depending on their bonding state. As a result of a comprehensive survey, more than 8000 polyphenol structures have been found [2]. Owing to their potential health advantages for humans, polyphenols are of tremendous interest in the functional food, nutraceutical, and pharmaceutical industries [3]. According to research and the use of polyphenols, olive leaves are known to be a good source of polyphenols [4]. One of the principal phenolic substances found in olive fruits and leaves is the o-dihydroxyphenol glycoside oleuropein [5].

Oleuropein is a bitter glycoside found throughout the olive tree, but primarily in the leaves [6,7], and studies suggest that this phenolic substance has significant anti-inflammatory [8], antimicrobial [9,10], and antiviral activities [11], among others. Consumers are increasingly seeking natural goods or products that incorporate natural chemicals in their composition, pushing researchers in the food and cosmetic fields to investigate replacing synthetic antioxidants with those derived from plants [12,13]. Phenolic ingredients can be extracted by several methods including the use of cold solvents [14], filtration [15], microwave [16], microfluidic system [17], pressurized fluid [18], Soxhlet [19,20], supercritical fluids [21], and ultrasound [22,23]. Soxhlet extraction is well known and preferred as an extraction method that offers much higher efficiency than other methods in obtaining the desired target compounds (antioxidant and phenolic compounds) [24]. In addition, it has some advantages over other methods, such as ease of operation, less costly, constant operating conditions (e.g., temperature), no need for additional

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filtration and product purification, and constant contact of solvent and sample [25,26]. On the other hand, various experimental parameters dependent on these approaches, such as time, temperature, and solvent type, affect the extraction of phenolic compounds such as oleuropein. To generate this molecule, researchers studied to optimize the process and identify "clean" technologies that use non-toxic solvents and are low-cost. Japón Luján et al. [27] investigated the identification and quantification of phenolic chemicals in olive tree material extracts (olive oil, olive pomace, leaves, olive pits, and branches). Oil was extracted using liquid-liquid extraction, leaves, stones, and twigs were filtered using micro-assisted filtration, and olive pomace was filtered using pressure-liquid filtration. Oleuropein, which has a concentration of 2% (w/w) in olive leaves, was the highest phenolic component in olive tree materials. Oleuropein concentrations in leaves and branches were around 19 mg g^{-1} and 0.6 mg g^{-1}, respectively. Yateem et al. [19] evaluated parameters such as pH, temperature, and solvent type for the extraction of oleuropein from olive leaves, and the highest content of oleuropein as 13 mg g^{-1} dry olive leaf was obtained using 80% ethanol followed by 20% acetonitrile as 10 mg g^{-1} dry olive leaf. In another study, to enhance extraction yield, oleuropein content, and antioxidant activity, olive leaves were subjected to a pressurized liquid extraction (PLE) using environmentally friendly solvents such as water and ethanol. An ethanolic extraction at 190°C for three consecutive cycles was determined to be best regarding extraction yield. In terms of extracted oleuropein content, 43:57 mixtures of H_2O/EtOH at 190°C for 1 extraction cycle produced the optimum results [28]. Zunqiu et al. [29] compared the yields of oleuropein in leaves harvested at various dates during the harvest season. According to the findings, oleuropein levels dropped during flower bud differentiation and olive fruit ripening, with January having the highest oleuropein concentration (19.58%) and July having the lowest (1.56%). Lamprou et al. [30] studied a new low-cost acid hydrolysis process for extracting phenolic components from olive leaves (H_2SO_4). After hydrolysis, the resultant extract yielded an optimal level of oleuropein of 43.2 mg g^{-1} (dry weight basis). Recently, Cho et al. [31] examined the impact of the extraction solvent type (water, aqueous acetone, ethanol, and methanol) on various extract parameters to find the best conditions for olive leaf conversion to obtain phenolic. With 90% (by volume) methanol, the greatest extraction yield of 20.41% was obtained. The olive tree (Olea europeae) and its by-products, such as a leaf, have also been studied by utilization of microwave-assisted extraction to create an extract high in total phenolics (TPI), flavonoids (TFI), and antioxidant activity (AA) using a variety of common solvents (ethanol, methanol, acetonitrile, and acetone solutions) (MAE). On the other hand, the response surface method (RSM) was used to establish the best experimental conditions of the parameters that were effective on a limited number of tests using a 3-factor and 3-level central composite design (CCD). Under ideal conditions, the best results for TPI, TFI, and AA were 10.45 mg GAE/g DL, 9.69 mg CE/g DL, and 96.34% (230 W, 1.5 min, and 63.16 mL of 30% acetonitrile solution) [32].

The main objective of this study is to investigate and compare oleuropein extraction yield in a wide range of solvents (acetonitrile, ethanol, and methanol) in pure and aqueous concentrations under different parameters (extraction time, particle size, and pretreatment) by Soxhlet technique. Since Turkey ranks fourth in world olive production after Spain, Italy, and Greece in terms of botanical properties, ecophysiology, and phytochemical aspects [33], obtaining extracts such as oleuropein from the leaves of olive trees grown in large quantities can contribute considerably to the economy of Turkey.

2. Experimental

2.1. Chemicals

The olive leaves employed as a raw material source in this study were obtained from olive trees on the Izmir Institute of Technology's campus (Izmir, Turkey). Oleuropein (98%) and sodium carbonate (99.5%) were purchased from Sigma Aldrich, while methanol (99.8%), ethanol (99.9%), acetonitrile (99.9%), acetic acid (99%), gallic acid (97.5%) and Folin-Ciocalteu's phenol reagent were provided from Merck.

2.2. Method

Before usage, olive leaves were thoroughly cleaned with tap water to remove dust and debris, sprinkled with deionized water, and dried for 24 hours in a vacuum oven (JSR JSVO-60T) at 55°C. After drying, the leaves were ground into a fine powder between 250 and 2000 microns using a laboratory-scale grinder. To avoid frictional heating of the sample, the grinding procedure was repeated every 5 minutes.

Oleuropein was extracted from olive leaves using a Wisd brand Soxhlet extraction apparatus (DH.WHM 12295). The filter paper was used to weigh 10 g of pulverized olive leaves, which were then put in an extractor with a 250 mL solvent capacity. In this context, to obtain the highest oleuropein yield, the effects of different parameters were studied, namely solvent type (methanol, ethanol, acetonitrile) and their aqueous forms, extraction period (4 cycles, 4 h, and 8h), particle size (250-500 μm, 900-2000 μm). In addition, the effect of pretreatment on the amount of oleuropein in the extract and the efficiency of extraction was studied in such a way that the particle size was set between 250 and 500 microns, and the solvents were chosen based on the best results of the preceding parameters: 80% methanol and 80% ethanol. The treatments were carried out in an ultrasonic bath at 40 kHz on 25-30°C for 1 hour. After the 1-hour pretreatment in the ultrasonic bath, the olive leaves were placed on filter paper and placed in the Soxhlet extractor. The extraction took place in 4 hours and 8 hours.

A rotary evaporator was used to separate the extract from the solvent when the extraction was completed (Laborota 4001, Heidolph). The rotary evaporator's water bath temperature was set at 40°C, and the rotation frequency was set to 60 rpm. The remaining solid residue, on the other hand, was placed in a vacuum oven

for 24 hours at 50°C to remove moisture. The overall olive leaf conversion (X, $wt\%$) was calculated based on the initial dry olive leaf amount (W_i, g) and the amount of remaining solid residue (W_s, g) according to the Eq. (1):

$$X\ (wt\%) = \frac{W_i - W_s}{W_i} \times 100 \qquad (1)$$

To assess the quantity of oleuropein in the olive leaf extract, a high-performance liquid chromatography (HPLC) instrument was used to evaluate the concentrated liquid product. A C18 Inerstil column (5 m, 250 mmx4.6 mm) and an Agilent 1100 series detector are included in the HPLC system. The mobile phase was acetonitrile/water (20:80, v/v) containing 0.1% acetic acid, and it was fed with a flow rate of 1 mL min^{-1} at a column temperature of 30°C.

The extracted amount of oleuropein was calculated by the Eq. (2):

$$q = \frac{C_{oleuropein} \times V}{W_i} \qquad (2)$$

where q is the amount of oleuropein per total gram of dry olive leaf (mg g^{-1} dry leaf), $C_{oleuropein}$ is the concentration of oleuropein transferred to the solvent phase (mg L^{-1}) and V is the extracted volume (L).

Folin-Ciocalteu's method was also used to determine the total phenolic content of the liquid extract. Folin-Ciocalteu's reagent was diluted 10-fold in this procedure, and a 7.5 percent (75 g L^{-1}) sodium carbonate solution was prepared. After combining 0.5 mL of Folin-Ciocalteu's reagent, 0.5 mL of liquid product, and 1 mL of saturated sodium carbonate solution, the volume was adjusted to 10 mL with distilled water. After mixing, the liquid was kept at room temperature for 45 minutes in the dark. Thermo's Multiscan UV spectrophotometer was used to detect the absorbance at 765 nm, and the phenolic content was defined in gallic acid equivalents (mg GAE/mL).

3. Results and Discussion

3.1. Effect of extraction solvent on conversion of olive leaf and oleuropein yield

Solvent selectivity has great importance in extraction processes to obtain the desired compound from the plant material. To obtain a high yield of the desired compound in the extraction process, the extracted compound and the solvent must have similar polar properties. Because oleuropein, the most abundant phenolic ingredient in olive leaf extract, is a polar substance, a solvent with a high polarity is required to get oleuropein efficiently [34,35]. Polar protic solvents are solvents with polar features that release hydrogen into the environment and feed hydrogen to the environment via -OH bonds, resulting in increased extraction efficiency [36,37].

The effect of solvent type on the conversion of olive leaf and oleuropein yield under 10 g of dry olive leaf with a particle size of 250-500 µm, in the presence of different solvents (pure, 80%, 70%, 50%, and 20% ethanol, pure, 80% and 50% methanol, 20% acetonitrile and water) and extraction time of 8 hours' conditions are shown in Fig. 1. The content of oleuropein varied from 0.48 to 13.35 mg g^{-1} dry olive leaf. The amount of oleuropein obtained in tests using methanol as a solvent was often higher than that obtained in experiments using other solvents. The highest amounts of oleuropein were found with 13.35 mg g^{-1} dry leaf with 80% methanol as solvent and 12.44 mg g^{-1} dry leaf with 80% ethanol as solvent. Meanwhile, 37.55 mg g^{-1} dry leaf was reported as the highest yield of oleuropein using pure methanol as solvent, while this value was found to be 18.58 mg g^{-1} dry leaf using methanol/hexane (3/2:v/v) as solvent [38].

The quantity of oleuropein normally increases as the polarity of the solvents utilized increases. When compared to water (1), the polarity of methanol (0.762) and ethanol (0.654) is lower, resulting in a drop in the solvent's dielectric constant, which improves the solubility and diffusion of the desired target molecules in the solvent. However, the use of solvents in their pure form leads to dehydration and the collapse of plant cells. Proteins and phenolic chemicals in the cell wall are also denaturized. The extraction of phenolic compounds becomes harder as a result of these factors [39]. In our case, the lowest extraction efficiency was 17.6% in water, while the highest extraction efficiency was 36% in 80% methanol and 29.8% in 20% acetonitrile by volume, respectively. Pure ethanol and its aqueous solutions produced nearly identical findings, however, tests using methanol as a solvent yielded higher oleuropein yields. This can be explained by the higher polarity value of methanol compared to other solvents. Moreover, there is a big difference in oleuropein yield between the solvents in pure form (ethanol and methanol) and their aqueous solvents. This can also be explained by the higher polarity value of aqueous solvents as compared to pure solvents. Although water is the most polar solvent used, it does not seem to perform well in the extraction of oleuropein. The reason could be that the Soxhlet method's long boiling period at high temperatures decreases the extract's oleuropein concentration. The absence of such a situation in methanol extracts may be explained by the fact that the boiling point of methanol (64.7°C) is lower than that of water.

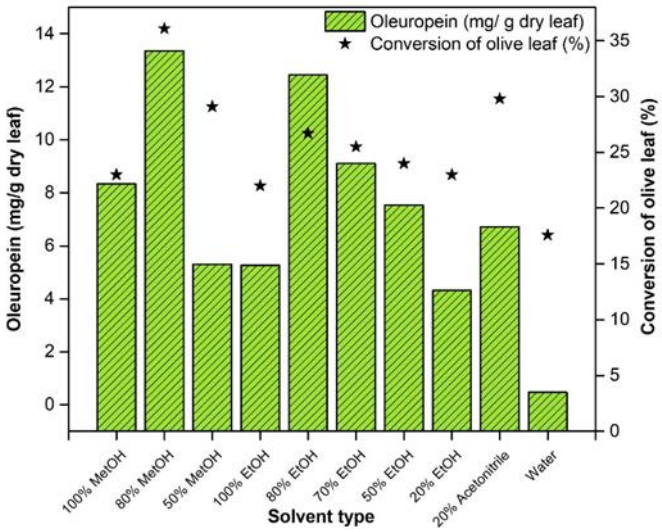

Figure 1. Effect of solvent type on the quantity of oleuropein extracted per gram of dry leaf and olive leaf conversion after an 8-hour Soxhlet extraction

3.2. Effect of extraction time on conversion of olive leaf and oleuropein yield

To minimize energy and cost, one of the most significant aspects to study in the extraction process is extraction time. The data obtained from 10 g of olive leaves with size 250-500 µm in 4 h and 8 h by Soxhlet extraction using pure, 80%, and 50% methanol, pure, 80%, 70%, 50% and 20% ethanol, 20% acetonitrile and water are shown in Figs. 2a and 2b.

The highest extracted amount of oleuropein was recorded as 13.35 mg g^{-1} of dry leaves after 8 hours of extraction with 80% methanol. The use of 80% ethanol as solvent for 8 hours of extraction resulted in a remarkable amount of oleuropein of 12.44 mg g^{-1} dry leaf as well. When comparing extraction times, 8-hour tests yielded significantly more oleuropein than 4-hour experiments. Methanol outperformed ethanol at different times (4.13 mg g^{-1} dry leaf for 4 h with pure methanol, 3.7 mg g^{-1} dry leaf for 4 h with pure ethanol). In addition, Pure solvents extracted less oleuropein from olive leaves than aqueous solvents, according to the findings. Xie et al. [40] also investigated the effect of varied ethanol-water mixture proportions on the yield of oleuropein extract. The maximum oleuropein yield was discovered to be between 55 and 75% ethanol, and the mixture of ethanol and water was shown to be a good solvent. Because it combines polarity and penetration properties, this feature is particularly essential. The affinity of the solvent and solute, as well as the increased surface area of contact between the solvent and solute, were also thought to boost the yield of the target molecule. Therefore, the oleuropein yield and ethanol concentration fell between 75 and 85% before remaining unchanged between 85 and 95%. The content of ethanol increased while its polarity reduced as a result of the observations. This property was discovered to be detrimental to oleuropein yield, and the optimum ethanol concentration was set at 75%.

Furthermore, both 80% methanol and 80% ethanol gave higher yields of oleuropein under different experimental conditions. The amounts of oleuropein in the solvent 20% acetonitrile were almost the same for different extraction times. For example, 6.4 mg g^{-1} dry leaf was obtained with 20% acetonitrile for 4 hours while 6.72 mg g^{-1} dry leaf was obtained with 8 hours. The oleuropein amount of 20% acetonitrile solvent gave better results than the 20% ethanol solvent in the 4- and 8-hours extraction experiments. Although acetonitrile gives good results in oleuropein amount, it was not used in other experiments because of its high boiling point and because it is an expensive solvent. The boiling temperature of acetonitrile is 81.6°C and similar oleuropein yields were obtained as it is close to the boiling point of water. Since the boiling points of ethanol or methanol are lower than the boiling temperature of water, higher oleuropein yields were observed in the presence of these solvents.

On the other hand, the highest extraction efficiency in terms of olive leaf conversion was found to be 36% using 80% methanol for 8 hours. The following highest conversion efficiencies were obtained using 80% ethanol and 70% ethanol for 4 hours' extraction with 30% and 32.3% respectively. The extraction yields at two different extraction times were very close even at 50% ethanol.

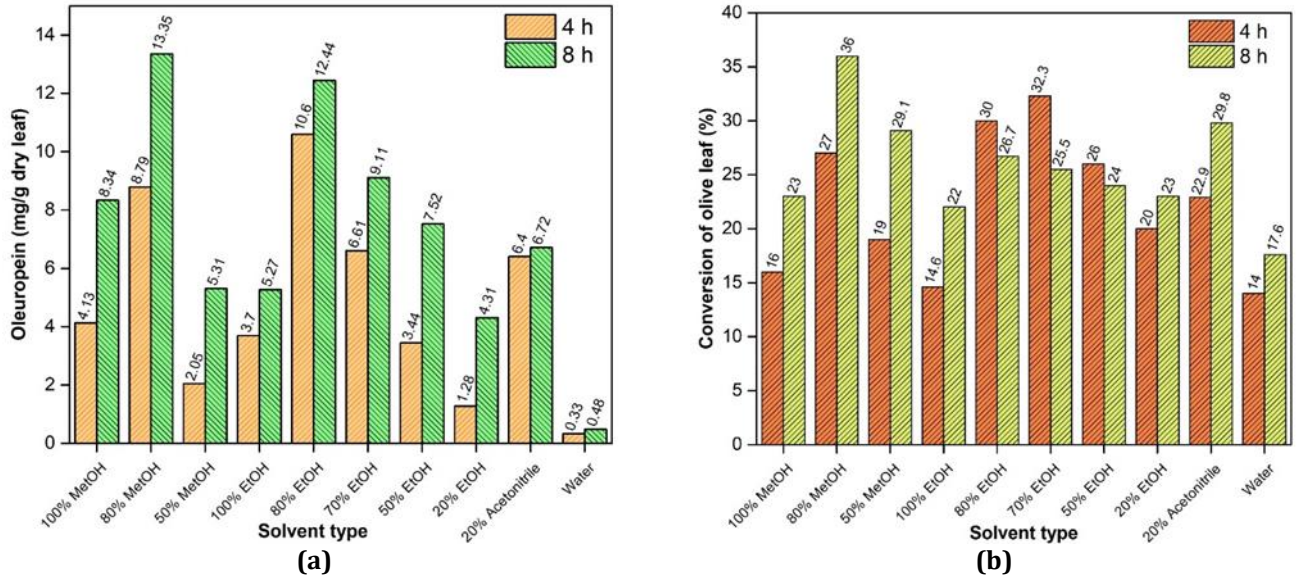

Figure 2. Effect of extraction time on **(a)** the amount of oleuropein extracted per gram of dry leaf and **(b)** the conversion of olive leaves by Soxhlet extraction

3.3. Effect of particle size on conversion of olive leaf and oleuropein yield

Pre-treatments, such as separation techniques or particle size reduction, could be applied to raw materials to achieve a great improvement in the extraction efficiency of phenolic compounds [41]. Physical processes such as drying and grinding are of great importance in obtaining herbal extracts. The values of oleuropein and extraction yield at various particle sizes were attempted to be determined in this section. Oleuropein was aimed to be extracted from 10 g of olive

leaves in four cycles using pure methanol and ethanol, 80% ethanol, and water solvents (1 cycle = 45 min for methanol, 40 min for ethanol, and 75 min for water). The operating conditions were kept the same in all experiments, the particle sizes of olive leaves (250-500 µm, 900-2000 µm) were changed to understand the effect of particle size on oleuropein yield. Figs. 3a and 3b show the determined oleuropein and extraction yields by Soxhlet extraction of particles of various sizes, respectively. Particle size was found to significantly affect oleuropein yield. In an 80% ethanol extract, the yield of oleuropein obtained with a size of 250-500 µm was about 5 times higher than that obtained with a size of 900-2000 µm. The highest oleuropein yield was 5.4 mg g^{-1} dry leaf with 80% ethanol, 250-500 µm, while the lowest oleuropein yield was 0.03 mg g^{-1} dry leaf with water, 900-2000 µm. This is due to the fact that as particle sizes are reduced, the surface area rises, making oleuropein extraction considerably easier and efficient. Additionally, the highest yield in terms of olive leaf conversion (26.7%) was also found when 80% ethanol was used as solvent and particle size was 250-500 µm, while the lowest yield (9.5%) was obtained when water was used with a particle size of 900-2000 µm of olive leaves. In general, it was observed that the solvents used (ethanol, methanol, and water) showed higher extraction efficiency for particles with size of 250-500 µm. Overall, the results are in good agreement with the literature, namely, Nagy & Simándi, (2008) studied the impact of particle size and moisture content on supercritical fluid extraction of chili peppers and found that using smaller particle sizes resulted in greater extraction yields [42].

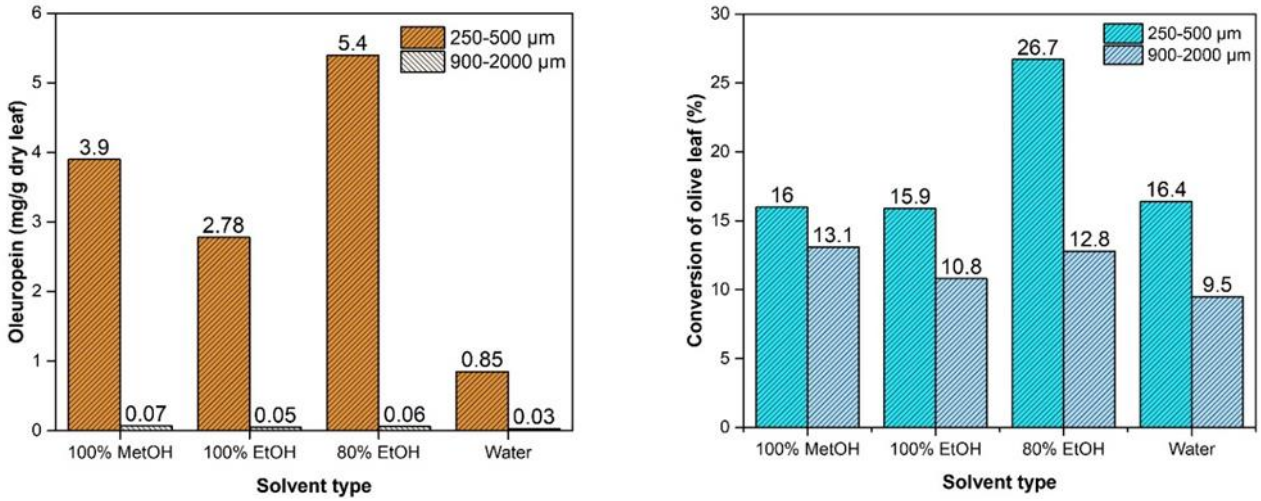

Figure 3. Effect of particle size on **(a)** the amount of oleuropein extracted per gram of dry leaf and **(b)** the conversion of olive leaves by Soxhlet extraction in four cycles extraction

3.4. The effect of pre-treatment on oleuropein yield and extraction efficiency

The data regarding the effect of pretreatment on oleuropein yield and extraction efficiency are presented in Table 1. For better differentiation, the results were compared with Soxhlet extraction experiments without pretreatment carried out under the same conditions (250-500 µm, 80% MetOH (4 h and 8 h) and 80% EtOH (4 h and 8 h)). As expected, a decrease in the extracted amount of oleuropein was observed due to the degradation of phenolic compounds in olive leaf during the sonication for 1 h. Nevertheless, among the pretreatment experiments, the highest oleuropein content was found to be 5.57 mg g^{-1} dry leaf when 80% MetOH was used for 8 h, while the lowest oleuropein content was 3.17 mg g^{-1} dry leaf when 80% EtOH was used for 4 h. According to a recent investigation of ultrasound-assisted extraction for the yield of oleuropein and hydroxytyrosol with extraction times of 10 min, 30 min, 60 min and 120 min, the highest yield of oleuropein, was obtained in the first 10 min of extraction with a value of 10.65 mg g^{-1} dry leaf. However, a steady decrease in the oleuropein yield was observed when extraction times of more than 10 min were applied in the experiment [43]. In another study, olive leaves were also extracted by ultrasound assisted and low-pressure extraction of 1, 2, 3, 4, 5, 10 and 15 min duration. The maximum oleuropein yield was obtained after 3 min but then stabilized with increasing time, indicating that oleuropein extraction was completed after 3 min [44]. Overall, pretreatment by sonication had a negative effect on oleuropein yield, as part of the phenolic content might have degraded by the temperature rise during heating in the ultrasonic bath.

Table 1. The effect of ultrasonic pre-treatment on oleuropein yield

Solvent Type	Extraction Time	Oleuropein Yield (mg/g dry leaf)
80% methanol (Without pre-treatment)	4 h	8.79
	8 h	13.35
80% methanol (With pre-treatment)	4 h	4.85
	8 h	5.57
80% ethanol (Without pre-treatment)	4 h	10.6
	8 h	12.44
80% ethanol (With pre-treatment)	4 h	3.17
	8 h	5.06

3.5. Total phenolic content assay (Folin-Ciocalteu's method)

The effect of pure ethanol and its aqueous forms such as 20%, 50%, 70% and 80% with different extraction times (4 h, 8 h) on total phenolic content and the comparison of extraction with ethanol and methanol with 4 h duration in terms of phenolic content is shown in Figs. 4a and 4b, respectively. The highest phenolic content was observed with 80% ethanol for 8 h (0.082 mg GAE mL^{-1}), while extraction for 4 h with pure ethanol gave the lowest value for phenolic content (0.02 mg GAE mL^{-1}). Pure ethanol did not show to be a viable solvent for olive leaf extraction for both the amount of oleuropein and total phenolic content, but 20% ethanol appears to produce better results than pure ethanol for both oleuropein and total phenolic content. This finding implies that water is required to improve polyphenol extraction from plant tissue diffusion, making extraction easier and more effective. The total phenolic content was almost comparable in terms of the amount of oleuropein when used ethanol and methanol and their aqueous solutions as solvent type. The highest yield was obtained using 80% ethanol solution and the phenolic content was found to be 0.068 mg GAE mL^{-1}.

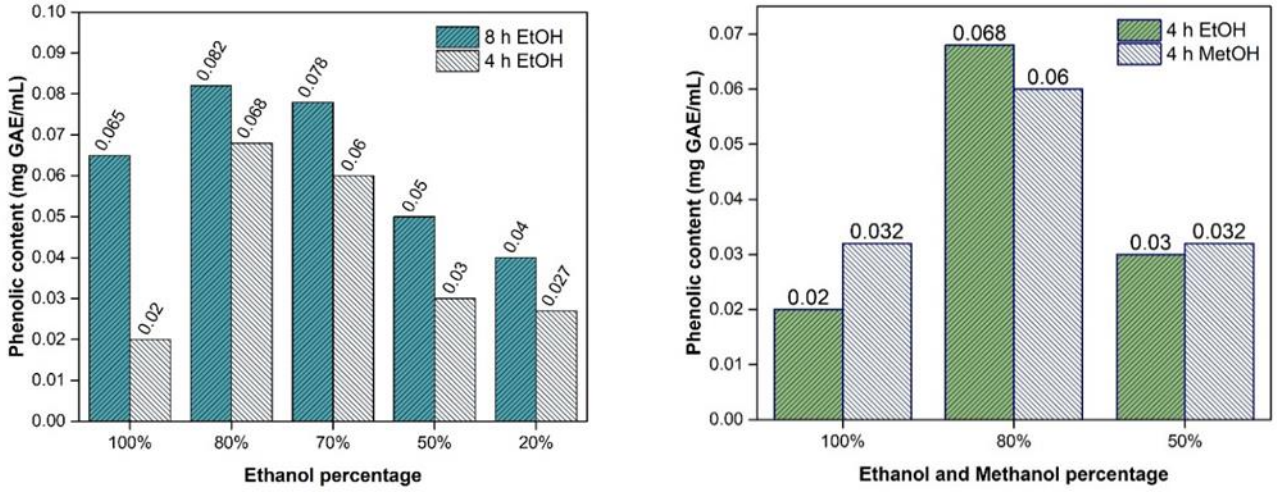

Figure 4. The influence of **(a)** extraction time (4 h, 8 h) with different ethanol content and **(b)** solvent type (ethanol, methanol) with 4 h extraction on total phenolic content.

4. Conclusion

This study contributes to the valorization of olive leaves by extracting polyphenols from them using different types of solvents and parameters. Oleuropein, the major phenolic compound in olive leaves, was obtained by Soxhlet extraction, which has long been known in the health field and is a crucial raw material with high availability in our country, as Turkey is the fourth largest olive producer in the world. In this context, the type of solvent and aqueous solutions (ethanol, acetonitrile, methanol, and water), extraction time (4-8 h), particle size (250-500 µm and 900-2000 µm) and pre-treatment of olive leaves on the amount of oleuropein and extraction yield were investigated. The summary of the experiments and obtained oleuropein amounts is given in Table 2.

When aqueous solvents were used instead of their pure forms, a larger quantity of oleuropein was produced. This is explained by the fact that water distends the cells of plants and facilitates diffusion. Higher oleuropein yield was obtained when the particle size of the raw material to be utilized for extraction was lowered. Increasing the extraction time and using 80% methanol as solvent resulted in significant improvement in oleuropein yield. In contrast, the use of pre-processed olive leaves in the extraction process resulted in a serious decrease in the oleuropein yield. The largest quantity of oleuropein and extraction efficiency were obtained from olive leaves with a particle size of 250-500 µm during an 8-h extraction with an 80 percent methanol solution as the solvent. Under these circumstances, the greatest oleuropein concentration was found to be 13.35 mg g^{-1} of dried leaves, with a 36% extraction efficiency.

Table 2. Comparison of oleuropein amounts obtained in different experiments

ExpNo.	Solvent type	Time	Particle size (μm)	Oleuropein amount (mg/g dry leaf)
1	80% MetOH	8 h	250–500	13.35
2	80% EtOH	8 h	250–500	12.44
3	80% EtOH	4 h	250–500	10.60
4	70% EtOH	8 h	250–500	9.11
5	80% MetOH	4 h	250–500	8.79
6	Pure MetOH	8 h	250–500	8.34
7	50% EtOH	8 h	250–500	7.52
8	20% ACN	8 h	250–500	6.72
9	70% EtOH	4 h	250–500	6.61
10	20% ACN	4 h	250–500	6.40
11	80% EtOH	4 cycles	250–500	5.40
12	50% MetOH	8 h	250–500	5.31
13	Pure EtOH	8 h	250–500	5.27
14	20% EtOH	8 h	250–500	4.31
15	Pure MetOH	4 h	250–500	4.13
16	Pure MetOH	4 cycles	250–500	3.90
17	Pure EtOH	4 h	250–500	3.70
18	50% EtOH	4 h	250–500	3.44
19	Pure EtOH	4 cycles	250–500	2.78
20	50% MetOH	4 h	250–500	2.05
21	20% EtOH	4 h	250–500	1.28
22	Water	4 cycles	250–500	0.85
23	Water	8 h	250–500	0.48
24	Water	4 h	250–500	0.33
25	Pure MetOH	4 cycles	900-2000	0.07
26	80% EtOH	4 cycles	900-2000	0.06
27	Pure EtOH	4 cycles	900-2000	0.05
28	Water	4 cycles	900-2000	0.03

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Author contributions

Yaşar Kemal Recepoğlu: Writing – Original draft preparation, Visualization. **Gülin Gümüşbulut:** Investigation, Writing-Original draft preparation. **Aslı Yüksel Özşen:** Supervision, Writing-Reviewing and Editing.

Conflicts of interest

The authors declare no conflicts of interest.

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