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Valuable Nutrients, Aroma Profile, and Functional Bioactives Extracted by Eco-Friendly Extraction Techniques from Wild Olive Fruits (*Olea europaea* L. var. *sylvestris*)

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Abstract: Wild olive tree, or oleaster (var. *sylvestris*), native to the Mediterranean region, is considered a traditional source of healthy food. Wild olive fruit (WOF) exhibits several biological properties associated with its chemical composition. Although Greece has important olive genetic resources, including oleaster populations, limited information is available on the chemical characterization of WOF. Therefore, the present investigation was undertaken to study the nutritional, bioactive (phenolics, tocopherols, and pigments), volatile profile, and antioxidant properties of WOF collected from Greece. Moreover, eco-friendly processes, including ultrasound-assisted extraction (UAE), microwave-assisted extraction (MAE), and accelerated solvent extraction (ASE), were applied to obtain oleuropein-rich extracts from WOF. Evaluation of phenolic composition in WOF extracts was carried out by LC-DAD-ESI-MS, while antioxidant activity was evaluated by applying DPPH, ABTS, and FRAP methods. Our outcomes show that Greek WOF is rich in oleic acid (71.55%), total phenolics (64.89 mg GAE/g dw), tocopherols (107.05 mg/kg dw), and carotenoids (85.90 mg/kg dw). Oleuropein (72.03 mg/g dw) was the main phenolic compound in the WOF extracts. Also, the WOF was characterized by an increased level of volatile compounds, mainly terpenoids (46.73%). UAE was more efficient than MAE and ASE for recovering oleuropein-rich extracts with high antioxidant activity. These results emphasized the high potential of WOF as an alternative bioactive ingredient for use in the food industry.

Keywords: oleaster; wild olive fruit; nutritional; bioactive compounds; volatile composition; oleuropein; green extraction; tocopherols; optimization; LC-MS analysis

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1. Introduction

The olive tree *(Olea europaea* L., Oleaceae family) is one of the world's most ancient crops that can be naturally distributed across a large area worldwide, with remarkable social, economic, and ecological importance [1]. The olive (*Olea europaea* subsp. *europaea* var. *europaea*) is the cultivated form, whereas oleaster (*Olea europaea* subsp. *europaea* var. *sylvestris*) is the wild form, the former which is found throughout the Mediterranean, while generally oleasters are mainly distributed into the western and eastern Mediterranean regions [2–4]. In general, wild olive trees are distinguished from cultivated ones, regarding spine scent juvenile shoots, little fruits with a thin pulp, and an individual's ability to survive in extreme environmental conditions [5]. Oleaster olive fruits represent a characteristic element of Mediterranean flora, with their ability to adjust to climate change and produce high-quality wild olive oil [6,7].

Even though oleaster is not a cultivated species, it is a potential source of genetic improvement for biotic and abiotic stress, playing an important role in olive breeding programs [8]. Several authors believe that minor genetic diversity exists between wild and cultivated olive plants [9,10]; however, it remains difficult to distinguish wild Mediterranean olives and cultivated olives [11]. Although genetic variation was observed between wild and cultivated olive tree populations in Italian regions [12], low genetic differentiation was exhibited among the wild olive populations in Greece [10].

It is known that oleaster has been considered of lower agronomical value than the cultivated olive, but, given the increased demand for edible oils under an increasing human population and facing the challenges of climatic change, alternative oil sources originated from unexploited wild trees are required [13]. Oleaster oil has been recognized as a valuable resource due to its good lipidic profile and great antioxidant capacity, which are related to its high content of bioactives, including phenolic compounds, tocopherols, sterols, and pigments, as compared to cultivated olive oil [7]. In addition, it has shown important antibacterial activity [14]. Regarding the organoleptic properties, oleaster oil is spicier, more bitter, and fruitier than olive oil extracted from cultivated olive trees.

Several reports have shown that wild olive and its different products are characterized as an ideal source of high nutritional and phytochemical value with beneficial biological properties for the consumer [15–17]. Epidemiological studies have shown the preventive effects of olive oil consumption against cardiovascular events, inflammatory diseases, cancer, and mortality, due to its antioxidant, anti-inflammatory, antifibrotic, and antitumoral properties [18]. Additionally, phytotherapy is widely used with traditional herbs against high blood pressure, high levels of LDL, diuresis, bacterial and viral infections, cancer, fever, hyperuricemia, diabetes, and myocarditis [19]. Various studies have investigated the quality [7,20–22] and phenolic [8,23] and aroma profile [6] of wild olive oil, highlighting its high potential to be used as a valuable natural and alternative resource for improving olive oil quality [7].

Although conventional extraction remains the main approach for recovering bioactive compounds, the application of eco-friendly extraction methods is continuously increasing, offering healthier and safer food products, cost reduction, and energy safety [24]. However, the bioactivity of a wild olive extract is depended on the extraction method and conditions. Therefore, it is important to evaluate different extraction methods to choose the suitable technique for maintaining high levels of phenolic compounds, while being environmentally friendly and economical.

Taking into account these circumstances, the applicability of green technologies is necessary to yield high extraction efficiencies for bioactive compounds from different olive parts. Although most of the reports focus on olive leaves [15,25], olive pomace [26], and *Olea europaea* olive fruit [27], very few studies have reported so far on phenolic extracts obtained from wild olive fruits (WOFs). Most surveys associated with WOF originate mainly from Mediterranean countries such as Spain [28], Portugal [29], Morocco [22], Tunisia [30], and Algeria [31]. To the best of our knowledge, no earlier studies addressing the characterization of bioactive compounds of WOF in Greece.

Hence, there is a need to explore the potential of WOF and establish more information about its bioactive characteristics and antioxidant properties. In this study, the evaluation of the nutritional (fatty acid composition), bioactive (phenolics, tocopherols, and pigments) and aroma profile (SPME-GC-MS), as well as the antioxidant activity of WOF originated in Greece, is carried out for the first time. As a promising source of valuable bioactives, these WOFs have been extracted using eco-friendly methods such as microwave- (MAE), ultrasonic-, (UAE) and accelerated-solvent extraction (ASE) to recover potent antioxidant phenolic compounds. In addition, a comparative study was carried out to establish the optimal extraction conditions for recovering phenolic extracts rich in oleuropein from WOF, followed by their analytical characterization using the liquid chromatography/mass spectrometry (LC-MS) technique.

2. Materials and Methods

2.1. Materials

Fresh WOFs (*Olea europaea* var. *sylvestris*) at a maturity stage were collected from "Volvi Estate" (23o 30′46″01/40o 41′48″13), which lies by the shores of the protected region of Volvi Lake in Greece, with around 90,000 wild olive trees. The region is characterized by a typical Mediterranean climate, and wild olive trees have been growing on the estate for centuries, unaffected by any human intervention, just with the protection of the mild microclimate of the lake. Wild olives grow and bear fruits completely unaided, in the most natural of processes, relying only on the sun and the rain. The collected WOF samples were rinsed with distilled water, the seeds were removed manually, and then the pulp was lyophilized using a laboratory freeze-dryer (Christ, Martin Christ, Gefriertrocknungsanlagen GmbH, Osterode am Harz, Germany). Then, the freeze-dried WOF sample was milled and homogenized in a laboratory mill (pulverisette 11, Fritsch, Idar-Oberstein, Germany) at 8000 rpm for 1 min, and kept at −25 °C until analysis.

Analytical standards of oleuropein (OLE), hydroxytyrosol (HTYR), and tyrosol (TYR) were supplied by Sigma-Aldrich (Steinheim, Germany), whereas luteolin-7-O-glucoside (LUTGL), verbascoside (VER), rutin (RUT), and luteolin (LUT) were obtained from Extrasynthese (Genay Cedex, France). α-, β-, γ- and δ-tocopherol standards were purchased by Supelco (Bellefonte, PA, USA). Analytical reagents of Folin-Ciocalteu, 1,1-diphenyl-2-picrylhydrazyl (DPPH), 2,2′-azinobis-(3-ethylbenzthiazolin-6-sulfonic acid) (ABTS), 6-hydroxyl-2,5,7,8-tetramethychromane-2-carboxylic acid (Trolox), and 2,4,6-tripyridyl-s-triazine (TPTZ) were from Sigma-Aldrich (Steinheim, Germany). All other solvents/chemicals obtained from Chem-Lab (Zedelgem, Belgium) were analytical or HPLC or LC-MS grade.

2.2. Proximate Chemical Composition

The chemical composition of the fresh WOP sample was determined using the Methods of Association of Official Agricultural Chemists (AOAC) International [32]. Moisture content was determined by drying the samples at 105 °C to constant weight, while ash content was determined based on the gravimetric loss by heating to 600 °C for a period of two hours. The total nitrogen value obtained by the Kjeldahl method was multiplied by a conversion factor to determine crude protein content, whereas crude fat content was measured by Soxhlet method using petroleum ether. The carbohydrate composition was determined by the difference.

2.3. Analysis of Fatty Acid Composition

Fat and fatty acids were extracted from the WOF into ether, then methylated to fatty acid methyl esters (FAMEs) using BF3 in methanol. FAMEs were quantitively measured by gas chromatography (GC) (Model Varian CP-3800, equipped with a flame ionization detector), against $C_{11:0}$ internal standard, according to AOAC 996.06 [33]. Individual fatty acids were identified by comparison of their retention times with those of an external standard (Supelco 37 Compone nt FAME Mix, Merck KGaA, Darmstadt, Germany). The amounts of fatty acids identified were expressed as percentages of the total fatty acid areas of the chromatograms identified. Each sample was analyzed in triplicate.

2.4. Determination of Chlorophylls and Carotenoids

The contents of chlorophylls and carotenoids were determined according to the spectrophotometry method as described by Lichtenthaler [34]. In brief, 0.35 g of the WOF sample was extracted with 20 mL of 95% ethanol (v/v) in an ultrasonic bath (FB 15051, Thermo Fisher Scientific Inc., Loughborough, UK) for 5 min, followed by centrifuging at 2680× g for 5 min. The above procedure was repeated until the extract became colorless. The extracts were combined, and the absorbances at 664.1, 649.6, and 470 nm were measured. Quantification of chlorophyll a (Chl a), chlorophyll b (Chl b), and total carotenoids was achieved by applying the following equations:

$$\text{Chlorophyll a (Chl a)} = 13.36 \times \text{A664.1} - 5.19 \times \text{A649.6}$$

$$\text{Chlorophyll b (Chl b)} = 27.43 \times \text{A664.1} - 8.12 \times \text{A649.6}$$

$$\text{Total carotenoids} = [1000 \times \text{A470} - (2.13 \times \text{Chl a} - 97.64 \times \text{Chl b})]/209$$

The results were expressed as mg/kg of dried WOF sample from three replications.

2.5. Tocopherols Analysis

The determination of tocopherols isomers was carried out using an Agilent 1200 HPLC system (Agilent Technology, Urdorf, Switzerland) equipped with a Nucleosil 100 C_{18} column (4.6 mm × 250 mm, 5 µm), and a fluorescence detector, as described previously by Irakli et al. [35] with some modifications. Briefly, 0.5 g of freeze-dried WOF was subjected to sonication with 5 mL of absolute ethanol, and the extract was collected after centrifugation at 1500× *g* for 10 min. The above procedure was repeated twice, and the combined extracts were evaporated until dryness under a gentle flow of nitrogen at 40 °C. The residue was reconstituted with 0.5 mL of methanol, and aliquots of 20 µL were injected into the HPLC column. The tocopherol isomers were separated, applying a linear gradient based on methanol and a mixture of methanol–isopropanol–acetonitrile. The results were expressed as mg/kg of dried WOF sample from three replications.

2.6. Extraction Procedures of Phenolic Compounds

In the present study, three 'green' extraction methods named ultrasound-assisted extraction (UAE), microwave-assisted extraction (MAE), and accelerated solvent extraction (ASE) were investigated to extract phenolic compounds from freeze-dried WOF at optimal levels, applying single factor experiments. After preliminary studies, extractions were carried out with ethanol/water (70:30, *v*/*v*) in all methods studied. Two factors were selected to optimize the extraction parameters, considering the specificity of each technique applied: time and temperature of extraction. The experimental procedures are described in the following sections.

2.6.1. Ultrasound-Assisted Extraction (UAE)

The UAE was performed in an ultrasonic bath (model FB 15051, Thermo Fisher Scientific Inc., Loughborough, UK), consisting of a stainless-steel jug and a maximum capacity of 1 L, with a frequency of 37 kHz, operated at an input power of 280 W. The experiments were designed in two groups as follows: (a) 0.5 g of dried WOF sample was mixed with 20 mL solvent in a 50 mL plastic centrifuge tube and was introduced into the ultrasonic reactor for varying extraction times (5, 10, 20, and 30 min) at a fixed temperature set at 30 °C; (b) the above procedure was followed by varying the temperature extraction at different levels (30, 45 and 60 °C) at constant time of 20 min.

2.6.2. Microwave-Assisted Extraction (MAE)

In the MAE experiment, the WOF samples were extracted using a closed microwave unit (Milestone, ETHOS X, Sorisole, Italy), consisting of an extraction rotor inside the sample chamber, a touchscreen controller, and two magnetrons of 950 W each connected with the module. The experiments were designed as follows: 0.5 g of sample was mixed with 20 mL solvent in the lid-covered TFM vessels, placed in rotor units and then extracted for 30 min at varying extraction temperatures (40, 60, and 90 °C) controlled by an infrared easyTEMP sensor adopted on the bottom of the microwave cavity.

2.6.3. Accelerated Solvent Extraction (ASE)

The ASE experiment was carried out using a Dionex ASE 350 extractor (Thermo Fisher Scientific Inc., Sunnyvale, CA, USA) containing stainless-steel cells of 22 mL and collection vials of 60 mL. The ASE unit was operated at 1500 psi, applying 5 min of preheating time, 90 s of purging time with nitrogen, 65% of flushing volume, and adopting three extraction

cycles with a total run time of 30 min. After mixing the dried WOF (1 g) with sand, the mixture was filled into extraction cells, extracted with approximately 50–70 mL of solvent, and then the extracts were collected into glass vials, depending on the temperature applied (60, 90, or 120 °C).

2.7. Total Phenolic and Total Flavonoid Contents

The Folin–Ciocalteu calorimetric reagent method was used to calculate the total phenolic content (TPC) of WOF extracts, according to the protocol of Singleton et al. [36]. Briefly, 0.2 mL of appropriately diluted phenolic extract was mixed with 0.8 mL of diluted (1:10 *v*/*v* in water) Folin–Ciocalteu reagent, followed by the addition of 2 mL of sodium carbonate (7.5% *w*/*v*) and filling up to 10 mL with distilled water. The absorption was measured at 725 nm after incubation of 60 min in a dark place, and the results were expressed as mg of gallic acid equivalents per g of dried WOF (mg GAE/g dw).

The total flavonoid content (TFC) of WOF extracts was estimated by the method described by Bao et al. [37], with some modifications. More specifically, 0.3 mL of $NaNO_2$ (5% *w*/*v*) was added to 0.3 mL of appropriately diluted WOF phenolic extract, and then 0.225 mL of $AlCl_3 \cdot H_2O$ (10% *w*/*v*) and 0.75 mL of NaOH (1 M) were added. After 30 min of incubation, the absorbance was measured at 415 nm, and the results were expressed as mg of catechin equivalents per g of dried WOF (mg CATE/g dw).

2.8. Antioxidant Activity Assays

2.8.1. DPPH Radical Scavenging Activity

WOF phenolic extracts (0.1 mL) were mixed with 2.85 mL of 0.1 mM DPPH in methanol, and the decrease in absorbance was measured at 516 nm after 5 min of reaction [38]. The calibration curve was constructed by plotting the Trolox concentration and % DPPH scavenging activity. The DPPH values were expressed as mg Trolox equivalents per g of dried WOF (mg TE/g dw).

2.8.2. ABTS Radical Scavenging Assay

ABTS radical scavenging activity was determined on WOF extracts according to the protocol of Re et al. [39]. The extracts (0.1 mL) were mixed with ABTS•+ solution (3.9 mL) with an absorbance of 0.70, and allowed to rest for 4 min at room temperature. Then, the absorbance was measured at 734 nm. The ABTS values were expressed as mg Trolox equivalents per g of dried WOF (mg TE/g dw).

2.8.3. Ferric Reducing Antioxidant Power Assay (FRAP)

A FRAP assay was performed according to Benzie and Strain 1999 [40]. The FRAP solution consisted of a mixture of 20 mM ferric chloride solution, 10 mM TPTZ (solution in 40 mM HCl, and 0.3 mM acetate buffer pH 3.6 in a proportion of 1:1:10, respectively). The extracts (0.1 mL) were mixed with fresh FRAP solution (3 mL) for 4 min at 37 °C, under dark conditions, and the absorption was measured at 593 nm. The results were expressed as mg Trolox equivalents per g of freeze-dried WOF (mg TE/g dw).

2.9. LC-DAD-MS Analysis of Phenolic Compounds

Identification of phenolic compounds were carried out on an HPLC system (Nexera, Shimadzu, Kyoto, Japan) consisting of a diode array detector (DAD) and a single quadrupole mass spectrometer (model LCMS-2020) operated with an electrospray ionization (ESI) interface in negative mode. Separation of phenolic compounds was performed on a Poroshell 120 EC-C_{18} column (4 µm, 4.6 × 150 mm, Agilent Technologies, Urdorf, Switzerland), thermostated at 35 °C. The solvents of mobile phases were 0.1% formic acid in water, *v*/*v* (A), and 100% acetonitrile (B), at a flow rate of 0.5 mL/min, adopting gradient elution: 0, 15% B; 5 min, 25% B; 10 min, 35% B; 28 min, 60% B; 35 min, 100% B; and 40.0 min, 15% B. The DAD detector was operated in the range of 200–400 nm, and the UV spectra were reordered at 280 nm.

The applied MS conditions were: scan range from m/z 100 to 1000, scan speed of 938 µ/s, event time of 1 s, nebulizing gas (N_2) flow rate of 1.5 L/min, drying gas (N_2) flow rate of 15 L/min, interface temperature of 350 °C, heat block temperature of 200 °C, DL (desolvation line) temperature of 250 °C, and interface and DL voltage of −4.5 kV and 1 V, respectively. The injection volume was 10 µL. Metabolite identification was based on comparing the retention time, UV, and MS spectra of each eluted compound with those of standard solutions. Quantification was performed using UV spectra at 280 nm based on a calibration curve ($R^2 \geq 0.996$) of eight phenolic standards. The quantification of secoiridoid derivatives was based on standard curves generated by oleuropein, due to the unavailability of commercial standards. Analyses were performed in triplicate, and the results were expressed as mg per g of dried extract (mg/g dw).

2.10. SPME-GC-MS Analysis

WOF sample composition analysis was performed using headspace solid-phase microextraction/gas chromatography–mass spectrometry (HS-SPME/GC–MS), as described by Nouska et al. [41]. Approximately 0.5 g of fresh WOF, after removing the seeds, was placed in a 15 mL glass vial, followed by the addition of 10 µL of 4-methyl-2-pentanol (0.05%) as an internal standard. Then, the closed vial was inserted in a water bath adjusted to 50 °C for 15 min, and the volatile compounds were extracted on a divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS) fiber (1 cm, 30/50 µm; Supelco Ltd., Bellefonte, PA, USA) during 30 min. Then, SPME fiber was desorbed at 240 °C in splitless mode for 10 min in the injector port of a GC interfaced with a mass detector using a Shimadzu GC-MS system (GCMS-QP2020, Shimadzu Group Company, Kyoto, Japan). Separation of the volatile compounds was carried out on a DB-5 MS capillary column (30 m × 0.32 mm i.d., film thickness 0.25 µm; Agilent Technologies, Santa Clara, CA, USA). The initial oven temperature was set at 40 °C for 5 min, then increased to 180 °C at a rate of 5 °C/min, to 240 °C at a rate of 30 °C/min, and held there for 5 min. The linear velocity of helium as carrier gas was 36 cm/s. The mass spectrometer was operated in the electron impact mode (EI) at 70 eV, scanning the range of 40–350 m/z at a scan rate of 2 scans/s. The temperature of the ion source and interface was set at 230 °C and 240 °C, respectively. Identification of the peaks was performed by comparing their mass spectra data with those from mass spectra databases (NIST 98 and Willey 7), using the accompanied Shimadzu Lab Solutions GC-MS software (version 4.52; Shimadzu, Kyoto, Japan). For the determination of the retention indices (RI), a C7–C22 n-alkanes series was injected under the same chromatographic conditions, and the values were compared, when available, with those reported in the literature for similar chromatographic columns. The relative content of each compound was calculated as a percent of the total chromatographic area, and the results were expressed as the means of three replicates.

2.11. Statistical Analysis

All the results are reported as mean ± standard deviations of three replications. A comparison of means was performed using one-way analysis of variance (ANOVA) according to Tukey's test (IBM SPSS statistical software, version 22.0, IBM Corp., Armonk, NY, USA). Significance was defined at $p \leq 0.05$.

3. Results and Discussion

3.1. Nutritional Profile

The chemical composition (moisture, crude protein, oil, and ash) is presented in Table 1. The moisture content of freshly collected WOF was 62.08%, which was within the range of 62–65% reported by Georgiou et al. [42], studying ninety-eight Kalamata olive fruit samples collected from different geographical regions in Greece. Regarding crude protein content, the present results (5.22% dw) were similar to those reported by Gagour et al. [22], studying eight Moroccan wild olive populations. Regarding to the oil content of WOF (21.44%), our results were found to be in the range of 12.25–31.81% for Moroccan WOF [22],

but higher compared to those of Tunisian oleaster cultivars (15.04%) [43], or Moroccan wild olives (5.0–7.7%) [44]. With regard to the ash content of the studied WOF (2.61%), our findings were in accordance with those of Gulfraz et al. [45], but lower than those reported by Hinnach et al. [43], measuring a mean value of 14.75% for two Tunisian oleaster samples. The variation in chemical composition of WOF samples is probably due to genetic and environmental factors and geographical variations [46].

Table 1. Nutritional profile and fatty acid composition of Greek WOF sample.

Composition	Units	Values	IOC 2019 *
Moisture	g/100 g fresh WOF	62.08 ± 1.10	
Crude protein	g/100 g WOF dw	5.22 ± 0.15	
Oil	g/100 g WOF dw	21.44 ± 0.25	
Ash	g/100 g WOF dw	2.61 ± 0.03	
Carbohydrates	g/100 g WOF dw	66.48 ± 0.35	
Fatty acids	g/100 g oil		
Myristic (C14:0)		0.07 ± 0.01	≤0.05
Palmitic (C16:0)		12.79 ± 0.41	7.5–20.0
Palmitoleic (C16:1)		1.00 ± 0.04	0.3–3.5
Heptadecanoic (C17:0)		0.08 ± 0.01	≤0.3
cis-10 Heptadecenoic(C17:1)		0.24 ± 0.01	≤0.3
Stearic (C18:0)		2.56 ± 0.11	0.5–5
Oleic (C18:1)		71.55 ± 0.22	55.0–83.0
Linoleic (C18:2)		8.26 ± 0.08	2.5–20.10
Linolenic acid (C18:3)		1.38 ± 0.06	≤1.0
Arachidic (C20:0)		0.42 ± 0.02	≤0.6
cis-11-Eicosenoic (C20:1)		0.26 ± 0.02	≤0.4
cis-11,14-Eicosadienoic (C20:2)		0.59 ± 0.01	-
Heneicosanoic (C21:0)		0.10 ± 0.01	-
Eicosapentaenoic (C20:5)		0.08 ± 0.01	-
Behenic (C22:0)		0.20 ± 0.01	≤0.2
Tricosanoic (C23:0)		0.07 ± 0.01	-
Lignoceric (C24:0)		0.33 ± 0.04	≤0.2
Saturated fatty acids		16.76 ± 0.08	
Monounsaturated fatty acids		72.80 ± 0.10	
Polyunsaturated fatty acids		10.81 ± 0.09	

* established limits by International Olive Council for virgin olive oil.

Fatty acid composition is an important quality parameter and authenticity indicator of olive oils, which is closely related to stability, nutritional, and cosmetic properties [22].

The studied WOF oil showed a fatty acid composition (Table 1) within the limits established by the IOC 2019 [47], except for linolenic acid and lignoceric acid. The total monosaturated fatty acid content level of the studied oleaster oil was the major subclass of fatty acids, comprising 72.80% of the total fatty acids quantified, followed by saturated fatty acids (16.76%) and polyunsaturated fatty acids (10.81%).

Oleic acid was the predominant monounsaturated fatty acid in the oleaster oil composition, constituting 71.55% of the total fatty acid content, with values within the range (64.7–76.15) found by Bouarroudj et al. [7], comparing four Algerian oleaster oils. Linoleic acid was the predominant polyunsaturated fatty acid in the studied WOF oil (8.26%), followed by linolenic acid (1.38%), which exceeds the 1% limit established by the IOC, 2019 [47]. Similar to our results, Bouarroudja et al. [7] and Anwar et al. [23] found greater levels of linolenic acid in oleaster oils. Palmitic acid was the main saturated fatty acid present in oleaster oil (12.79%), followed by stearic acid (2.56%) and palmitoleic acid (1.00%). It has been reported that oleaster oils contain higher levels of stearic acid than virgin olive oils, so it could be considered a marker of varietal characterization [23]. In addition, oil from WOF also showed lower levels of myristic, heptadecanoic, arachidic, eicosenoic, heneicosanoic, behenic, and lignoceric acids. Similar fatty acid profiles were also reported

for var. *sylvestris* oil in earlier studies in Algeria [7], Tunisia [6], Pakistan [23], Spain [48], and Italy [49].

3.2. Chlorophyls and Carotenoids Contents

Chlorophylls and carotenoids are the only pigments that are responsible for the characteristic yellowish-green color of the virgin olive oil. Chlorophyll is considered a basic attribute related to the quality of olive oil. The total chlorophyll content in the WOF sample was 148.41 mg/kg dw of olive fruit, whereas the total carotenoids was 85.90 mg/kg dw of olive fruit (Table 2). The chlorophyllic fraction is mainly composed of chlorophyll a (86.61 mg/kg dw of olive fruit) and chlorophyll b (61.80 mg/kg dw of olive fruit). The concentrations of both chlorophylls and carotenoids decrease progressively during olive fruit ripening [50].

Table 2. Pigments and tocopherol composition (mg/kg) of Greek WOF sample.

	Chemical Composition	Value (mg/kg dw of Olive Fruit)
Chlorophylls	Chlorophyll a	86.61 ± 0.99
	Chlorophyll b	61.80 ± 2.34
	Total Chlorophylls	148.41 ± 3.33
Carotenoids	Total carotenoids	85.90 ± 1.22
Tocopherols	δ-tocopherol	0.99 ± 0.01
	(β+γ)-tocopherol	1.68 ± 0.12
	α-tocopherol	104.39 ± 1.26
	Total tocopherols	107.05 ± 1.38

As presented in Table 2, α-tocopherol was the predominant tocopherol fraction of the WOF sample, whereas (β+γ)-tocopherol and δ-tocopherol were minor isomers. It is known that α-tocopherol plays a protective role against oil stability and shelf life, preserving its quality [51]. The total tocopherol content quantified in the present study (107.05 mg/kg dw of WOF or 497.07 mg/kg of oil) was within the range reported for seven populations of Tunisian oleaster oils (309.5–781.8 mg/kg of oil) [52], and slightly higher for those of three Portuguese oleaster oils (392.5–467.6 mg/kg oil) [29]. However, Algerian wild oils had much lower total tocopherol content (87–182 mg/kg oil) compared to our results [7]. These differences could be associated with genetic factors or other factors involved during the transportation, storage, and processing of the fruit [53]. The (β+γ)- and δ-isomers were minor constituents, accounting for less than 3% of the total tocopherols, which was slightly lower than those found in virgin olive oils (approximately 10%). Their contents differ among cultivars and modify during fruit ripening, while environmental factors can affect their contents [54]. According to Baldioli et al. [55], olive oils with total tocopherol contents within the range of 100 and 300 mg/kg are characterized by a good quality.

3.3. Preliminary Studies for Phenolic Extraction

There are four major phenolic classes of metabolites in olive fruits, including phenolic acids, flavonoids, lignans, and secoiridoids. Secoiridoids, e.g., oleuropein, ligstroside, and simple phenolics, are the main phenolics of olive fruits [56]. Due to their antioxidant and antimicrobial activities, they are considered an abundant source of bioactive ingredients in the food industry. So, the recovery of phenolic extracts using alternative approaches to the classical maceration method can promote sustainable and eco-friendly practices, often leading to energy cost savings and developing value-added products. Extraction is the most critical step to isolating bioactive extracts with high yields, if designed properly through optimization studies. The extraction parameters, including solvent type, extraction time, and temperature, can affect the extraction yield and the functionality of the final extract [57].

Hence, one of the main goals of this current study was to study the effects of various extraction conditions, including solvent type, extraction time, and temperature on

the phenolic composition and antioxidant activity of WOF extracts, by using eco-friendly extraction processes like UAE, MAE, and ASE. Firstly, the efficiency of different extraction solvents, including hexane, ethyl acetate, ethanol/water (70:30), methanol/water (70:30), and acetone/water (70:30) was studied by using UAE for determining the TPC of WOF extracts. UAE was selected as it is one of the simplest and inexpensive extraction systems for phenolic compounds in common laboratory equipment [58]. As illustrated in Figure 1, the highest TPC value was detected in both ethanol/water (70:30, *v*/*v*) and methanol/water (70:30, *v*/*v*) extracts (63.98 and 63.07 mg GAE/g dw, respectively), followed by acetone/water (70:30, *v*/*v*) and ethyl acetate extracts (55.80 and 13.57 mg GAE/g dw). Hexane extract (1.75 mg GAE/g dw) was the least efficient solvent to extract phenolic compounds from WOF, which is in agreement with the values (2.12 mg GAE/g dw) reported by Ghorbel et al. [17], who isolated phenolic compounds from oleaster fruit by maceration. According to our preliminary results, ethanol/water (70:30, *v*/*v*) was selected for optimization studies as it is recommended as a "green solvent" due to its low toxicity and its potential enhancement of environmental sustainability and social well-being.

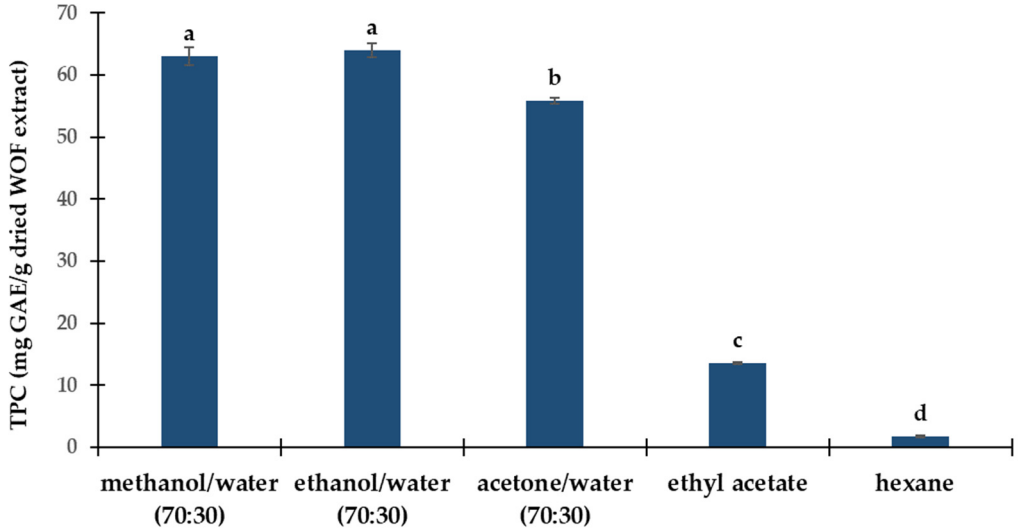

Figure 1. Total phenolic contents in wild olive fruit extracts obtained by ultrasound-assisted extraction (UAE) using different extraction solvents. Means followed by the same letter within a column are not significantly different ($p > 0.05$).

3.4. Identification of Phenolic Extracts

The liquid chromatography–mass spectrometry (LC-MS) technique is widely used in the structural characterization of the phenolic compounds in WOF [17,31,59]. In the current study, the identification of the phenolic compounds in the WOF extract was performed by LC-MS-ESI in negative ionization mode, accompanied by a DAD detector. A representative UV chromatogram at 280 nm of phenolic compounds in the WOP extract is illustrated in Figure 2. A total of 18 major phenolic compounds were successfully identified in the WOF extract, which include secoiridoids (10), flavonoids (3), simple phenols (3), and organic acids (2), as listed in Table 3. The phenolic compounds were tentatively identified and characterized based on their retention times, UV spectra, and MS spectra ($[M\text{-}H]^-$ ions), as confirmed by using commercial standards. However, secoiridoid derivatives (peaks 5, 7, 8, 12, 13, 15, 16, and 18) were identified based on the literature information concerning olive fruit characterization, due to the limitation of available standards [17,30,60–64].

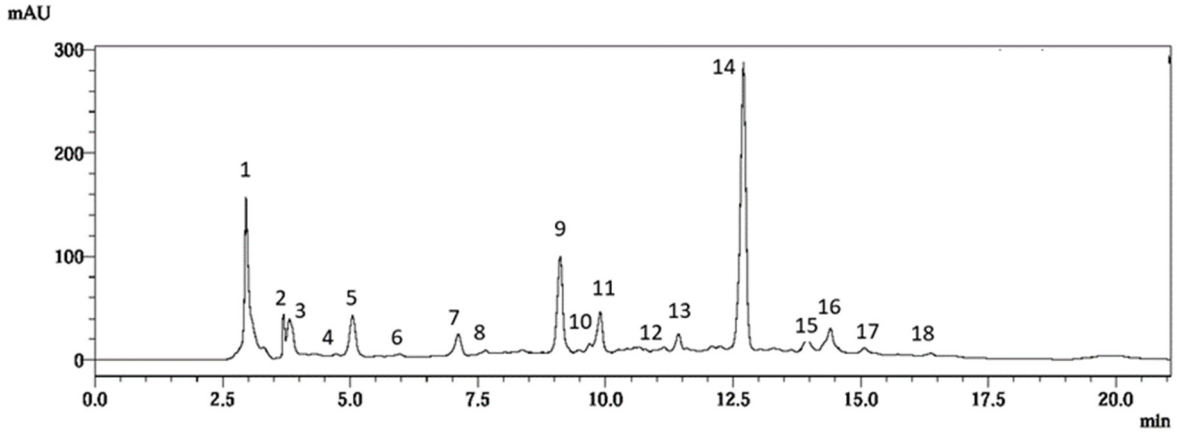

Figure 2. Chromatographic profile reordered at 280 nm for ethanolic extract of wild olive fruit after ultrasound-assisted extraction for 20 min at 45 °C. For peak identification, see Table 3.

Table 3. List of tentative major phenolic compounds identified by LC-DAD-MS in the WOF extracts.

Peak	Rt (min)	UV (nm)	[M-H]$^-$	Compound	Family	Reference
1	2.96	280	191	**quinic acid**	organic acid	standard solution
2	3.68	280	191	**citric acid**	organic acid	standard solution
3	3.86	278	315 (153)	**hydroxytyrosol glucoside**	simple phenols	[17,60,64]
4	4.81	281	153	**hydroxytyrosol**	simple phenols	standard solution
5	5.00	282	389	**oleoside/secologanoside (A)**	secoiridoid	[17,60,61]
6	6.03	278	137	**tyrosol**	simple phenols	standard solution
7	7.02	240, 280	403	**elenolic acid glucoside**	secoiridoid	[61,62]
8	7.52	283, 321	377	**oleuropein aglycone (A)**	secoiridoid	[30,60,61]
9	9.02	290, 353	609	**rutin**	flavonoid	standard solution
10	9.71	290, 336	623	**verbascoside**	secoiridoid	standard solution
11	9.92	250, 345	447	**luteolin-7-glucoside**	flavonoid	standard solution
12	10.72	237, 280	509	**dimethyl ligstroside**	secoiridoid	[60]
13	11.41	278	389	**oleoside/secologanoside (B)**	secoiridoid	[60,61]
14	12.68	281	539	**oleuropein**	secoiridoid	standard solution
15	14.00	282	583 (537)	**lucidumoside C**	secoiridoid	[61]
16	14.50	238, 278	523 (377)	**ligstroside**	secoiridoid	[17,60]
17	15.21	253, 366	285	**luteolin**	flavonoid	standard solution
18	15.81	283, 321	377 (307)	**oleuropein aglycone (B)**	secoiridoid	[30,60,61]

Secoiridoids were the major category of phenolic compounds identified in the WOF phenolic extract, belonging to oleuropein derivatives (peaks 8 and 18), ligstroside derivatives (peaks 12 and 16), oleoside-type (5 and 13), and elenolic acid derivatives (peak 7). Oleuropein was the major peak identified, showing a pseudo-molecular ion [M-H]$^-$ at m/z 539 (peak 14) (Figure 1). Peaks 5 and 13 presented a similar profile with maximum absorbance at ~280 nm and the same molecular ion [M-H]$^-$ at m/z 389, and were characterized as oleoside/secologanoside isomers (A and B), according to the literature data [17,60]. Other related compounds with the same UV max at ~280 nm were elenolic acid glucoside (peak 7) with m/z 403, oleuropein aglycone isomers (peaks 8 and 18) with m/z 377,

dimethyl ligstroside (peak 12) with m/z 509, lucidumoside C (peak 15) with m/z 583 [61], and ligstroside (peak 16) with m/z 523 [30,60].

Regarding the flavonoids, the most abundant compounds were rutin (peak 9, m/z 609), luteolin 7-O-glucoside (peak 11, m/z 447), and luteolin (peak 17, m/z 285), as confirmed by authentic standards. Within the category of simple phenols, two compounds were identified in trace: hydroxytyrosol (m/z 153), and tyrosol (m/z 137), as confirmed by their reference substances. However, peak 3 with m/z 315 was identified as hydroxytyrosol glucoside [17,60,64]. Compounds belonging to the chemical classes of organic acids were quinic acid and citric acid with a similar m/z 191 (peaks 1 and 2). To the best of our knowledge, there are few papers focused on the identification of phenolic compounds in wild olive fruits [17,64], while most of the studies are related to wild olive oils [29,31] or wild olive leaves [8,62,63].

3.5. Optimization Studies of Different 'Green' Extraction Methods

3.5.1. Process Optimization of Ultrasound-Assisted Extraction (UAE)

The effect of varying UAE times and temperatures on the antioxidant properties and phenolic composition of WOF extracts is presented in Table 4. Notably, the TPC appeared to increase significantly ($p \leq 0.05$) up to 20 min at a constant temperature of 30 °C, reaching the highest value of 63.98 mg GAE/g dw; however, an insignificant effect of extraction time within the range of 20–40 min was observed. Similarly, the highest level of antioxidant activity, as estimated by ABTS, DPPH, and FRAP values, is attained at 20 min, while the TFC values appeared to be independent of time extraction.

Table 4. Effect of extraction parameters on total phenolic content (TPC), total flavonoid content (TFC), oleuropein content, and antioxidant activity using ultrasound-assisted extraction (UAE), microwave-assisted extraction (MAE), and accelerated-solvent extraction (ASE) for recovering WOF extracts. Different letters in a column of each treatment indicate a significant difference ($p \leq 0.05$).

Parameters		Phenolic Components			Antioxidant Activity (mg TE/g dw)		
Time (min)	Temp. (°C)	TPC (mg GAE/g dw)	TFC (mg CATE/g dw)	Oleuropein (mg/g dw)	ABTS	DPPH	FRAP
Ultrasound-assisted extraction (UAE)							
10	30	60.23 ± 0.64 ^{c}	116.14 ± 5.46 ^{a}	65.43 ± 0.81 ^{c}	96.74 ± 1.14 ^{c}	106.22 ± 1.57 ^{b}	49.89 ± 0.40 ^{b}
20	30	63.98 ± 1.12 ^{ab}	116.82 ± 3.86 ^{a}	67.27 ± 1.03 ^{bc}	109.66 ± 2.28 ^{ab}	109.70 ± 2.95 ^{ab}	60.81 ± 0.56 ^{a}
30	30	63.07 ± 0.16 ^{b}	122.50 ± 2.25 ^{a}	67.31 ± 0.98 ^{bc}	110.88 ± 2.86 ^{ab}	111.92 ± 1.38 ^{ab}	60.92 ± 0.24 ^{a}
40	30	63.64 ± 0.00 ^{ab}	120.45 ± 2.57 ^{a}	69.27 ± 1.03 ^{b}	110.88 ± 2.86 ^{ab}	113.73 ± 2.75 ^{a}	62.29 ± 0.08 ^{a}
20	45	64.89 ± 0.16 ^{a}	116.59 ± 3.54 ^{a}	72.00 ± 1.41 ^{a}	118.69 ± 1.88 ^{a}	111.37 ± 5.31 ^{ab}	62.17 ± 2.49 ^{a}
20	60	63.52 ± 0.16 ^{ab}	116.14 ± 0.32 ^{a}	69.47 ± 0.75 ^{b}	104.01 ± 1.14 ^{bc}	109.84 ± 0.79 ^{ab}	62.86 ± 0.72 ^{a}
Microwave-assisted extraction (MAE)							
30	40	58.00 ± 0.56 ^{b}	123.00 ± 0.36 ^{b}	46.05 ± 1.36 ^{b}	95.03 ± 2.36 ^{b}	102.56 ± 0.65 ^{b}	53.60 ± 0.45 ^{b}
30	60	59.91 ± 0.13 ^{a}	128.00 ± 1.54 ^{a}	48.90 ± 1.56 ^{a}	99.36 ± 0.46 ^{b}	100.31 ± 9.47 ^{a}	55.84 ± 0.58 ^{a}
30	90	61.73 ± 0.90 ^{a}	131.82 ± 0.26 ^{a}	52.68 ± 1.87 ^{a}	112.93 ± 3.20 ^{a}	107.17 ± 9.87 ^{a}	55.52 ± 0.39 ^{a}
Accelerated-solvent extraction (ASE)							
30	60	56.82 ± 0.96 ^{a}	114.55 ± 1.93 ^{a}	44.49 ± 2.11 ^{a}	99.77 ± 0.29 ^{a}	98.80 ± 5.38 ^{ab}	55.23 ± 0.56 ^{a}
30	90	59.18 ± 1.54 ^{a}	111.00 ± 0.39 ^{a}	43.68 ± 1.87 ^{a}	100.10 ± 2.74 ^{a}	103.72 ± 3.85 ^{ab}	53.79 ± 0.77 ^{a}
30	120	43.27 ± 3.15 ^{b}	81.77 ± 1.35 ^{b}	25.25 ± 1.06 ^{b}	71.56 ± 1.60 ^{b}	89.59 ± 1.58 ^{b}	35.60 ± 0.79 ^{b}

Regarding oleuropein content, the predominant phenolic compound identified in WOF extracts, it was noticed that at higher extraction times, a higher value of oleuropein appeared, reaching the greatest value (69.27 mg/g dw) at 40 min; however, an insignificant effect was noticed at duration times of 20–40 min. Concerning the temperature effect in UAE, the increased temperature up to 45 °C favored the removal of the phenolic compounds present in the WOF, while longer increased temperatures until 60 °C showed a significant decrease. These results suggest that a sonication time of 20 min at 65 °C was sufficient to maximize TPC, TFC, and antioxidant activity as well as the oleuropein content, potentially

leading to the enhancement of its nutritional factors, and health and well-being benefits. These findings align with the literature data emphasizing the importance of sonication time and temperature in optimizing phenolic compound extraction from freeze-dried olive fruit material by ultrasonic probe accompanied with a three-step extraction of 20 min at 45 °C [64]. Similarly, Ullah et al. [65] appraised optimal extraction conditions to be a 32 min time and 43 °C temperature for the best recovery of TPC and antioxidant potential of Gemlik olive fruit.

3.5.2. Process Optimization of Microwave-Assisted Extraction (MAE)

MAE experiments were performed at a fixed time of 30 min, which shows an effective yield of phenolic compounds, when using microwave irradiation [66], while the extraction temperatures were varied from 40 to 90 °C. For the three temperature levels evaluated, the only statistically significant difference for phenolic compounds and antioxidant potential appeared at 30 °C (Table 4). High temperatures (>40 °C) appeared to have a positive effect on phenolic extraction, which means that the main phenolic compound in the WOF extract, named oleuropein, presented a thermolabile behavior under MAE up to 60 °C. Although insignificant differences were noticed at temperatures of 60 and 90 °C, for most of the phenolic-based components, the higher temperature was selected to extract the phenolic compounds from the WOF. According to the results of da Rosa et al. [67], high MAE temperatures (86 °C) do not cause the degradation of antioxidant compounds from olive tree leaves. Similar optimized conditions were determined for olive pomace using applied MAE conditions at 113 °C for 26 min, yielding values of 67.4 mg/g for oleuropein [68]. In another study, it was found that MAE at a higher temperature (86 °C) was more efficient in terms of TPC yield of olive leaves with a short extraction time (3 min) [69].

3.5.3. Process Optimization of Accelerated-Assisted Extraction (ASE)

ASE is gaining wider attention nowadays due to its environment-friendly processes and sustainability. The WOF sample was extracted with ASE using three different temperatures (60, 90, and 120 °C) for a total time of 30 min, applying three extraction cycles. The use of static cycles helps to maintain a favorable extraction equilibrium by adding fresh solvent during the extraction process. According to Culina et al. [70], the addition of a fresh solvent positively affects the extraction of phenolic compounds from sea buckthorn leaf and berry extracts when three extraction cycles are applied.

The phenolic compounds in the WOF extracted by ASE revealed a decrease with an increase in temperature. This means that the use of low temperatures under ASE results in high antioxidant activity in WOF extracts with a superior yield of oleuropein. Similarly, other authors found that the extraction temperature had a negative effect on the lignan glucoside contents extracted by ASE equipment [71]. The optimum temperature for ASE phenolic extraction from WOF was achieved at 60 °C. Similar to our findings, most of the available literature reports about olive-related products apply low temperatures. Specifically, Cepo et al. [72] applied an ASE temperature of 70 °C for the recovery of an oleuropein-rich extract from olive pomace, while Chaji et al. [73] used a much lower temperature of 30 °C for olive tree leaves. However, Ahmad et al. [27] reported that temperature showed no significant correlation for phenolics when studying the effect of solvent and temperature on ASE extraction of phenolics in fresh olive fruit (*Olea europaea*).

3.6. Comparison of UAE, MAE, and ASE

Phenolic compounds, along with tocopherols, are the main components that are related to the antioxidant capacity of olive oil and are also associated with its bitter taste [74]. In addition, several health-promoting properties have been attributed to the phenolic content of olive fruits. A comparative study of all extracts was carried out in order to gain a better insight into the extraction efficiency of the tested methods (UAE, MAE, and ASE), The values of TPC, TFC, and the antioxidant activity of the WOF extracts obtained under UAE, MAE, and ASE optimized extraction conditions are shown in Figure 3. It is noticed that

TPC yield was the highest when extracted by UAE, while that obtained by ASE was much lower (Figure 3a). Similar to the TPC yields, WOF extracts under UAE had the highest antioxidant activity, followed by MAE and ASE extracts (Figure 3b). Comparing the results in Figure 3a, much higher concentrations of TPC were achieved with UAE (64.89 mg/g dw) and MAE (61.73 mg/g dw) than with ASE (56.82 mg/g dw). Kabach et al. [75] found that the methanolic extract had a higher TPC in oleaster fruit from Morocco, with a value of 116.94 mg GAE/g dw; however, Ghorbel et al. [17] found that the ethyl acetate extract of wild olive fruit from Tunisia had a lower TPC of 17.04 mg GAE/g dw, while Hannachi et al. [16] reported a much lower value of 2.12 mg GAE/g dw for the methanolic extract of WOF. Differences in TPC values could be attributed to different extraction parameters applied, as well as to genetic factors, environmental and geographic conditions, agronomic practices, ripeness, and post-harvest processing [76].

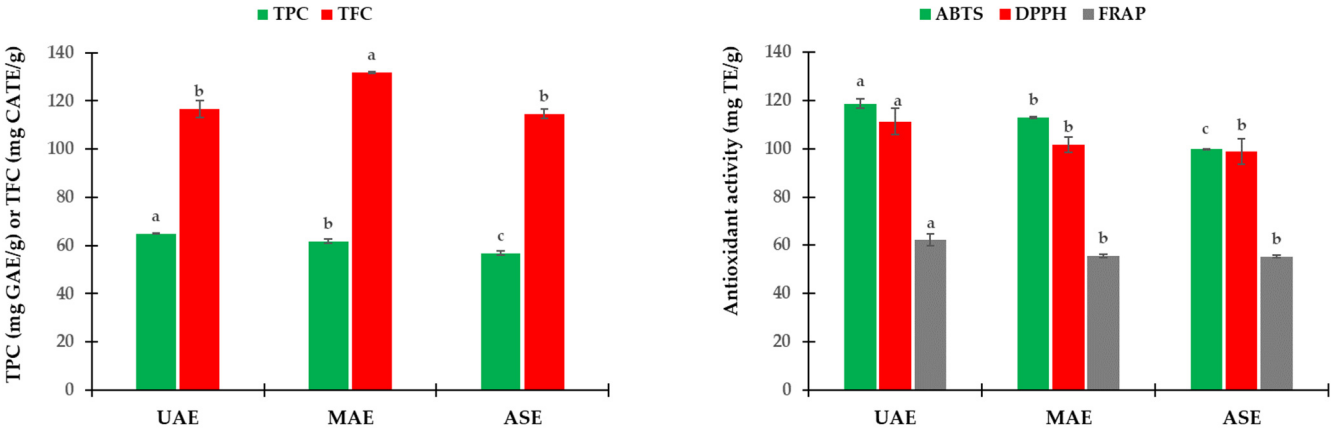

Figure 3. Total phenolic content (TPC) and total flavonoid content (TFC) (**a**) as well as the antioxidant activity as evaluated by DPPH, ABTS, and FRAP tests (**b**) of wild olive fruit extracts obtained by ultrasound-assisted extraction (UAE), microwave-assisted extraction (MAE), and accelerated-solvent extraction (ASE); different letters in a column of the same color indicate a significant difference ($p \leq 0.05$).

On the other hand, under MAE, higher yields of TFC were obtained compared to UAE and ASE (Figure 3a), demonstrating the advantages of MAE for the extraction of flavonoids. Similarly, Culina et al. [70] showed that MAE was more suitable for the extraction of flavonols from berries. A similar trend was observed for the antioxidant activity of the WOF extracts as evaluated by ABTS, DPPH, and FRAP values under optimal conditions, with UAE extracts presenting the highest antioxidant activity, followed by MAE and ASE extracts (Figure 3b).

Generally, a high correlation was observed between TPC and antioxidant capacity, while no correlation was found between TFC and antioxidant activity, which suggests that the contribution of flavonoids, as minor phenolic components, was little to antioxidant power. Positive correlations between TPC and antioxidant activity of phenolic extracts from wild olive oils have been reported in agreement with our findings, suggesting that WOF phenolic extracts may contribute to the oxidative stability of olive oil in a higher proportion than α-tocopherol [77].

The phenolic composition of WOF extracts obtained at optimal UAE, MAE, and ASE conditions was analyzed using HPLC coupled to a mass spectrometer with an ESI source. The data listed in Table 5 revealed that secoiridoids represent 76 to 84% of the total individual phenolic compounds of the WOF extracts. Oleuropein was the most abundant secoiridoid in the WOF extracts, contributing 55–67% of the total phenolic compounds quantified. According to the study of Jerman et al. [64], oleuropein was the

most abundant phenolic compound (more than 85% of the total phenolic compounds) identified in cultivated olive fruit extracts.

Table 5. Phenolic compounds in wild olive fruit extracts obtained via ultrasound-assisted extraction (UAE), microwave-assisted extraction (MAE), and accelerated solvent extraction (ASE). Different letters in a line indicate a significant difference ($p \leq 0.05$).

Phenolic Compound	UAE	MAE	ASE
	secoiridoids		
oleoside/secologanoside (A)	3.23 ± 0.06	3.23 ± 0.09	3.24 ± 0.12
oleoside/secologanoside (B)	4.94 ± 0.20	6.01 ± 0.13	5.03 ± 0.10
oleuropein aglycone (A)	0.40 ± 0.01	0.64 ± 0.02	0.71 ± 0.01
oleuropein aglycone (B)	4.31 ± 0.22	4.29 ± 0.20	3.89 ± 0.11
elenolic acid glucoside	1.12 ± 0.07	1.08 ± 0.05	1.08 ± 0.04
verbascoside	1.27 ± 0.02	1.09 ± 0.03	0.77 ± 0.03
oleuropein	72.03 ± 0.71	52.68 ± 0.93	44.49 ± 1.05
dimethyl ligstroside	1.63 ± 0.07	1.36 ± 0.12	0.62 ± 0.01
ligstroside	1.14 ± 0.03	0.56 ± 0.03	0.53 ± 0.01
lucidumoside C	0.39 ± 0.01	0.40 ± 0.02	0.24 ± 0.01
total secoiridoids	89.83 ± 0.51 [a]	71.34 ± 0.53 [b]	61.89 ± 0.93 [c]
	flavonoids		
rutin	3.75 ± 0.07	4.69 ± 0.08	3.29 ± 0.07
luteolin-7-glucoside	13.40 ± 0.09	18.35 ± 0.25	14.78 ± 0.15
luteolin	0.11 ± 0.00	0.05 ± 0.00	0.07 ± 0.00
total flavonoids	17.25 ± 0.16 [b]	23.08 ± 0.17 [a]	18.15 ± 0.22 [b]
	simple phenols		
hydroxytyrosol glucoside	1.92 ± 0.06	2.34 ± 0.08	2.68 ± 0.05
	organic acids		
quinic acid	1.27 ± 0.03	0.90 ± 0.02	1.27 ± 0,02
citric acid	0.08 ± 0.01	0.11 ± 0.01	0.15 ± 0.02
total organic acids	1.35 ± 0.02	1.01 ± 0.01	1.42 ± 0.05

Among the WOF extracts, the highest concentration was found in the UAE extracts (72.03 mg/g dw), followed by the MAE (52.68 mg/g dw) and ASE (44.49 mg/g dw) extracts. Similar to our results, in the study of Ghorbel et al. [17], oleuropein was found to be the most abundant compound in oleaster olive fruits, with a concentration of 61.93 mg/kg, applying maceration as the extraction method. However, there are no available literature data about the application of 'green' extraction methods of phenols from the wild olive fruit matrix in order to compare our data. Concerning olive leaves, Xie et al. [78] found that UAE was an extremely useful and important extraction method for extracting oleuropein. However, de Rosa et al. [69] reported that the oleuropein content in olive leaves for the MAE had a two-fold increase compared to the UAE.

Oleoside and secologanoside derivative forms (A and B) were the second most represented group of secoiridoids and showed a higher content in the MAE extract (9.23 mg/kg dw) than that quantified in the UAE and ASE extracts (8.18 and 8.27 mg/kg dw, respectively). However, the third most abundant compounds, oleuropein aglycon derivatives, showed similar values in all extracts (4.61–4.93 mg/kg dw). Elenolic acid glucoside and lucidumoside C compounds appeared to be independent of the extraction method applied, while verbascoside, dimethyl ligstroside, and ligstroside contents were highest in the UAE among the other extracts. Regarding simple phenols, hydroxytyrosol glucoside was the major constituent varying from 1.92 to 2.68 mg/kg dw in the WOF extracts, whereas hydroxytyrosol was quantified in traces.

In the group of flavonoids, luteolin-7-glucoside was the most abundant compound in WOF, appearing to have the highest value in the MAE extract (18.35 mg/g dw), whereas no significant differences were observed in the UAE and ASE extracts. Among the other

identified flavonoids, rutin (3.29–4.69 mg/kg dw) showed higher values compared to luteolin (0.05–0.11 mg/kg dw). These flavonoids have previously been found in WOF grown in Tunisia [17]. Generally, it can be concluded that UAE should be preferred over MAE, since it gives similar extraction performances at a lower energy density.

3.7. Volatile Compounds

Volatile compounds are responsible for the aroma profile of olive fruits. Consequently, the volatile compounds profile is considered a suitable tool for authentication of virgin olive oils. In total, 60 volatile compounds were identified in the WOF used, composed of 21 terpenoids, 15 hydrocarbons, 9 aldehydes, 8 alcohols, 2 ketones, 2 esters, and 3 miscellaneous compounds (Table 6).

Most of the volatile compounds in the WOF were terpenoids (46.84%), followed by hydrocarbons (20.21%) and aldehydes (10.95%), whereas the content of the rest of the classes was slightly lower (<12%). Regarding ketones, their percentage was recorded at low levels (5.09%), with acetoin and 6-methyl-5-heptene-2-one being the predominant compounds. The main volatile compounds of unripe fruits were β-ocimene (13.63%), followed by α-copaene (9.64%), α-farnesene (8.03%), and α-phellandrene (5.04%), all of them belonging to terpenoids. β-ocimene (floral, herbal, sweet note) along with α-farnesene (woody, green, floral, herbal note) and α-copaene (woody, spicy note) have already been identified as volatile markers in Greek olive oils [79]. The presence of many mono- and sequiterpenes, even if in low amounts, in the unripe fruits has also been mentioned by Lamini et al. [80].

Table 6. Volatile composition (% of total area of identified compounds) of wild olive fruit as determined by solid-phase microextraction/gas chromatography-mass spectrometry (SPME-GC-MS) method.

No.	Volatile Compound	RI (exp) [1]	RI (lit) [2]	% Area [3]	Odor Description [4]
	Aldehydes				
1	**2-Methyl-butanal**	646	664	0.07 ± 0.01	Musty, coffee, nutty
2	**Hexanal**	803	801	3.80 ± 0.25	Green, apple
3	**(E)-2-Hexenal**	861	853	4.78 ± 0.02	Apple-like, green, leaf
4	**Heptanal**	904	902	0.21 ± 0.02	Fresh, fatty, green, herb, wine
5	**(E,E)-2,4-heptadienal**	1001	998	0.24 ± 0.01	Fatty, nut
6	**Octanal**	1005	1005	0.27 ± 0.01	Fatty, lemon
7	**Benzene acetaldehyde**	1053	1050	0.45 ± 0.04	Pungent, honey, fruity,
8	**Nonanal**	1106	1102	1.00 ± 0.07	Rose, fresh, orris, orange, fatty
9	**Decanal**	1208	1204	0.17 ± 0.01	Soap, orange peel, tallow
	Total Aldehydes			10.95 ± 0.14	
	Ketones				
10	**Acetoin**	695	709	3.31 ± 0.18	Creamy, fatty
11	**6-Methyl-5-Heptene-2-one**	989	986	1.79 ± 0.02	Citrus-like, fruity
	Total Ketones			5.09 ± 0.20	
	Alcohols				
12	**1-Methoxy-2-propanol**	649	672	0.24 ± 0.03	-
13	**3-Methyl-1-butanol**	721	727	0.19 ± 0.01	Fermented
14	**2,3-Butanediol**	785	785	2.42 ± 0.05	Creamy
15	**2,3-Butanediol isomer**	797	806	0.67 ± 0.01	Creamy
16	**1-Hexanol**	875	867	0.62 ± 0.01	Herbal, pungent, alcoholic
17	**1-Hexanol 2-ethyl-**	1032	1029	0.25 ± 0.01	Herbal, pungent, alcoholic
18	**Benzyl alcohol**	1043	1034	0.49 ± 0.02	Floral, sweet, phenolic
19	**Phenethyl alcohol**	1119	1110	0.47 ± 0.06	Floral, sweet, bready

Table 6. *Cont.*

No.	Volatile Compound	RI (exp) [1]	RI (lit) [2]	% Area [3]	Odor Description [4]
	Total Alcohols			5.32 ± 0.01	
	Terpenoids				
20	**α-Thujene**	926	931	0.33 ± 0.01	Woody, green, herbal
21	**α-Pinene**	933	939	0.80 ± 0.01	Fresh, herbal, pine
22	**Camphene**	951	953	0.08 ± 0.00	Camphor, citrus, green, spicy
23	**m-Cymene**	964	961	0.14 ± 0.01	-
24	**Sabinene**	974	976	0.22 ± 0.01	Pepper, wood
25	**α-Phellandrene**	1005	1005	5.04 ± 0.07	Citrus, herbal, terpenic, green
26	**α-Terpinene**	1018	1018	0.09 ± 0.01	Woody, terpenic, citrus
27	**p-Cymene**	1027	1025	1.50 ± 0.02	Solvent, citrus
28	**Limonene**	1029	1031	1.58 ± 0.01	Citrus, lemon, orange
29	**1,8-Cineole**	1035	1033	1.23 ± 0.01	Herbal, medicinal
30	**cis-β-Ocimene**	1038	1040	0.46 ± 0.04	Floral, herbal, sweet
31	**β-Ocimene**	1050	1050	13.63 ± 0.13	Floral, herbal, sweet
32	**γ-Terpinene**	1060	1062	0.31 ± 0.01	Terpy, citrus, lime-like, oily
33	**Linalool**	1101	1098	0.47 ± 0.04	Floral, citrus, terpenic
34	**allo-Ocimene**	1130	1129	0.10 ± 0.00	Terpenic, sweet, fresh floral
35	**Neo-Allo-Ocimene**	1142	1138	0.30 ± 0.01	-
36	**Carvacrol**	1301	1298	0.15 ± 0.04	Spicy, woody, herbal
37	**α-Copaene**	1376	1376	9.64 ± 0.08	Woody, spicy
38	**β-Caryophyllene**	1422	1418	0.81 ± 0.01	Spicy, woody, terpenic
39	**α-Muurolene**	1498	1500	1.90 ± 0.03	-
40	**α-Farnesene**	1502	1508	8.03 ± 0.32	Woody, green, floral, herbal
	Total Terpenoids			46.84 ± 0.25	
	Hydrocarbons				
41	**Toluene**	755	756	8.33 ± 0.27	Sweet
42	**Octane**	793	801	0.12 ± 0.01	Gasoline-like
43	**m-Xylene**	873	867	0.30 ± 0.02	Plastic
44	**p-Xylene**	873	875	0.31 ± 0.00	-
45	**o-Xylene**	893	892	0.52 ± 0.01	Geranium
46	**n-Nonane**	896	900	0.26 ± 0.00	Gasoline-like
47	**Benzene, 1-ethyl-4-methyl-**	968	969	0.11 ± 0.01	-
48	**1,3,5-Trimethy-lbenzene**	994	996	0.31± 0.02	-
49	**Decane**	997	1000	0.40 ± 0.01	-
50	**Undecane**	1097	1100	0.39 ± 0.01	-
51	**4,8-dimethyl-(E)-Nona-1,3,7-triene**	1113	1114	8.09 ± 0.14	-
52	**Cosmene**	1130	1130	0.33 ± 0.01	-
53	**Dodecane**	1196	1199	0.23 ± 0.00	-
54	**Cyclosativene**	1370	1368	0.45 ± 0.00	-
55	**Heptadecane**	1396	1400	0.09 ± 0.01	-
	Total Hydrocarbons			20.21 ± 0.44	
	Esters				
56	**Methyl hexanoate**	930	932	0.12 ± 0.01	Fruity, pineapple, ether
57	**Isopropyl myristate**	1823	1825	0.26 ± 0.01	-
	Total Esters			0.38 ± 0.03	
	Miscellaneous Compounds				
58	**2-Butoxyethanol**	905	909	0.72 ± 0.08	-
59	**epi-ligulyl oxide**	1542	1544	0.30 ± 0.07	-
60	**n-Octyl ether**	1596	1657	0.14 ± 0.00	-
	Total Miscellaneous Compounds			1.16 ± 0.01	

[1]: RI(lit): literature retention index, [2]: RI(exp):experimental retention index (NIST MS search), [3]: Mean value of three replications, [4]: Odor description was obtained from https://www.thegoodscentscompany.com/ (accessed on 10 May 2024).

According to previous reports, the percentages of terpenoids that exist in olive oils depend on both the olive variety and geographical origin [81]; actually, α-farnesene was often reported to be the most representative terpenoid in virgin olive oils from different geographic locations [81,82]. Other related compounds identified in unripe olive fruits in quite low amounts (<1%) were a-murolene, p-cymene, limonene, 1,8-cineole, β-caryophyllene, α-pinene, and linalool. The role of terpenoids in the definition of flavor is not clear.

In addition, hydrocarbons were the second most abundant chemical class in the WOF, with toluene (8.33%) and 4,8-dimethyl-(E)-nona-1,3,7-triene (8.09%) being the major volatile constituents. The last hydrocarbon has also been reported in black ripe table olives [83]. Regarding the aldehydes, which represented the most abundant volatile class in olive oils, being the products of the lipoxygenase pathway, (E)-2-hexenal (4.78%), followed by hexanal (3.80%), was the most abundant aldehyde identified in the WOF tested, which is responsible for green, apple-like, fatty, and grass sensory notes. These results agree with previous findings, suggesting that (E)-2-hexenal was the most prominent compound in two Tunisian wild oils [6].

Other minor aldehydes observed were nonanal, benzeneacetaldehyde, (E,E)-2,4-heptadienal, octanal, heptanal, decanal, and 2-methyl butanal. Of the eight identified alcohols, 2,3-butanediol, 1-hexanol, benzyl alcohol, and phenethyl alcohol were found in appreciable amounts. Generally, WOF is characterized by low levels of total alcohols [6]. The level of esters (0.38%), such as methyl hexanoate and isopropyl myristate, which are responsible for the floral sensory note [84], was found to be much lower compared to the levels of aldehydes and alcohols. Olive oil derived from wild *Olea europaea* ssp. *europaea* var. *sylvestris* is characterized by a pungent taste due to the higher phenolic content when compared to the oil extracted from cultivars [6,29].

4. Conclusions

This study investigated, for the first time, in a holistic approach, the nutritional, bioactive, and volatile profile of wild olive fruits, or oleaster (*Olea europaea* var. *sylvestris*), collected from Greece, to promote their sustainable use in the Mediterranean diet. According to the findings, wild olive fruits are a promising source of nutrients and bioactives from green extraction technologies that can be valorized for food, pharmaceutical, and cosmetic purposes. The findings from this work revealed that wild olive oil contained a high quality of fatty acids, with oleic acid being the predominant fatty acid detected, followed by linoleic acid. Due to their richness in extracting phenolic compounds, eco-friendly extraction methods were applied to recover natural extracts with enhanced antioxidant activity. The HPLC analysis revealed that the oleaster extracts were predominated by oleuropein as the major phenolic compound. Based on the obtained results, UAE for 20 min at 45 °C was the best extraction technique among UAE, MAE and ASE to recover phenolic compounds using ethanol/water (70:30, *v*/*v*) as a solvent, whereas ASE proved to be the least powerful tool to isolate oleuropein-rich extracts. Finally, the emerging knowledge about the health-promoting effects of wild-type foods should encourage further basic and clinical research on human health benefits and explore fundamental principles for industrial applications, aiming to promote a powerful nutraceutical.

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