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Review

Exploring the Influence of Extraction Methods, Solvents, and Temperature on Total Phenolic Recovery and Antioxidant Capacity in Olive Leaf Extracts: A Systematic Review with Quantitative Synthesis

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Abstract

Background: Olive leaves are a rich source of bioactive phenolic compounds, but extraction yields vary depending on methodological choices. The aim was to identify optimal parameters for maximizing recovery and preserving antioxidant activity. Methods: Fourteen studies (149 samples) were included, following predefined eligibility criteria and PRISMA guidelines for systematic review. Data on TPC, TFC, and antioxidant assays (DPPH, FRAP, ABTS) were extracted and analyzed according to extraction method, solvent type, and processing conditions. Results: Soxhlet extraction and shaking achieved the highest TPC and antioxidant capacity, whereas ultrasound-assisted and high-voltage electrical discharge extractions showed lower averages unless intensity or duration was increased. Solvent polarity was critical: $\geq$75% aqueous methanol provided the highest TPC and FRAP, while $\geq$75% ethanol yielded the greatest TFC and ABTS activity. Pure water consistently gave the lowest yields. Extractions at >50 °C increased TPC up to fivefold compared to room temperature but did not proportionally improve radical-scavenging capacity. Most phenolic compounds were recovered within $\leq$1 h under optimized, heated, or assisted conditions, with longer times offering no significant advantage. Conclusions: Optimizing solvent composition, temperature, and extraction time is essential for maximizing yield and maintaining antioxidant quality in olive leaf extracts, and standardized protocols are needed to enable direct comparisons across studies.

Keywords: olive leaves; solvent; extraction time; phenols; oleuropein; by-products; Soxhlet; ultrasound

1. Introduction

Olive leaves (OL) are among the most abundant by-products of the olive oil industry, generated in huge quantities during olive harvesting and pruning [1]. Major olive-producing countries in the Mediterranean (Spain, Italy, Greece, and Portugal) account for nearly all OL production, amounting to hundreds of thousands of tonnes annually. Traditional disposal of OL (e.g., agricultural waste, low-value animal feed, or incineration) imposes environmental burdens due to the energy-intensive removal processes and biomass waste generation [2]. However, OLs are rich in bioactive phytochemicals, notably phenolic compounds such as the secoiridoid oleuropein (the predominant bitter glycoside in olive leaves) and its hydrolysis product hydroxytyrosol, alongside various flavonoids and phenolic acids [2,3]. These constituents endow OLs with significant nutraceutical value. Indeed, OL extracts and their polyphenols have demonstrated a broad spectrum of health-promoting effects, including potent antioxidant, anti-inflammatory, antimicrobial, and cardioprotective activities, as well as neuroprotective benefits [4–7]. Given these bioactivities, there is growing scientific and commercial interest in repurposing olive leaf waste into value-added products. Researchers and industry stakeholders are exploring OLs as a readily available source of functional food ingredients and nutraceutical supplements [4–7].

Critically, the phytochemical profile and yield of bioactive compounds from OLs depend on numerous variables. Factors such as the olive cultivar, geographic origin, growth climate, pruning/harvest season, and post-harvest processing (drying) all modulate the leaf's phenolic composition. Additionally, extraction conditions, including solvent type, temperature, and technique, have a profound influence on the recovery of oleuropein, hydroxytyrosol, and other nutraceuticals [8,9]. Recent comparative studies of extraction methodologies (e.g., conventional Soxhlet vs. pressurized liquid extraction, and ultrasound-assisted extraction) highlight significant variability in total phenolic yields and antioxidant activities of OL extracts [5,8]. Equally important is the choice of detection method, since different analytical approaches (e.g., colorimetric assays, chromatographic and hyphenated techniques) can influence the quantification and apparent distribution of phenolics, thereby contributing to discrepancies between studies [10]. This variability underscores the need to identify optimal extraction conditions and to move toward standardizing extraction protocols. Therefore, the present review aims to systematically summarize current OL extraction methods and outcomes, determine the ideal extraction parameters for maximizing phenolic and flavonoid recovery, and support the development of standardized, efficient extraction procedures.

2. Materials and Methods

2.1. Search Strategy

In this systematic review, two authors (M.C.-C. and C.M.-H.) systematically searched the PubMed database for in vitro assays up to 23 November 2024. The search string combined various terms related to olive leaves with terms related to phenolic and flavonoid content. Both Medical Subject Headings (*MeSH*), including "olive leaf", and free-text search terms were utilized. The search did not include any limitations regarding the publication date. The following search strategy was employed: ("olive leaf" OR "olive leaves") AND (TPC OR TFC OR "Total flavonoid content" OR "Total phenolic content") in the title or abstract.

2.2. In- and Exclusion Criteria

The following inclusion criteria were applied to select relevant studies: (1) studies evaluating the phenolic content of dried olive leaves or their extracts; (2) studies reporting results per unit of dry extract.

Exclusion criteria were defined as follows: (1) studies that combined olive leaves with other by-products or compounds; (2) studies using solvents such as natural eutectic solvents or similar; (3) studies presenting data per unit of fresh product or per milliliter; (4) studies with an unclear extraction procedure description; (5) studies that remained unavailable after contacting the corresponding authors. Discrepancies were resolved through consensus among the authors.

2.3. Outcomes

The primary outcome measured was the total phenolic content (TPC) of olive leaf extracts. Secondary outcomes included the total flavonoid content (TFC) and antioxidant capacity tests, such as 2,2-Diphenyl-1-picrylhydrazyl (DPPH), Ferric Reducing Antioxidant Power (FRAP), and 2,2′-Azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS). Studies were required to evaluate at least the primary outcome to be included. Secondary analyses that duplicated primary or secondary outcomes reported in different articles were excluded by consensus among the authors. In the results section, TPC and TFC were expressed as milligrams of gallic acid equivalents (GAE) and catechin equivalents (CAE) per gram of dry weight (DW), respectively. Antioxidant capacities measured by DPPH, FRAP, and ABTS assays were expressed as micromoles (µmol) of Trolox equivalents (TE) per gram of DW.

2.4. Data Extraction and Statistical Analysis

Two authors (M.C.-C. and C.M.-H.) independently reviewed and extracted data from the studies into a predesigned database, including publication details, olive leaf extraction characteristics, and primary and secondary outcomes. Discrepancies were resolved through discussion or, if necessary, by a third researcher (M.D.N.-H.).

For the quantitative analysis, data from the different articles were aggregated into the categories studied (extraction method, solvent, time, and temperature) to enable a better understanding and facilitate comparative evaluation. While Table 1 shows the ranges reported in each individual study, the quantitative synthesis refers to the aggregated values across studies.

Table 1. Summary of selected articles on olive leaf extraction methods and phenolic content.

Olive Variety	Solvent	Extraction Time	Extraction Temp.	Additional Methods	TPC (mg GAE/g)	Refs.
Arbequina and not specified OL varieties	Pure water, ethanol/water (80/20, *v*/*v*), ethanol/water (50/50, *v*/*v*), and ethanol/water (25/75 *v*/*v*)	30 s, 45 s, 3 min, 9 min, 24 h, and 48 h	35–40 °C and 22 °C	HAE, HVED, and shaking	15.88–53.00	[11]
Koroneiki	Ethanol/water (80/20, *v*/*v*)	5 h	30 °C	Shaking	1.97	[12]
Not specified OL varieties	Water, ethanol/water (25/75 *v*/*v*) and ethanol/water (50/50, *v*/*v*)	3 and 9 min	22 °C	Shaking	5.32–20.61	[13]
Apollo, Ascolana tenera, Carolea, Cellina, Cipressino, Itrana, Maurino, Minerva, Moraio, Nociara, Ogliarola, Pendolino, Sant Agostino, Ravece, and Taggiasca	Ethanol/water (60/40, *v*/*v*)	2 h	25 °C	Shaking	12.00–47.00	[14]

Table 1. *Cont.*

Olive Variety	Solvent	Extraction Time	Extraction Temp.	Additional Methods	TPC (mg GAE/g)	Refs.
Ascolana, Ayvalik, Cekiste, Esek Zeytini, Gemlik, Kilis Yaglik, Memecik, Saurani, and Uslu	Methanol/water (80/20, v/v)	45 min	65 °C	Shaking	110.00–268.00	[15]
Picual, Hojiblanca, Arbequina, and OL mixtures	Ethanol/water (80/20, v/v)	2 h	25 °C	Shaking	27.19–52.78	[3]
Mousaabi, Khouderi, Zaity, and Nipali	Ethanol/water (80/20, v/v)	4 h	60 °C	Soxhlet extraction	38.39–72.78	[16]
Hojiblanca	Ethanol/water (50/50, v/v) and ethanol/water (75/25 v/v)	5 h	80 °C	Soxhlet extraction	71.90–76.10	[17]
Not specified OL varieties	Water	25 min	<75 °C	UAE	162.00	[18]
Rosinjola and Istarska bjelica	Ethanol 100%	30 min	<30 °C	UAE	10.80–14.15	[19]
Koroneiki	Methanol 100%	30 min	50 °C	UAE	15.40–41.80	[20]
Not specified OL varieties	Ethanol 70% (1/10 w/v)	30 min	50 °C	UAE	79.43	[21]
Nocellara del Belice and Carolea	Ethanol/water (50/50 v/v)	3 h	25 °C	UAE	10.83–92.93	[22]
Lastovka, Levantinka, Oblica, Moraiolo, Frantoio, Nostrana di Brisighella, and Arbequina	Ethanol/water (80/20, v/v)	45 s, 30 min and 1 h	25 °C and 35–40 °C	UAE	16.50–44.00	[23]

Abbreviations: UAE: ultrasound-assisted extraction; GAE: galic acid equivalents; v: volume; s: second; min: minute; h: hour; HAE: heat-assisted extraction; HVED: high-voltage electrical discharge; OL: olive leaf.

Statistical analyses, including normality, variance homogeneity, and ANOVA, were conducted using IBM SPSS 25 (Chicago, IL, USA), with post hoc Duncan's Multiple Range test, considering significance at $p < 0.05$. Data reported only in figures were digitized using the online version of Plot Digitizer (https://plotdigitizer.com, accessed on 13 May 2025). All data extraction and analyses were reviewed by the corresponding authors, with disagreements resolved through panel discussions.

3. Results and Discussion

3.1. Study Selection and Characteristics

A total of 52 records were identified through PubMed. In addition, by screening the references cited within the articles retrieved from PubMed, one additional record was identified through handsearching. After removing duplicates and applying the inclusion and exclusion criteria, 14 studies were included in the systematic review (Figure 1).

According to the exclusion criteria, 13 studies combining olive leaves with other products, 11 studies without TPC measurements, seven articles that were not accessible through open access or the University of Granada license, and one article that did not clearly describe the extraction procedure, as well as six studies excluded because their thematic content was beyond the scope of the present review, were excluded. Ultimately, 14 studies with 149 olive leaf samples were included in the quantitative synthesis. The average values of the primary and secondary outcomes identified in the 14 eligible studies are shown in Table 1.

The 14 included studies provide a broad dataset on olive leaf extract composition and antioxidant activity (Table 2). All 14 studies reported TPC, encompassing 149 samples with an average TPC of 45 mg GAE/g DW. This indicates a moderate phenolic yield on average, though individual extracts varied widely, from only a few tens of mg GAE/g DW in milder conditions to well over 100 mg GAE/g DW in the most optimized extractions. In contrast, far fewer studies (5 of 14) quantified TFC, yielding 67 total observations with an average of 17 mg CAE/g DW.

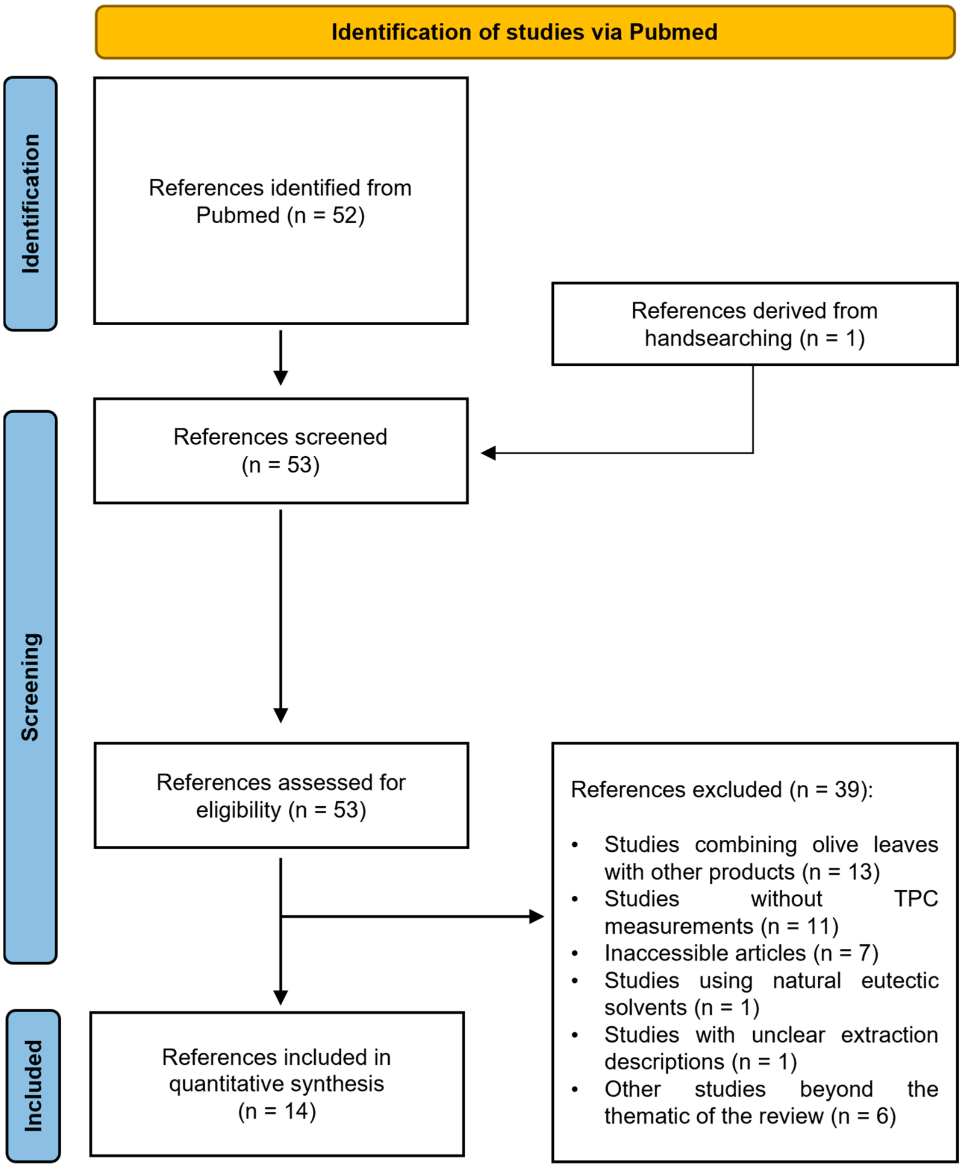

Figure 1. PRISMA flow diagram of study selection process.

Table 2. Average of the primary and secondary outcomes of the included studies.

Measurement	Studies	Samples	Mean	SEM	Refs.
Total phenolic content (mg GAE/g DW)	14	149	45	5	[3,11–23]
Total flavonoid content (mg CAE/g DW)	5	67	17	2	[3,19–22]
DPPH (µmol TE/g)	5	105	121	11	[3,11,13–15]
FRAP (µmol TE/g)	5	91	458	44	[3,11,13,15,18,21]
ABTS (µmol TE/g)	4	61	438	19	[3,11,15,21]

Abbreviations: ABTS: 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid); CAE: catechin equivalents; DPPH: 2,2-diphenyl-1-picrylhydrazyl; DW: dry weight; FRAP: ferric reducing antioxidant power; GAE: gallic acid equivalents; SEM: standard error of the mean; TE: Trolox equivalents.

Antioxidant capacity was commonly assessed by free-radical scavenging and reducing power assays, though not every study applied all methods. Five studies (105 total samples) reported DPPH radical scavenging capacity, with a mean of 121 µmol TE/g DW and substantial between-sample variability. Similarly, FRAP was measured in five studies (91 samples) and averaged 458 µmol TE/g DW, reflecting the generally high reducing capacity of olive leaf extracts. ABTS radical scavenging capacity was slightly less frequently reported (four studies, 61 samples) but showed a comparably high mean (438 µmol TE/g DW). These summary values underscore the strong antioxidant nature of olive leaf phenolic compounds, while the sizeable error ranges indicate heterogeneity: indeed, some extracts exhibited extremely high FRAP or ABTS values in the hundreds to over a thousand µmol TE/g DW, whereas others were an order of magnitude lower. This diversity reflects differences in olive leaf cultivars, extraction conditions, and analyte reporting. For example, certain studies focused on specific olive genotypes or growing conditions, analyzing 15 Italian cultivars and reporting TPC ranging from 11 to 49 mg GAE/g DW among them, whereas others used mixed or unspecified cultivars [14].

3.2. Influence of Extraction Method in TPC, TFC and Antioxidant Capacity

Extraction methods proved to be a key determinant of phenolic and flavonoid recovery. Broadly, the studies employed conventional solvent extractions (simple shaking/maceration, sometimes with heat or extended time) versus modern assisted techniques (e.g., ultrasound, high-voltage electrical discharge, homogenizer-assisted, or Soxhlet reflux). These methods differ in energy input and mass transfer efficiency, which in turn affect compound release from the olive leaf matrix. For instance, ultrasound can disrupt cell walls and enhance solvent penetration, while Soxhlet reflux provides continuous percolation of hot solvent, driving near-exhaustive extraction of soluble compounds. High-voltage electrical discharge (HVED) is a newer green technology that creates plasma sparks in the solvent; it can both extract phenolic compounds and nano-formulate them, although it operates on very short timescales (minutes). The relevance of these differences is reflected in the quantitative outcomes compiled in Table 3.

Table 3. Comparison of extraction methods based on TPC, TFC, and antioxidant capacity.

Extraction Method	Samples	TPC	TFC	DPPH	FRAP	ABTS	Refs.
Heat-Assisted Extraction	3	46 (5) ^{a}	-	-	-	-	[11]
High Voltage Electrical Discharge-assisted extraction	24	31 (5) ^{b}	-	30 (1) ^{a}	273 (25) ^{a}	-	[11]
Shaking	85	52 (6) ^{ac}	21 (8) ^{a}	150 (63) ^{b}	527 (277) ^{b}	449 (81) ^{a}	[3,11–15]
Soxhlet extraction	6	63 (8) ^{c}	-	-	-	-	[16,17]
Ultrasound-assisted extraction	30	34 (6) ^{b}	6 (3) ^{b}	-	421 (357) ^{ab}	119 (47) ^{b}	[18–23]

Abbreviations: ABTS: 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid); DPPH: 2,2-diphenyl-1-picrylhydrazyl; FRAP: ferric reducing antioxidant power; TFC: total flavonoid content; TPC: total phenolic content; -: insufficient data for quantitative analysis. Results are expressed as mean (SEM). TPC and TFC are expressed in mg of GAE or CAE/g of DW, respectively. DPPH, FRAP, and ABTS assays are expressed as µmol TE/g DW. Different superscript letters indicate statistically significant differences between extraction methods (ANOVA with post hoc Duncan's Multiple Range test $p < 0.05$).

Based on the aggregated data, Soxhlet extraction seems to be the most effective method for phenolic recovery. On average, Soxhlet yielded the highest TPC (63 mg GAE/g DW) among the methods compared. In practical terms, Soxhlet's vigorous conditions likely explain its strong performance, as continuous hot solvent cycling dissolves phenolic compounds thoroughly, albeit at the risk of extended heat exposure. Conventional shaking or stirring extraction (room-temperature or modest heating, without special assistance) gave similar phenolic yields. The mean TPC for shaking/maceration methods was about 52 mg GAE/g DW, not significantly different from the Soxhlet outcome in the pooled analysis. This suggests that, given sufficient time or repeated solvent renewals, even simple solvent extraction can approach a similar recovery to Soxhlet. Indeed, Orak et al. achieved TPC values of 110–268 mg GAE/g DW using a straightforward hot solvent extraction repeated in three short 15-min batches. By splitting the process into multiple brief, heated extractions, they maximized yield without continuous boiling, illustrating that shaking methods can be pushed to high efficiency [15].

In contrast, the non-thermal-assisted methods (HVED and ultrasound) tended to yield lower TPC in aggregate. HVED extracts averaged 31 mg GAE/g DW, and ultrasound-assisted extracts averaged 34 mg GAE/g DW, both significantly below the Soxhlet and heated maceration levels. Part of this gap arises because those advanced techniques were often applied for short durations. For example, Žuntar et al. (2019) used HVED treatments of only 3–9 min; while HVED dramatically outperformed a 9-min conventional extraction (tripling the TPC), its absolute yields (roughly 20–50 mg GAE/g DW under various settings) remained lower than those achieved with longer extractions [13]. Similarly, Martín-García et al. optimized an 8-min ultrasound protocol (sonotrode) and obtained about 40–50 mg GAE/g DW across olive cultivars, statistically comparable to a conventional ultrasonic bath extraction. In other words, when extraction time was held very short, modern methods did not necessarily exceed the phenolic yields of longer traditional methods [24]. These results suggest that the lower yields observed in aggregate are largely due to the reduced extraction times typically employed, which may not allow full release of phenolic compounds. Moreover, HVED and ultrasound may preferentially extract more polar or structurally labile compounds, which do not always dominate the TPC values [24]. Nonetheless, under optimized conditions (e.g., prolonged sonication, higher intensities, or the combination with suitable solvent systems), both HVED and ultrasound could enhance extraction efficiency and preserve thermolabile compounds, potentially making them more effective than conventional methods in specific contexts. Notably, Márquez et al. directly compared techniques and found that high-shear homogenizer-assisted extraction (HAE) was superior to both ultrasound and conventional shaking. At 18,000 rpm for 30 s, HAE yielded the highest TPC and antioxidant activity of all methods tested [11]. In fact, the selected HAE conditions produced 51.96 mg GAE/g DW and 579 µmol TE/g DW antioxidant capacity, beating out their ultrasound and static maceration trials [11]. This underscores that more intense mechanical methods can improve compound release, provided the conditions (e.g., solvent choice, time) are optimized.

Total flavonoid content appears to follow a similar pattern, though data are sparse. Table 3 suggests that conventional extraction recovered more flavonoids on average (21 mg CAE/g DW with shaking) than ultrasound (6 mg CAE/g DW). This large difference should be interpreted cautiously: only a few studies reported TFC, and they did not all use the same extraction methods. It is possible that prolonged or repeated solvent extraction allows more thorough leaching of flavonoid compounds, whereas short sonication may favor the release of very polar phenolic compounds over less soluble flavonoids, resulting in lower TFC. For example, the study with the highest TFC (82.9 mg CAE/g DW) used an extended solvent process yielding a very pure extract with fewer non-phenolic impurities [24]. In contrast, studies employing brief ultrasonic treatments did not focus on flavonoid yields, and in one case the reported TFC was under 10 mg CAE/g DW [3]. Thus, while the extraction method clearly influences flavonoid recovery, the absolute TFC values should be compared within similar experimental contexts.

The choice of extraction method also affected antioxidant assay results, although not always a linear correspondence with TPC. Generally, methods yielding higher TPC tended to produce extracts with higher radical scavenging and reducing power. For instance, shaking extraction (150 µmol TE/g DW) gave much stronger DPPH scavenging on average than the HVED method (30 µmol TE/g DW). The difference is striking, as conventional extracts scavenged DPPH about five times more effectively than HVED extracts in the compiled data, and likely reflects their higher phenolic content, including a broader array of radical-scavenging compounds. However, there are notable nuances. Žuntar et al. observed that despite HVED dramatically increasing TPC, the antioxidant capacity (by DPPH and FRAP assays) showed no significant differences compared to the control extraction [13]. This indicates that HVED may extract phenolic compounds that are less efficient radical quenchers (e.g., large glycosides) or even cause subtle changes (like nanoemulsion formation) that alter the assays without truly boosting free-radical scavenging. Indeed, composition matters: Orak et al. found strong correlations between oleuropein content and FRAP, DPPH, and ABTS values, suggesting that methods that enriching oleuropein (a potent reducing agent) will excel in FRAP, whereas methods extracting smaller phenolic compounds (hydroxytyrosol, etc.) might excel in DPPH/ABTS [15]. In Table 3, Soxhlet and shaking methods (which gave high TPC) also produced the highest FRAP (527 µmol TE/g DW) and ABTS (449 µmol TE/g DW) values on average. Ultrasound and HVED methods, with lower phenolic yields, showed significantly lower FRAP and ABTS activity. One caveat is that not all methods were represented in all assays. Several studies focused on one or two antioxidant tests, so the comparisons involve some cross-study interpolation. Nevertheless, the integrated trend is that more intensive methods (long hot extraction or Soxhlet) gave extracts with greater overall antioxidant power, in line with their higher total phenolic content. More innovative methods (HVED or short ultrasound) can achieve comparable antioxidant effects only when optimized.

Several methodological limitations complicate the interpretation of the observed differences between extraction methods. Key variables such as solvent type, solvent-to-solid ratio, and sample pretreatment were not standardized across studies, making it difficult to isolate the effect of the extraction technique. For example, the HVED study showed lower yields partly due to its short extraction time, but longer treatments notably increased phenolic recovery [11]. Additionally, some methods, like HVED and ultrasound, were only represented by single or limited studies, and their average results may not reflect optimized conditions. Variations in ultrasound equipment (bath vs. probe) and energy also affect outcomes. Furthermore, extended heating in aggressive methods like Soxhlet may degrade or transform phenolic compounds, altering antioxidant profiles without necessarily reducing TPC. Overall, while extraction methods do influence TPC, TFC, and antioxidant capacity, outcomes heavily depend on specific implementation details. Both mild and aggressive techniques can yield high recoveries if properly optimized, but careful control is needed to preserve compound integrity.

3.3. Influence of Solvent Used in TPC, TFC and Antioxidant Capacity

Solvent polarity and composition play a crucial role in extracting phenolic compounds and flavonoids from olive leaves. Phenolic compounds in olive leaves range from highly polar (e.g., hydroxytyrosol or simple phenolic acids) to moderately polar glycosides (oleuropein and flavonoid glucosides) [25]. Thus, a solvent's polarity determines which of these molecules it can dissolve effectively. In general, water alone is a poor solvent for many phenolic compounds despite its ability to swell plant tissues, whereas mixtures of water with alcohols (methanol or ethanol) can significantly improve extraction yield by balancing polarity and breaking phenolic–matrix interactions [26]. The summary in Table 4 reflects these principles: the lowest phenolic yields were obtained with 100% water, and the highest with high-percentage alcohol (especially methanol). Pure water extracts had an average TPC of 35 mg GAE/g DW, versus 103 mg GAE/g DW with $\geq$75% methanol, nearly a threefold increase. Orak et al. directly observed this disparity: their hot-water extracts of olive leaves contained 1.4–2.6 times fewer total phenolic compounds than extracts made with organic solvents [15]. In practical terms, the high polarity of water limits its ability to solubilize less-polar polyphenols like oleuropein; adding an alcohol improves solubility and disrupts the binding of phenolic compounds to leaf proteins and fibers. Žuntar et al. explicitly noted that using ethanol in HVED disrupted the linkage between phenolic compounds and components of the plant material to which they are bound, enhancing release [13].

Table 4. Comparison of extraction solvent based on TPC, TFC, and antioxidant capacity.

Solvent	Samples	TPC	TFC	DPPH	FRAP	ABTS	Refs.
Water 100%	12	35 (12) ^{a}	-	29 (1) ^{a}	270 (53) ^{a}	-	[11,13,18]
Methanol $\geq$ 75% in water	22	103 (20) ^{b}	4 (1) ^{a}	48 (4) ^{b}	1606 (117) ^{b}	223 (10) ^{a}	[15,20]
Ethanol $\leq$ 25% in water	11	33 (5) ^{a}	-	29 (1) ^{a}	263 (19) ^{a}	-	[11,13]
Ethanol 25–75% in water	28	34 (4) ^{a}	-	23 (2) ^{a}	246 (26) ^{a}	-	[11,13,14,17,21]
Ethanol > 75% in water	75	36 (1) ^{a}	20 (2) ^{b}	222 (10) ^{c}	378 (23) ^{c}	490 (16) ^{b}	[3,11,12,19,23]

Abbreviations: ABTS: 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid); DPPH: 2,2-diphenyl-1-picrylhydrazyl; FRAP: ferric reducing antioxidant power; TFC: total flavonoid content; TPC: total phenolic content; -: insufficient data for quantitative analysis. Results are expressed as mean (SEM). TPC and TFC are expressed in mg of GAE or CAE/g of DW, respectively. DPPH, FRAP, and ABTS assays are expressed as μmol TE/g DW. Different superscript letters indicate statistically significant differences between extraction methods (ANOVA with post hoc Duncan's Multiple Range test $p < 0.05$).

Ethanol–water mixtures are commonly used for edible extracts, so it is notable that moderate ethanol fractions (25–50% v/v) did not dramatically outperform water in the aggregated data. Table 4 shows TPC averages of 33–34 mg GAE/g DW for 25–75% ethanol, all statistically on par with water. In contrast, very high concentrations of ethanol (>75%) gave a slight uptick. This pattern likely arises from the specific studies included: many of the water or 25–75% ethanol extractions were part of short-duration experiments, whereas the methanol data came from studies that pushed conditions harder. For example, Žuntar's conventional extraction at 25% ethanol for 9 min achieved 16 mg GAE/g DW, essentially the same as what 9 min of water yielded (15.9 mg GAE/g DW), insufficient time for the solvent advantage to manifest [13]. However, when extraction time was extended or assisted, ethanol mixtures did show benefits. Žuntar reported that at 3 min, switching from pure water to 50% ethanol tripled the TPC (from 5.3 to 15.8 mg/g DW), and even at 9 min, 50% ethanol gave 20.6 mg GAE/g DW vs. 15.9 mg GAE/g DW with water [13]. In fact, hydroalcoholic solvents are more efficacious, but their effect is most pronounced when other factors (time or method) are not limiting. Methanol appears highly efficient for olive leaf phenolic extraction. Two studies that employed 80% methanol as the solvent obtained the highest TPC values: Orak et al. (2019) used 80% methanol at 65 °C and measured TPC in the range 127–315 mg GAE/g DW extract [15], and Ibrahim et al. used 80% methanol (30 °C, 5 h) to extract crude olive leaf material, which had a greater phenolic content than even the original leaf powder [12]. By contrast, studies using diluted ethanol often reported lower yields. Nicolì et al. (2019) extracted leaves in aqueous ethanol and found TPC between 11 and 49 mg/g DW across cultivars [14]; those results align with Table 4 reports. It appears that methanol is a stronger solvent for olive leaf phenolic compounds than ethanol, likely because of its slightly higher polarity and smaller molecular size, though for practical and regulatory reasons ethanol–water is preferred in food applications [27].

The solvent polarity also affects which phenolic types are recovered, which in turn influences antioxidant capacity. Water tends to pull out very polar substances (e.g., dihydroxyphenols or glycosides) but may leave behind moderately polar flavonoids, adding alcohol improves flavonoid extraction. In our data, the only solvent category with appreciable flavonoid content was the high ethanol > 75% group, which averaged 20 mg CAE/g DW. Lower-polarity mixtures and water had too few TFC data points to report, implying either very low flavonoid yields or that those studies did not measure TFC at all. At least one included article suggests why water is suboptimal for flavonoids. Orak and co-workers reported that hot-water extracts had drastically less oleuropein and flavonoids than methanolic extracts of the same leaves. The underlying mechanism is that many flavones and oleuropein itself are better solubilized in 50–80% aqueous alcohol [28]. Indeed, Žuntar's HVED work found that increasing ethanol concentration led to higher oleuropein and hydroxytyrosol yields, correlating with greater antioxidant power [13].

Trends in antioxidant assays mirror the solvent effects on TPC, with some interesting exceptions. Pure water extracts had the weakest DPPH scavenging (29 µmol TE/g DW) and FRAP (270 µmol TE/g DW) on average. Adding even a little ethanol boosted these values; for example 25% ethanol extracts showed similar DPPH (29 µmol TE/g DW) and FRAP (263 µmol TE/g DW). The most polar (methanol-rich) solvents stand out for FRAP, as the ≥75% methanol category achieved an enormous average FRAP of 1606 µmol TE/g DW. This is an order of magnitude higher reducing power, reflecting that methanol pulled out a high load of phenolic compounds (like oleuropein, which strongly reduces Fe^{3+} to Fe^{2+}) [3]. Ethanol-based solvents showed a more moderate antioxidant extraction. For DPPH scavenging, ≥75% ethanol yielded the highest average (48 µmol TE/g DW) among the ethanol groups, significantly above water's 29 µmol TE/g DW. This suggests that a high ethanol concentration helps extract certain radical-scavenging compounds (flavonoid aglycones or secoiridoid aglycones) that water alone does not [28]. ABTS scavenging capacity was only reported for a couple of categories, but notably the >75% ethanol extracts had an average ABTS of 490 µmol TE/g DW, more than double that of methanol extracts (223 µmol TE/g DW). This may indicate that some antioxidants measured by ABTS (possibly lipophilic phenolic compounds or vitamin E-like compounds) were better extracted by ethanol than methanol [29]. In sum, higher organic solvent content generally correlates with higher antioxidant capacity in the extracts, due to higher phenolic loading. However, the differing responses of DPPH vs. ABTS vs. FRAP highlight that solvent choice can tilt the composition of phenolic compounds: methanol might extract more reducing substances (boosting FRAP), while a high ethanol proportion might favor compounds that excel in ABTS radical quenching (perhaps certain flavonoids or tocopherols) [29].

Several specific observations from the literature illustrate possible mechanisms. Benčić et al. maintained a low extraction temperature (<30 °C) specifically to preserve phenolic integrity while using 100% ethanol as the solvent. Despite the lack of water, their ultrasonic ethanol extraction was quite effective, yielding leaf extracts rich in oleuropein and showing strong bioactivities [19]. On the other hand, Ibrahim et al. chose 80% methanol, reasoning that a mixture can better solubilize a broad range of phenolic compounds [12]. Martín-García et al. found the optimal solvent for olive leaf sonication was 55/45 ethanol/water mixture, at this ratio, they obtained the highest total phenolic compounds, oleuropein, and hydroxytyrosol content. Pure ethanol (or pure water) gave lower yields in their optimization, indicating that a mid-polarity mix was ideal [24]. This is consistent with the "like dissolves like" principle: mid-polar solvents can penetrate the leaf matrix (which contains water) better than absolute alcohol, while still dissolving moderately polar phenolic compounds better than water alone [30]. Indeed, Žuntar et al. kept 50% ethanol in all HVED runs based on preliminary trials, and their highest phenolic yield occurred at that composition [13].

From a mechanistic standpoint, polar alcohols disrupt the hydrogen bonding network in plant tissues and can precipitate proteins that otherwise bind phenolic compounds, freeing those compounds into solution [31]. Water by itself lacks this precipitating power and can leave phenolic compounds hydrogen-bonded within the cellulose/protein matrix [31]. This explains why adding ethanol or methanol not only increases the quantity of phenolic compounds extracted but can also change the quality. For example, some tannins or flavonols might remain in water extracts in bound form but are released when alcohol is present [31]. In olive leaves, many phenolic compounds (oleuropein, verbascoside, and rutin) are glycosylated and fairly polar, so a small amount of water is useful to keep them in solution, whereas pure methanol can be too non-polar for glycosides [30]. The aggregated data suggests methanol was exceptionally effective, but it's worth noting that methanol has a higher dielectric constant (more polar) than ethanol, and many protocols use methanol for analytical extraction. Ethanol, being slightly less polar, may extract a bit less of the very polar phenols but is still adequate for most (especially when hot). Crucially, any organic solvent in the mixture greatly improves extraction compared to water alone.

However, some experimental limitations should be mentioned. Most studies did not directly compare multiple solvents, instead selecting one based on prior knowledge or small trials. Only a few [13,24] systematically tested different solvent compositions. As a result, trends in Table 4 partly reflect differences between separate studies rather than true head-to-head comparisons, introducing confounding factors such as extraction time and temperature. Methanol studies often used longer or hotter extractions, boosting yields, while water studies tended to be shorter and gentler. Although it is clear that a polar organic solvent is essential for maximizing phenolic compound recovery, the exact magnitude of solvent effects is hard to isolate. Solvent selectivity also matters, as harsher solvents could extract other compounds [32], but the included studies focused on water, methanol, and ethanol. Within that range, 50–80% aqueous alcohol gives the highest total phenolic content and antioxidant activity by recovering both polar and moderately non-polar phenolic compounds. Absolute alcohol can reduce yield by excluding ionic compounds, and pure water excludes less-polar phenolic compounds. Overall, solvent polarity is a key driver of efficiency, with higher alcohol content, especially methanol, producing extracts richer in phenolic compounds, flavonoids, and antioxidant capacity, while water alone is suboptimal for recovering oleuropein and flavonoid glycosides from olive leaves.

3.4. Influence of Extraction Time and Temperature in TPC, TFC and Antioxidant Capacity

Extraction time and temperature are critical parameters because they influence both the extent of compound recovery and the stability of those compounds. Phenolic compounds can degrade, isomerize, or polymerize if exposed to heat or reactive conditions for too long [33]. On the other hand, insufficient time or low temperature can lead to incomplete release of phenolic compounds from the plant matrix [2]. Optimizing these factors is therefore a balancing act: higher temperatures and longer times generally increase phenolic solubility and diffusion rates (boosting TPC and TFC initially), but beyond a certain point they may cause thermal decomposition or oxidation of the very compounds being sought. The compiled data in Tables 5 and 6 illustrate these dynamics.

Table 5. Comparison of extraction temperature based on TPC, TFC, and antioxidant capacity.

Extraction Temperature	Samples	TPC	TFC	DPPH	FRAP	ABTS	Refs.
≤25 °C	103	32 (1) ^{a}	20 (2) ^{a}	129 (11) ^{a}	330 (17) ^{a}	490 (16) ^{a}	[3,11,13,14,22,23]
25–50 °C	33	33 (3) ^{a}	7 (3) ^{b}	-	-	-	[11,12,19–21,23]
>50 °C	16	152 (20) ^{b}	-	48 (4) ^{b}	1523 (134) ^{b}	217 (10) ^{b}	[15–18]

Abbreviations: ABTS: 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid); DPPH: 2,2-diphenyl-1-picrylhydrazyl; FRAP: ferric reducing antioxidant power; TFC: total flavonoid content; TPC: total phenolic content; -: insufficient data for quantitative analysis. Results are expressed as mean (SEM). TPC and TFC are expressed in mg of GAE or CAE/g of DW, respectively. DPPH, FRAP, and ABTS assays are expressed as µmol TE/g DW. Different superscript letters indicate statistically significant differences between extraction methods (ANOVA with post hoc Duncan's Multiple Range test $p < 0.05$).

Table 6. Comparison of extraction time based on TPC, TFC, and antioxidant capacity.

Extraction Time	Samples	TPC	TFC	DPPH	FRAP	ABTS	Refs.
≤1 h	70	57 (8) ^{a}	7 (3) ^{a}	34 (2) ^{a}	556 (93) ^{a}	204 (16) ^{a}	[11,13,15,18,20,23]
1–2 h	65	34 (1) ^{b}	21 (2) ^{b}	175 (13) ^{b}	378 (23) ^{b}	490 (16) ^{b}	[3,14]
>2–5 h	9	54 (10) ^{a}	-	-	-	-	[12,16,17,22]
>24 h	4	43 (1) ^{c}	-	-	-	-	[11]

Abbreviations: ABTS: 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid); DPPH: 2,2-diphenyl-1-picrylhydrazyl; FRAP: ferric reducing antioxidant power; TFC: total flavonoid content; TPC: total phenolic content; -: insufficient data for quantitative analysis. Results are expressed as mean (SEM). TPC and TFC are expressed in mg of GAE or CAE/g of DW, respectively. DPPH, FRAP, and ABTS assays are expressed as µmol TE/g DW. Different superscript letters indicate statistically significant differences between extraction methods (ANOVA with post hoc Duncan's Multiple Range test $p < 0.05$).

As a general trend, raising the extraction temperature greatly enhanced phenolic yield up to the highest levels tested in these studies. Extracts performed at or below room temperature (≤25 °C) had an average TPC of only 32 mg GAE/g DW [3,11,13,14,22,23], whereas extractions at >50 °C yielded an average TPC of about 152 mg GAE/g DW, nearly five times higher [11,12,19–21,23]. This dramatic increase is reflected in antioxidant measures as well. For example, FRAP capacity jumped from 330 µmol TE/g DW at ≤25 °C to 1523 µmol TE/g DW at >50 °C. Such gains are not surprising, since heat accelerates solvent penetration and solubilizes phenolic compounds by softening cell walls and lowering solvent viscosity. Alhakim et al. (2024) explicitly compared extraction at 60 °C with room temperature and found that the hot extraction yielded much higher oleuropein content [16]. The data show that moderate heating (25–50 °C) did not produce a significant improvement over room temperature on average (33 mg vs. 32 mg GAE/g DW). It was the jump to high temperatures (>50 °C) that unlocked substantially more phenolic compounds. This is likely to reflect that olive leaf tissues are relatively recalcitrant and benefit from near-boiling conditions to release compounds. Some phenolic compounds may also be present in bound forms (e.g., esterified to cell-wall components) that are cleaved at higher heat, increasing measurable TPC [20,34]. Interestingly, Pyrka et al. found that drying olive leaves at 140 °C (as opposed to 70 °C) increased the extractable oleuropein and TPC in the leaves. They posited that the high-temperature drying inactivated polyphenol oxidases and perhaps broke certain complexes, thereby preserving or freeing phenolic compounds that would otherwise be lost [20]. This aligns with the extraction findings: higher temperature extraction can give higher yields not just by solubility, but by preventing enzymatic oxidation and releasing bound phenolic compounds. However, there is an upper limit, beyond a certain temperature, actual thermal degradation can occur. While none of the studies boiled the solvent to dryness or exceeded 100 °C (most >50 °C studies were in the 60–80 °C range), there is literature evidence that prolonged exposure to >100 °C can destroy oleuropein, and flavonoids [35]. Benčić et al. reported that oven-drying olive leaves at high heat significantly reduced TPC, oleuropein, and hydroxytyrosol compared to gentle drying, highlighting that the effect of temperature can be negative if applied too long [19]. In our dataset, we see a hint of this trade-off in the antioxidant data. DPPH and ABTS results for the >50 °C category were lower on average than those for the ≤25 °C category. For example, DPPH scavenging averaged 129 µmol TE/g DW at low temperature, but only 48 µmol TE/g DW at >50 °C. This seems counterintuitive given the huge rise in TPC with high temperature, but it might indicate that extreme conditions favor the extraction of certain phenolic compounds that contribute more to FRAP (reducing power) than to radical scavenging. A plausible explanation is that oleuropein (which is abundant in high- temperature extracts) has strong reducing ability (high FRAP) but is not as effective a DPPH scavenger as simpler phenolic compounds; meanwhile, very heat-sensitive radical-scavengers (like some flavonols) might partially degrade at 70–80 °C, lowering the

DPPH capacity, as evidenced by partial least squares discriminant analyses in a 50 olive leaf samples research [3]. In essence, high temperature pulls out more phenolic compounds (hence higher TPC/FRAP) but could slightly change the composition in ways such that DPPH and ABTS do not increase proportionally.

On the other hand, the effect of extraction time is somewhat analogous to temperature. In this context, increasing time initially boosts phenolic compounds yield but eventually can reach a plateau or even decline if degradation occurs. The compiled results (Table 6) show that most phenolic compounds were extracted within the first hour in many cases.

Extractions ≤ 1 h long had the highest mean TPC (57 mg GAE/g DW) among the time categories. By contrast, the 1–2 h category averaged only 34 mg GAE/g DW, and very long extractions (>24 h) gave around 43 mg GAE/g DW (though that was based on a single study). On the face value, this suggests a non-monotonic trend where TPC peaks with short (1 h or less) extraction and can drop with extended time. In reality, this outcome is influenced by co-variation with method and temperature: many of the ≤1 h extractions were performed at higher temperatures or with assistance [11,13,15,18,20,23], whereas some 1–2 h extractions were at lower temperature or used less efficient stirring [3,14]. For example, the highest TPC values in ≤1 h likely came from studies like Orak's (45 min total at 65 °C) or Martin-García's optimized 8–10 min sonication (with strong solvent), both of which achieved high yields quickly [15,24]. In contrast, a gentle 2 h room-temperature steep might yield less than a 30 min hot extraction. Thus, time cannot be viewed in isolation: effective extraction time depends on what is happening during that time (temperature, agitation, etc.). Generally, if temperature is high, most phenolic compounds leach out rapidly (diminishing returns after 30–60 min). This is supported by Farah Alhakim et al., who noted that extending a 60 °C extraction from 1 h to 4 h (Soxhlet) did improve yields, but going far beyond that gave no further significant gain [16]. Indeed, one included study that ran >24 h extraction achieved a TPC (43 mg CAE/g DW) not much higher than a 1 h extraction [11]. Likely, the solvent had long reached equilibrium by then, and phenolic compounds may have even started to oxidize in air over the prolonged exposure. Some authors explicitly avoided very long extractions for this reason. Benčić et al. remarked that prolonged contact can influence to the effect of plant material and extraction method on the yield and purity, hinting that extended time might extract more impurities or cause phenolic breakdown, affecting extract purity. Instead, they sonicated for 30 min and stopped, limiting oxidative exposure [19]. Similarly, Márquez et al. used only 30 s for their homogenizer method to prevent any heat build-up or oxidation, yet achieved excellent yields in that burst [11].

The antioxidant data shows a pattern consistent with that mentioned above. In Table 6, extracts obtained within ≤1 h had the highest FRAP (556 µmol TE/g DW) among the time groups. However, extractions lasting 1–2 h showed higher DPPH (175 µmol TE/g DW) and ABTS (490 µmol TE/g DW) on average. This could be explained by the confounding factors mentioned earlier. Studies lasting ≤1 h were performed at higher temperatures than those in the 1–2 h subgroup. As mentioned, oleuropein (which is abundant in high-temperature extracts) has strong reducing ability (high FRAP) but is not as effective a DPPH or ABTS scavenger as simpler phenolic compounds, while low temperature extracts might partially conserve sensitive radical scavengers (like some flavonols) and increase DPPH and ABTS capacity. These results must be interpreted with caution due to the high risk of bias identified in the included studies, which may limit the reliability of direct comparisons.

Thus, the consensus from these 14 studies is to use sufficient time/temperature conditions to extract the bulk of phenolic compounds (often a matter of minutes to an hour with heating or sonication), but not to vastly exceed that. Practically, a fast, hot extraction appears to be the most favorable. It leverages solubility and diffusion gains from heat, yet curtails the window for thermal or oxidative degradation of sensitive flavonoids and polyphenols. Each study's methodological choices reflect this balance, for instance, 65 °C for 15 min × 3 [15], 80 °C Soxhlet for 4 h followed by immediate cooling [16], 30 °C shaking for 5 h to avoid heat [12], or room temperature and 30 min ultrasound to prevent heat build-up [19]. Where researchers suspected degradation (e.g., in drying or very long soaks), they explicitly noted the negative impact of excessive heat/time on phenolic content. Taken together, these findings underscore the need to optimize time and temperature in tandem: high temperature dramatically boosts phenolic extraction, but the extraction should be stopped once yields plateau to preserve maximum flavonoid content and antioxidant efficacy. This ensures robust recovery of TPC and TFC while maintaining the integrity of bioactive compounds.

This review has several limitations that should be acknowledged. First, some of the included studies provided truncated or incomplete datasets, limiting the comparability of results across outcomes. Second, there was substantial variability among olive cultivars, growing conditions, and experimental protocols, which introduced heterogeneity and prevented the performance of a full meta-analysis. Third, certain extraction methods, such as Soxhlet, HVED, or ultrasound-assisted techniques, were represented by a limited number of samples, restricting the generalizability of their outcomes. Future research should therefore focus on standardized experimental designs, with systematic optimization of solvents, time, and temperature, to reduce confounding variables. Randomized controlled extraction trials and compound-specific analyses are also needed to strengthen reproducibility, enhance comparability, and clarify the functional implications of different extraction protocols.

4. Conclusions

This systematic review and quantitative synthesis evaluated the influence of extraction method, solvent type, and extraction conditions (time and temperature) on the recovery of TPC, TFC, and antioxidant capacity of olive leaf extracts. Fourteen studies encompassing 149 samples were analyzed. The findings highlight that both conventional and assisted extraction methods can achieve high phenolic yields when parameters are optimized, but significant variability exists due to methodological heterogeneity. Soxhlet and prolonged heated shaking generally produced the highest TPC and antioxidant capacity, while non-thermal methods such as ultrasound and high-voltage electrical discharge yielded lower averages unless extraction time or intensity was increased.

Solvent choice emerged as a major determinant of extraction efficiency. Aqueous methanol ($\geq$75%) consistently provided the highest TPC and FRAP values, likely due to its optimal polarity and penetration capacity. High-ethanol mixtures (>75%) achieved the greatest TFC and ABTS activity, suggesting selective enrichment of certain antioxidant compounds. Pure water was consistently the least effective solvent for phenolic recovery.

Temperature strongly influenced extraction outcomes. Extractions at >50 °C produced TPC values up to five times higher than those at room temperature, with corresponding increases in FRAP. However, elevated temperatures did not proportionally enhance DPPH or ABTS, possibly reflecting compositional shifts toward compounds with greater reducing power but lower radical-scavenging efficiency. Extraction time showed an optimal window: most phenolic compounds were recovered within $\leq$1 h under heated or assisted conditions, while extended durations did not improve yields and may have increased the risk of heat-sensitive compounds.

Future research should prioritize standardized experimental designs to reduce confounding variables and facilitate direct comparisons across methods. Systematic optimization studies (testing solvent composition, temperature, and time in factorial combinations) would help establish universally applicable protocols. Additionally, integrating compound-specific analyses with bioactivity assessments could clarify how extraction parameters shape not only total phenolic yield but also the functional antioxidant profile of olive leaf extracts.

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