

Solvent Polarity-Dependent *In Vitro* Antioxidant and Enzyme Inhibition Profile of *Heracleum persicum*: A Preliminary Comparative Screening

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Abstract: *Heracleum persicum*, an aromatic Apiaceae species rich in phenolic compounds, has attracted growing interest, yet comparative data on solvent-dependent bioactivity remain limited. In the present study, the aerial parts of *Heracleum persicum* were subjected to maceration with solvents of graded polarity (water, ethanol, methanol, and *n*-hexane), and the resulting extracts were evaluated for antioxidant capacity and for inhibitory activity against acetylcholinesterase (AChE), butyrylcholinesterase (BChE), and urease. Antioxidant activity was determined by the ABTS, FRAP, and CUPRAC assays. All measurements were performed in triplicate ($n = 3$) and expressed as mean $\pm$ standard deviation. Data were compared by one-way ANOVA with Tukey's post hoc test, while Pearson correlation analysis and principal component analysis (PCA) were employed for exploratory purposes. Polar extracts exhibited markedly higher antioxidant activity; the aqueous extract showed the strongest urease inhibition, whereas the *n*-hexane extract was most active against AChE. Antioxidant assays correlated strongly, while AChE inhibition was inversely related to antioxidant parameters. These findings demonstrate that solvent polarity is a decisive determinant of the antioxidant and enzyme inhibitory profile of *Heracleum persicum* extracts and provide a rational basis for selecting solvent systems in subsequent bioactivity-guided studies.

Keywords: *Heracleum persicum*; solvent polarity; antioxidant activity; cholinesterase inhibition; urease inhibition.

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1. Introduction

Heracleum L., one of the largest genera in the Apiaceae family with more than 120 species, is widely distributed throughout the Northern Hemisphere [1]. Among these, *Heracleum persicum* Desf. ex Fisch., an aromatic perennial native to Türkiye, Iran, and Iraq, holds a distinctive position because of its considerable medicinal and economic value [2]. The plant produces aromatic volatile constituents [3] and has traditionally been used, particularly in Iranian ethnomedicine, as both a food and a remedy for diverse ailments; its aromatic fruits are widely used as a spice and food additive for their characteristic flavor and preservative qualities [4]. In folk medicine, the plant has been administered as a carminative and tonic for gastrointestinal disorders, as well as for its anti-inflammatory, analgesic, antiseptic, and anticonvulsant effects [5,6]. Modern pharmacological investigations have further substantiated

these traditional uses, revealing a broad spectrum of biological activities including antioxidant, immunomodulatory, antibacterial, antifungal, antiparasitic, anticonvulsant, anti-inflammatory, analgesic, anticancer, and antidiabetic properties [4,5,7-13]. These activities are largely attributable to the rich and diverse secondary metabolite profile of the species, which comprises volatile oils, furanocoumarins, flavonoids, and phenolic compounds. Hexyl butyrate and octyl acetate dominate the volatile fraction of the seeds, while *trans*-anethole is the leading volatile of the leaves and flowers [14,15]. Furanocoumarins (isopimpinellin, pimpinellin, bergapten, and bakuchicin), phenolic acids (cinnamic acid, *p*-coumaric acid, *p*-hydroxybenzoic acid, ferulic acid, and rosmarinic acid), and various flavonoids have also been reported in different parts of the plant [7,16,17].

Reactive oxygen species (ROS) and reactive nitrogen species (RNS) participate in numerous biochemical reactions under normal physiological conditions, and at low concentrations they sustain redox homeostasis, regulate cellular signaling and modulate immune responses [18]. This balance is easily disturbed. Poor diet, smoking, alcohol use, and physical inactivity, acting alongside aging and genetic predisposition, may increase the formation of these species or weaken antioxidant defense capacity [19]. The oxidative burden that follows damages cells at the molecular level through lipid peroxidation, protein oxidation and DNA damage, and eventually results in cellular dysfunction and more severe pathophysiological outcomes [20]. The brain is especially vulnerable in this respect, combining a high oxygen demand with membranes rich in polyunsaturated fatty acids, while its antioxidant defenses remain comparatively limited [21]. When oxidative stress persists, mitochondrial function deteriorates, neuroinflammation develops, and neurons are lost; these processes are regarded as a central pathogenic feature of neurodegenerative disorders such as Alzheimer's and Parkinson's diseases [22]. A comparable association is seen in the gastrointestinal tract, where *Helicobacter pylori* infection elicits an inflammatory response that in turn drives oxidative stress [23]. Neutrophils and macrophages activated during *Helicobacter pylori* colonization enhance the generation of reactive oxygen and nitrogen species [24], which worsen mucosal barrier damage and mediate gastric inflammation and ulcerogenesis, contributing to gastric carcinogenesis in advanced stages [25,26].

Within this context, cholinesterases (AChE and BChE) and urease have emerged as therapeutically relevant enzyme targets. Inhibition of AChE and BChE remains a principal pharmacological strategy in the symptomatic management of Alzheimer's disease, and plant-derived inhibitors have continued to attract attention as scaffolds for next-generation cholinesterase modulators [27]. Likewise, urease inhibition is an established approach to limiting *Helicobacter pylori* colonization and associated gastric pathology, and phenolic constituents of medicinal plants have increasingly been reported as effective urease inhibitors [28]. Solvent polarity, in turn, is a well-recognized determinant of the qualitative and quantitative composition of plant extracts and, consequently, of their bioactivity profile [29].

Although *Heracleum persicum* has been investigated for several biological activities, to the best of our knowledge, no study has systematically compared extracts obtained with solvents spanning a broad polarity range, evaluated simultaneously across complementary antioxidant assays and against pharmacologically relevant enzyme targets. The present study was therefore designed to address this gap by extracting the aerial parts of *Heracleum persicum* with water, ethanol, methanol, and *n*-hexane and by assessing the resulting extracts through three mechanistically distinct antioxidant assays (ABTS, FRAP, and CUPRAC) together with inhibition assays against AChE, BChE, and urease. The data were further integrated by means

of correlation and principal component analyses to elucidate solvent-dependent patterns of activity. The work is thus intended to provide a rational basis for selecting solvent systems in subsequent bioactivity-guided studies on *Heracleum persicum*.

2. Materials and Methods

2.1. Plant material and preparation of extracts.

The aerial parts of *Heracleum persicum* Desf. ex Fisch. were collected during the flowering period (1-8 June 2022) from Sariçiçek Neighborhood, Gürpınar District, Van Province, Türkiye (37°58'4.40" N, 43°16'54.69" E). Taxonomic authentication was carried out at the Department of Pharmacognosy, Faculty of Pharmacy, Ege University, where a voucher specimen was deposited (herbarium no. 1262/4). A single homogeneous plant batch was used throughout all procedures to eliminate inter-batch variability. The material was shade-dried at room temperature (22 ± 2 °C) for 14 days, ground in a mechanical grinder, passed through a 0.5 mm sieve, and prepared for extraction.

Four extracts with varying solvent polarities were prepared from the same batch under comparable conditions. For the organic extracts (ethanol, methanol, and *n*-hexane), 5 g of powder was combined with 100 mL of solvent (1:20, w/v), subjected to ultrasound-assisted pre-extraction (2 h, room temperature) and subsequent maceration (8 h, intermittent shaking), then filtered through Whatman No. 1 paper. The residue was re-extracted under identical conditions for a further 8 h, and the combined filtrates were concentrated under reduced pressure (rotary evaporator, 40 °C). The aqueous extract was prepared as a 5% (w/v) infusion: 5 g of powder was steeped in 100 mL of boiling distilled water for 10 min in a closed vessel, and filtered. The filtrate, being a thermal infusion unsuited to rotary evaporation, was frozen and lyophilized directly. The organic extracts were likewise lyophilized (72 h) to obtain dry extracts of comparable physical state.

The resulting water (HPWE), ethanol (HPEE), methanol (HPME), and *n*-hexane (HPHE) extracts were weighed, and the extraction yield of each was calculated gravimetrically as the percentage (w/w) of dry extract per gram of dry plant material (Table 1). The dry extracts were stored at -20 °C until analysis. Immediately prior to each assay, lyophilized extracts were reconstituted in DMSO to the required stock concentrations, from which working dilutions were freshly prepared in the appropriate assay medium.

Table 1. The extraction yield percentages of *Heracleum persicum* extracts.

Extracts	Extraction yield %
HPWE	25.26
HPEE	20.60
HPME	20.16
HPHE	2.52

2.2. ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)) radical (ABTS^{•+}) scavenging activity.

ABTS radical scavenging activity was determined according to Re et al. [30], with minor modifications. The ABTS radical cation (ABTS^{•+}) was generated by reacting 7 mM ABTS with 2.45 mM potassium persulfate (1:1, v/v) and incubating the mixture in the dark at room temperature for 16 h. The resulting solution was diluted with phosphate buffer (pH 7.4) to an absorbance of 0.70 ± 0.02 at 734 nm. 50 µL of extract (dissolved in DMSO; final concentration 25 µg/mL) was mixed with 950 µL of ABTS^{•+} solution. After 30 min of

incubation in the dark at 25 ± 1 °C, the absorbance was measured at 734 nm on a spectrophotometer (AELab S60 UV-Vis spectrophotometer). Solvent and sample blanks were subtracted from all readings to correct for the absorbance of the solvent and the intrinsic absorbance of each extract at 734 nm. Results were expressed as Trolox equivalents (mg TE/g extract) from a Trolox calibration curve ($0.156\text{--}5$ µg/mL; $R^2 = 0.9943$). All measurements were performed in triplicate ($n = 3$) and reported as mean $\pm$ standard deviation.

2.3. Ferric reducing antioxidant power (FRAP) activity.

The ferric reducing antioxidant power was determined according to Benzie and Strain [31], with minor modifications. The FRAP reagent was freshly prepared by mixing acetate buffer (0.3 M, pH 3.6), 2,4,6-tripyridyl-s-triazine (TPTZ) solution (10 mM in 40 mM HCl), and FeCl₃ solution (20 mM) at a 10:1:1 (v/v/v) ratio, and was warmed to 37 °C before use. 50 µL of extract (dissolved in DMSO; final concentration 50 µg/mL) was mixed with 950 µL of FRAP reagent. The mixtures were incubated at room temperature (25 ± 1 °C) for 30 min, and absorbance was measured at 593 nm using a spectrophotometer. Solvent and sample blanks were subtracted from all readings to correct for the absorbance of the solvent and the intrinsic absorbance of each extract at 593 nm. Results were expressed as Trolox equivalents (mg TE/g extract) from a Trolox calibration curve ($0.156\text{--}10$ µg/mL; $R^2 = 0.9913$). All measurements were performed in triplicate ($n = 3$) and reported as mean $\pm$ standard deviation.

2.4. Cupric ion reducing antioxidant capacity (CUPRAC) activity.

The cupric ion reducing antioxidant capacity was determined according to Apak et al. [32], with minor modifications. For the assay, 50 µL of extract (dissolved in DMSO; final concentration 50 µg/mL) was mixed with 250 µL CuCl₂ (10 mM), 250 µL neocuproine (7.5 mM), 250 µL ammonium acetate buffer (1 M, pH 7.0), and 200 µL distilled water. After incubation at room temperature (25 ± 1 °C) for 30 min, the absorbance was read at 450 nm on a spectrophotometer. Solvent and sample blanks were subtracted from all readings to correct for the absorbance of the solvent and the intrinsic absorbance of each extract at 450 nm. Results were expressed as Trolox equivalents (mg TE/g extract) from a Trolox calibration curve ($0.781\text{--}50$ µg/mL; $R^2 = 0.9992$). All measurements were performed in triplicate ($n = 3$) and reported as mean $\pm$ standard deviation.

2.5. Cholinesterase (AChE and BChE) inhibition.

The inhibitory activity of the extracts on cholinesterases was determined according to Ellman et al. [33], with minor modifications. Briefly, 10 µL of extract (final concentration 100 µg/mL; final DMSO content $\leq 1\%$, v/v) was mixed with 830 µL phosphate buffer (pH 8.0, 0.1 M) and 10 µL of enzyme solution (AChE (from *Electrophorus electricus*) or BChE (from equine serum), each at a final activity of ~ 0.005 U/mL) and pre-incubated at 25 ± 1 °C for 15 min. The reaction was initiated by adding 100 µL of 5,5'-dithiobis(2-nitrobenzoic acid) (DTNB, 10 mM) and 50 µL of substrate (acetylthiocholine iodide or butyrylthiocholine iodide, 2 mM), and the increase in absorbance was monitored at 412 nm for 5 min on a spectrophotometer. Solvent and sample blanks (extract without enzyme) were subtracted from all readings to correct for the absorbance of the solvent and the intrinsic absorbance of each extract at 412 nm. Donepezil served as the positive control, and inhibitory activity was expressed as donepezil equivalents (mg DNPE/g extract) from donepezil calibration curves

constructed separately for each enzyme (AChE: 5-80 ng/mL, $R^2 = 0.9920$; BChE: 0.400-2 $\mu\text{g/mL}$, $R^2 = 0.9980$). All measurements were performed in triplicate ($n = 3$) and reported as mean $\pm$ standard deviation.

2.6. Urease inhibition.

The inhibitory activity of the extracts on urease was determined using a phenol-hypochlorite colorimetric method [34], with minor modifications. Briefly, 10 μL of extract (final concentration 100 $\mu\text{g/mL}$; final DMSO content $\leq 1\%$, v/v) was mixed with 880 μL phosphate buffer (pH 8.0, 0.1 M) and 10 μL of jack bean (*Canavalia ensiformis*) urease (final activity ~ 0.1 U/mL), and pre-incubated at 37 $^{\circ}\text{C}$ for 15 min. Urea (100 μL , 20 mM) was then added, and the mixture was incubated at 37 $^{\circ}\text{C}$ for a further 15 min. The reaction was stopped by adding 250 μL of phenol reagent (1% w/v phenol, 0.005% w/v sodium nitroprusside) and 250 μL of alkali reagent (0.5% w/v sodium hydroxide, 0.1% w/v sodium hypochlorite), and the absorbance was recorded at 630 nm after 30 min on a spectrophotometer. Solvent and sample blanks (extract without enzyme) were subtracted from all readings to correct for the absorbance of the solvent and the intrinsic absorbance of each extract at 630 nm. Thiourea served as the positive control, and inhibitory activity was expressed as thiourea equivalents (mg THUE/g extract) from a thiourea calibration curve (0.5-3.5 $\mu\text{g/mL}$; $R^2 = 0.9946$). All measurements were performed in triplicate ($n = 3$) and reported as mean $\pm$ standard deviation.

2.7. Statistical analysis.

Statistical analyses were performed using OriginPro 2024 software. Results are expressed as mean $\pm$ standard deviation (SD) ($n = 3$). Differences between groups were evaluated by one-way ANOVA followed, where significant, by the post hoc Tukey test, with $p < 0.05$ considered statistically significant. Given the limited number of extract groups ($n = 4$), we used Pearson correlation analysis and principal component analysis (PCA) only for exploratory purposes to visualize patterns of association, not to support inferential conclusions. Linear relationships between variables were assessed by Pearson correlation analysis and presented descriptively as a heat map and scatter plots, while the multivariate structure of the antioxidant and enzyme-inhibitory data was examined by PCA and illustrated using biplots.

3. Results and Discussion

3.1. Antioxidant activity.

Antioxidant capacity cannot be fully evaluated with a single analytical method because it involves multiple mechanisms of action; therefore, it is recommended to use multiple analytical approaches in combination for reliable determination. In this context, the antioxidant capacities of different extracts of the *Heracleum persicum* plant were evaluated using ABTS $\bullet^+$ scavenging activity, FRAP, and CUPRAC assays, and the results are shown in Table 2.

A significant difference was found among the extracts in terms of ABTS $\bullet^+$ scavenging activity ($p < 0.05$). The highest ABTS $\bullet^+$ scavenging activity was detected in HPEE (251.978 ± 14.995 mg TE/g extract), followed by HPME, HPWE, and HPHE. The radical scavenging activity of phenolic and flavonoid compounds is related to the number and position of their hydroxyl groups; these groups stabilize radicals through hydrogen atom or electron transfer [35]. In the ABTS test, this mechanism neutralizes both hydrophilic and lipophilic antioxidants

in a sensitive manner [36]. However, since the solvent polarity determines which compound groups dissolve during extraction, it is well known that it directly affects the antioxidant capacity of the extract [37].

Table 2. Radical scavenging and reducing power activities of the different extracts of *Heracleum persicum* (mean $\pm$ SD, $n = 3$).

	Radical Scavenging Activity	Reducing Power Activity	
	ABTS* ⁺ (mg TE/ g extract)	FRAP (mg TE/ g extract)	CUPRAC (mg TE/ g extract)
HPWE	215.320 $\pm$ 7.136 ^a	54.111 $\pm$ 1.393 ^a	129.049 $\pm$ 3.230 ^a
HPEE	251.978 $\pm$ 14.995 ^b	49.970 $\pm$ 1.306 ^b	136.984 $\pm$ 1.953 ^a
HPME	235.592 $\pm$ 8.013 ^{ab}	47.404 $\pm$ 0.679 ^b	153.683 $\pm$ 4.735 ^b
HPHE	115.780 $\pm$ 5.229 ^c	23.049 $\pm$ 2.368 ^c	79.560 $\pm$ 1.869 ^c

Different superscript letters in the same column indicate significant differences ($p < 0.05$); TE: Trolox equivalent; HPWE, HPEE, HPME, HPHE: water, ethanol, methanol, and *n*-hexane extracts of *Heracleum persicum*, respectively.

In the study where the antioxidant capacity of extracts obtained from the aerial parts of the *Heracleum persicum* plant was evaluated using the DPPH method depending on the solvent polarity, it was reported that the highest activity was found in the ethyl acetate extract, followed by the methanol and hexane extracts, in parallel with the phenolic content [38]. The IC₅₀ values of the extracts obtained from the aerial parts of *Heracleum platytaenium* Boiss. with different polarities for their ABTS radical scavenging activities were found to be 3.98 $\pm$ 0.09 μ g/mL for the EtOAc extract, 17.06 $\pm$ 1.02 μ g/mL for the MeOH extract, and 35.90 $\pm$ 0.68 μ g/mL for the hexane extract, in proportion to their total phenolic and total flavonoid contents [39]. In the content analysis of *Heracleum persicum* fruit extracts prepared with solvents of different polarities using GC-MS, it has generally been reported that polar solvents (acetonitrile, ethanol, methanol) provide significantly more components and higher extraction efficiency compared to non-polar hexane; meanwhile, non-polar hexane extracts the fewest components (a single compound) [40]. This pattern observed in our study is consistent with the general literature, which shows that antioxidant activity largely depends on the solvent polarity and, consequently, the class of phytochemicals extracted.

In terms of FRAP reducing power, the highest activity was observed in HPWE (54.111 $\pm$ 1.393 mg TE/g extract), with HPEE, HPME, and HPHE showing lower activities compared to HPWE ($p < 0.05$). For CUPRAC reducing capacity, HPME (153.683 $\pm$ 4.735 mg TE/g extract) exhibited the best activity, followed by HPEE, HPWE, and HPHE ($p < 0.05$). When evaluating the FRAP and CUPRAC results together, it is observed that the metal-ion reduction capacity of the extracts varies with solvent. Although both methods are based on an electron-transfer mechanism, the redox potentials of the metal-ion pairs used (Fe³⁺/Fe²⁺ and Cu²⁺/Cu⁺) differ [41]. Since the FRAP reagent's chromophore is charged with (2+), it shows high affinity for the aqueous phase; in contrast, the CUPRAC chromophore is monovalent, allowing it to dissolve in both aqueous and organic solvents, thereby enabling the simultaneous evaluation of hydrophilic and lipophilic antioxidants [42]. The CUPRAC activity of 80% acidified ethanol extract of *Heracleum persicum* was found to be 49.7 $\pm$ 1.7 μ mol TE/g [43]. In the study evaluating the metal reducing power of *Heracleum humile* extracts obtained by ultrasound-assisted extraction, the CUPRAC capacity was found to be 58.66 $\pm$ 4.88 mg TE/g in ethyl acetate, 52.68 $\pm$ 0.59 mg TE/g in water, and 47.15 $\pm$ 0.18 mg TE/g in methanol, while the FRAP capacity was found to be 47.85 $\pm$ 0.22 mg TE/g in water, 36.00 $\pm$ 0.44 mg TE/g in methanol, and 29.61 $\pm$ 1.11 mg TE/g in ethyl acetate [44]. This difference is primarily due to extraction;

the polarity of the solvent determines which compounds are dissolved, and each extract has a different antioxidant profile [45]. In the FRAP method, which is more specific to hydrophilic compounds, the highest activity was observed in the water (HPWE) extract; thus, hydrophilic compounds stood out. In CUPRAC, which is more sensitive to compounds soluble in both aqueous and organic solvents, the higher activity of the methanol (HPME) extract reflects this solvent's ability to extract a broader and relatively non-polar content profile. Although studies systematically examining the effect of solvent polarity on antioxidant capacity in *Heracleum* species are limited, it has been reported that the highest activity in water-based methods, such as FRAP, is obtained in more polar extracts in studies conducted on different species; this supports the findings of our study [46,47].

3.2. Enzyme inhibitory activity.

The inhibitory effects of the extracts on AChE, BChE, and urease enzymes were determined using spectrophotometric methods and are presented in Table 3.

Table 3. Enzyme inhibitory activity of the different extracts of *Heracleum persicum* (mean $\pm$ SD, $n = 3$).

	AChE (mg DNPE/g extract)	BChE (mg DNPE/g extract)	Urease (mg THUE/g extract)
HPWE	0.044 $\pm$ 0.004 ^a	N.A.	25.665 $\pm$ 1.073 ^a
HPEE	0.039 $\pm$ 0.003 ^a	2.803 $\pm$ 0.201 ^a	20.957 $\pm$ 1.036 ^b
HPME	0.063 $\pm$ 0.004 ^b	4.108 $\pm$ 0.215 ^b	22.487 $\pm$ 1.904 ^{ab}
HPHE	0.072 $\pm$ 0.005 ^b	0.968 $\pm$ 0.081 ^c	5.531 $\pm$ 0.818 ^c

Different superscript letters in the same column indicate significant differences ($p < 0.05$); N.A.: not active under the tested conditions; DNPE: donepezil equivalent; THUE: thiourea equivalent; HPWE, HPEE, HPME, HPHE: water, ethanol, methanol, and *n*-hexane extracts of *Heracleum persicum*, respectively.

The highest inhibition of AChE was observed with HPHE (0.072 $\pm$ 0.005 mg DNPE/g extract), followed by HPME, HPWE, and HPEE ($p < 0.05$). For the other cholinesterase, BChE, HPME (4.108 $\pm$ 0.215 mg DNPE/g extract) exhibited the highest inhibitory activity, followed by HPEE and HPHE. HPWE did not show inhibitory activity on BChE ($p < 0.05$). There are very few studies evaluating the inhibitory effects of *Heracleum persicum* extracts on AChE and BChE. It has been shown that the methanol extract of *Heracleum persicum* fruit inhibits AChE by only 6.5% at a concentration of approximately 50 μ g/mL [48]. It has been reported that the essential oils of some plants from the Apiaceae family, including *Heracleum persicum*, exhibit AChE inhibition, which is attributed to *trans*-anethole in their structures [49]. Additionally, furanocoumarins such as psoralen, bergapten, xanthotoxin, isopimpinellin, angelicin, isobergapten, sphondin, pimpinellin, heratomin, 5-methoxyheratomin, moellendorffiline, and fraxetin have been isolated from the hexane extract of *Heracleum persicum* roots [11]. It has been reported that furanocoumarin mixtures obtained from *Heracleum* species show 68.8% inhibition of AChE and 76.7% inhibition of BChE at a concentration of 1 mg/mL. When individual furanocoumarins are examined, it has been determined that bergapten (1 mg/mL) inhibits AChE by 62.4% and BChE by 29.1%, while 8-methoxypsoralen (1 mg/mL) inhibits AChE by 33.5% and BChE by 67.1% [50]. In our study, extracts with moderate polarity and lipophilic character were generally effective at inhibiting AChE, whereas moderately polar compounds were effective at inhibiting BChE. The active sites of AChE and BChE have similar architectures, including the peripheral anionic site (PAS), a hydrophobic aromatic gorge approximately 20 Å deep, and the catalytic anionic site (CAS) located at the bottom of this gorge [51]. The active-site gorge of BChE is wider than that of

AChE but more rigid [52]. This situation may result from differences in the structures of the two ChEs and in the phytochemicals across extract types.

Jack bean (*Canavalia ensiformis*) urease shows more than 50% similarity with bacterial enzymes, and in this context, it is important for creating a model system to study the physiological aspects of these ureases [53]. Finally, in our study, when we evaluated the extracts for urease inhibition, HPWE showed the highest inhibitory effect (25.665 ± 1.073 mg THUE/g extract), followed by HPME, HPEE, and HPHE, respectively ($p < 0.05$). Similarly, studies evaluating the inhibition of urease enzyme by extracts of *Heracleum persicum* are quite limited. A study showed that the methanolic extract of *Heracleum persicum* seeds inhibits jack bean urease by 58.87% at a concentration of 330 μ g/mL [54]. Additionally, another study found that the methanol extract of *Heracleum persicum* fruits showed strong antibacterial activity against *Helicobacter pylori* in a disk diffusion test [55]. In our study, the highest urease-inhibitory activity was observed in extracts rich in polar components (HPWE, HPME, and HPEE), suggesting that phenolic compounds concentrated in these polar fractions are most likely responsible for this effect. In a mechanistic study, it was reported that phenolics inhibit by interacting with the reactive cysteine residue in the flap region that covers the enzyme's active site and by chelating metal ions (Ni^{2+}) [56]. This mechanism supports the findings obtained in our study. Indeed, differences in solvent polarity and selectivity determine the variety and amount of phytochemicals transferred to the extracts; as a result, each extract exhibits a unique compound profile and consequently exerts different effects on various targets.

3.3. Correlation analysis.

Linear relationships between variables were evaluated using Pearson correlation analysis, and the results are presented in Figure 1 as a heat map and scatter plot. Strong and positive correlations were identified among the antioxidant parameters. A strong positive relationship was found between ABTS and CUPRAC ($r = 0.947$) and between ABTS and FRAP ($r = 0.918$). A strong positive correlation was also observed between FRAP and CUPRAC ($r = 0.868$). Strong positive correlations were also identified between antioxidant activities (FRAP, ABTS, and CUPRAC) and urease enzyme inhibition ($r = 0.989$, $r = 0.893$, and $r = 0.889$, respectively). Conversely, a strong negative relationship was observed between the inhibition of extracts on AChE and FRAP ($r = -0.822$) and ABTS ($r = -0.741$), while a moderate negative correlation was found with CUPRAC ($r = -0.518$). A moderate positive correlation was detected between the inhibition of extracts on BChE and CUPRAC ($r = 0.619$) and ABTS ($r = 0.515$), whereas the relationship with FRAP remained weak ($r = 0.173$). A strong negative correlation was also identified between AChE and urease ($r = -0.734$). The strong positive correlation observed among antioxidant tests (ABTS, FRAP, and CUPRAC) is consistent with the fact that these methods rely largely on electron-transfer mechanisms. The literature also reports that these tests measure similar redox capacities and often show high correlation [57,58]. The opposing directions of the AChE and urease correlations with the antioxidant parameters most likely reflect the solvent-dependent partitioning of distinct constituent classes (non-polar furanocoumarin-type compounds favoring AChE inhibition and hydrophilic phenolics favoring both antioxidant and urease activity) rather than any direct mechanistic link between these activities. Given the limited number of extract groups, these associations are best regarded as exploratory, descriptive patterns that warrant confirmation in a larger sample set.

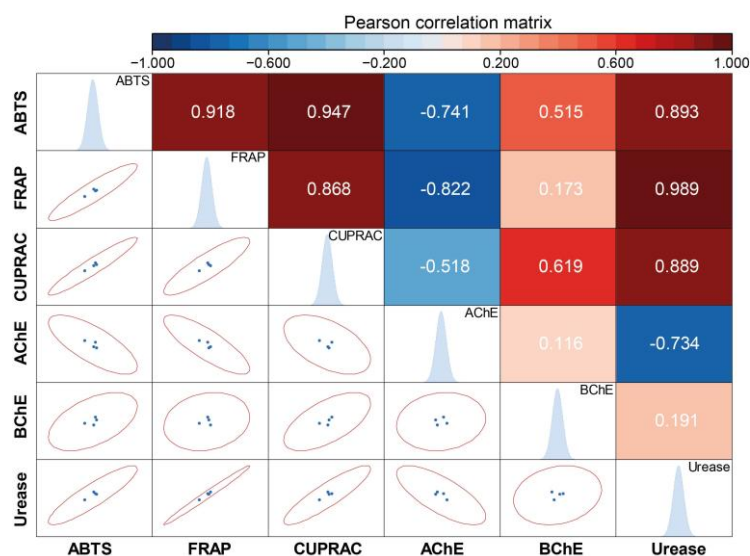


Figure 1. Pearson correlation matrix among the antioxidant activities (ABTS, FRAP, CUPRAC) and the enzyme inhibitory activities (AChE, BChE, urease) of *Heracleum persicum* extracts. The upper triangle presents the correlation coefficients (r) as a heat map, in which the color scale ranges from dark blue (strong negative correlation, $r = -1$) through white (no correlation, $r = 0$) to dark red (strong positive correlation, $r = +1$); the lower triangle displays the corresponding scatter plots for each variable pair. Correlations were computed across the four extracts (HPWE, HPEE, HPME, HPHE) using the mean activity value of each extract.

3.4. Principal component analysis (PCA).

Multivariate relationships between antioxidant and enzyme-inhibitory activities were evaluated using PCA (Figure 2). PC1 explains 74.45% of the total variance, while PC2 accounts for 21.19%, together representing 95.64% of the total variance. On the PC1 axis, ABTS, FRAP, CUPRAC, and urease are positioned on the positive side, while AChE is located on the opposite side. This distribution indicates that AChE varies in the opposite direction to antioxidant parameters and urease. On the PC2 axis, BChE distinctly separates on the positive side. When examining the distribution of samples, HPWE and HPEE are located on the positive side of PC1, positioned similarly to antioxidant parameters and urease. HPME, located on the positive side of PC2, is positioned similarly to BChE; meanwhile, HPHE, on the negative side of PC1, shows a distribution in the same direction as AChE. The clustering of these parameters in PCA along the same direction indicates that the extracts' reducing power is grouped along a common biochemical axis. Conversely, the fact that AChE inhibition does not parallel antioxidant parameters suggests that cholinesterase inhibition cannot be explained solely by redox capacity. It is known that AChE inhibition largely depends on specific ligand interactions with the enzyme's active site [59,60]. Therefore, extracts with high antioxidant capacity are not necessarily strong AChE inhibitors. The closer alignment of urease inhibition with antioxidant tests can be explained by compounds that bind to the enzyme's nickel center or interact with the metal [61,62]. This suggests that redox-active and phenolic content may contribute to urease inhibition. Overall, the results indicate that chemical differences across solvent polarities manifest differently in biological targets, and antioxidant capacity does not always correlate with enzyme inhibition. As with the correlation analysis, and given that the present PCA was derived from four extract groups, this ordination is interpreted as an exploratory description of the patterns separating the extracts rather than as a statistically robust multivariate model.

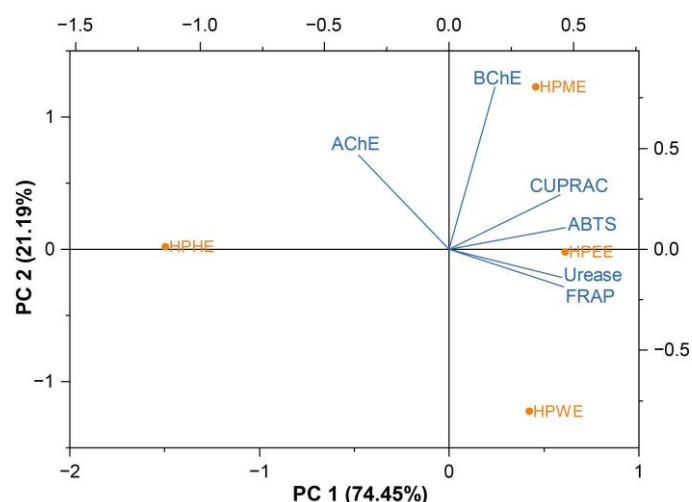


Figure 2. Principal component analysis (PCA) biplot of the antioxidant (ABTS, FRAP, CUPRAC) and enzyme inhibitory (AChE, BChE, urease) activities of *Heracleum persicum* extracts. PC1 and PC2 together explain 95.64% of the total variance. Blue vectors represent the variables, and orange points denote the four extracts (HPWE, HPEE, HPME, HPHE).

4. Conclusions

In this study, the antioxidant and enzyme-inhibitory activities of extracts from *Heracleum persicum* obtained with solvents of different polarities were compared. The findings showed that the extraction solvent significantly affected the biological activity profile. Polar extracts (water, ethanol, and methanol) exhibited higher antioxidant capacity, while the water extract was prominent in urease inhibition, and the *n*-hexane extract was prominent in AChE inhibition. A strong positive correlation was observed among the antioxidant tests, whereas AChE inhibition did not correlate with antioxidant capacity, indicating that redox-based activities and enzyme-inhibitory effects depend on different classes of constituents partitioned according to solvent polarity.

In conclusion, these findings show that solvent polarity decisively determines the *in vitro* antioxidant and enzyme-inhibitory profiles of *Heracleum persicum* and provide a rational basis for selecting solvent systems in subsequent bioactivity-guided studies. As these results are from preliminary *in vitro* screening, they offer a promising foundation for further phytochemical, mechanistic, and biological studies to explore the therapeutic potential of *Heracleum persicum*.

Author Contributions

Conceptualization, F.D. and H.K.; methodology, F.D. and H.K.; software, F.D.; validation, F.D. and H.K.; formal analysis, F.D. and H.K.; investigation, F.D. and H.K.; resources, F.D. and H.K.; data curation, F.D. and H.K.; writing-original draft preparation, F.D.; writing-review and editing, F.D. and H.K.; visualization, F.D.; supervision, F.D. All authors have read and agreed to the published version of the manuscript.

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The authors declare no conflict of interest.

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The authors used artificial intelligence-based tools solely for language editing.

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