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Phenolic Profile and Biological Potential of Olive Leaves from Organically Cultivated Cultivars

D. Čulum,[a*] *A. Nuić*,[a] *Ž. Šimović*,[b] and *R. Vučina* [b]

[a] University of Sarajevo, Faculty of Science, Zmaja od Bosne 33-35, 71 000 Sarajevo, Bosnia and Herzegovina
[b] AgroHerc Organic, Bišće polje b.b., 88 000 Mostar, Bosnia and Herzegovina

Abstract

Olive cultivation, besides yielding fruit, generates significant amounts of by-products such as olive leaves, which often remain unused. Although the presence of valuable phenolic compounds such as oleuropein and flavonoids, with proven benefits for human health, is well known, this by-product remains underutilised.

In this study, the leaves of four olive cultivars, *Levantinka*, *Leccino*, *Istarska bjelica*, and *Oblica*, grown under organic agricultural conditions, were collected in June 2024, and their chemical composition and biological activity were analysed. Total phenolic content ranged from 1392.15 to 2476.87 mg GAE/100 g dry material, while the content of total flavonoids varied from 181.60 to 265.30 mg QUE/100 g dry material. Five phenolic compounds (oleuropein, hydroxytyrosol, luteolin, luteolin-7-*O*-glucoside, and quercetin) were identified. Oleuropein was the most abundant compound, with its content varying from 673.32 to 2362.04 mg/100 g dry material. The extract of the *Leccino* showed the best ability to scavenge free radicals, while the extract from *Oblica* exhibited the strongest metal-chelating ability. Enzyme inhibitory activity against acetylcholinesterase (AChE) ranged from 26.58 to 33.13 %, while the results for inhibition of butyrylcholinesterase (BChE) varied from 11.11 to 32.57 %. The photoprotective activity of the extract solutions was determined spectrophotometrically using the Mansur mathematical equation. The obtained SPF values ranged from 15.57 to 16.87, corresponding to a blockage of over 93 % of UV radiation. The results of this research provide valuable data supporting the use of olive leaves as a potentially low-cost, renewable, and abundant source of bioactive compounds.

Keywords

Biological activity, oleuropein, olive leaves, phenolic compounds, photoprotective activity, antioxidant activity, enzyme inhibitory activity

1 Introduction

The olive tree (*Olea europaea* L.) is one of the most important crops in the Mediterranean region.[1] Since ancient times, olive oil and other olive-derived products have been used to treat various ailments and for skincare. Historical records provide strong evidence of olive oil's medicinal use in ancient Egypt, Greece, and Persia.[2] To this day, olive products are an integral part of the Mediterranean diet and are widely used in modern pharmacy and cosmetics, having demonstrated beneficial effects on human health, as proven by extensive scientific literature.[3–7] Due to their polyphenol abundance, olive products exhibit anti-cancer, anti-inflammatory, and antioxidant effects.[8] Researching new uses for by-products from the olive and olive oil industry is not only economically valuable but also environmentally beneficial in regions where olives are cultivated, and advantageous for human health. In addition to the benefits of olives and olive oil, this line of research could lead to significant positive outcomes.[9] Olive and olive oil production generates substantial amounts of waste, including leaves and pomace.[10] Olive leaves constitute the largest proportion of these by-products; pruning during the year results in about 25 kg of leaves per tree, while leaves also make up around 10 % of the total olive weight harvested for pressing.[11,12] The leaves are the primary site of plant metabolism of secondary metabolites and can therefore be considered a good source of bioactive compounds.[13] Numerous studies confirm that olive leaves contain high quantities of phenolic compounds and, given the large quantities of leaves as a by-product, they could represent a profitable and significant source of bioactive compounds.[9–11] The most common bioactive phenolic compounds found in olive leaf extracts include oleuropein, hydroxytyrosol, luteolin-7-*O*-glucoside, apigenin-7-*O*-glucoside, and verbascoside.[11,14] Olive leaf extracts have been shown to reduce endoplasmic reticulum stress, myocardial oxidative damage, and lipid peroxidation, indicating great potential in managing diabetes and cardiovascular diseases.[15] Furthermore, bioactive compounds from olive leaf extracts inhibit bacterial growth and possess potent antioxidant activity.[16] They also reduce amyloid-β formation and may serve as an adjuvant in the treatment of Alzheimer's disease. Commercial leaf extracts rich in hydroxytyrosol and oleuropein have also been found to effectively inhibit both AChE and BChE.[17]

Olive cultivation in Bosnia and Herzegovina is on the rise, as indicated by state agency figures. According to the Agency for Statistics of B&H, in 2021, there were 82.614 fruit-bearing trees over an area of 350 hectares.[18] In light of this, the present study aimed to evaluate the phytochemical profile of phenolic compounds in olive leaves and their

* Corresponding author: Dušan Čulum, PhD
Email: dusan.culum@pmf.unsa.ba

Fig. 1 – Investigated *Olea europaea* cultivars

biological activity, specifically their antioxidant, enzyme inhibitory, and photoprotective properties. The primary objective was to investigate the phenolic and biological potential of olive leaves, one of the main by-products in olive cultivation, and to direct its application for various purposes, such as medicine, pharmaceuticals, cosmetics, etc. From an economic perspective, olive leaves represent a low-cost, renewable, and abundant source of bioactive substances with broad biological potential. Moreover, the efficient utilisation of all by-products would yield positive environmental benefits.

2 Experimental

2.1 Chemicals, reagents, and instruments

All reagents, solvents, and standards used for HPLC analysis, antioxidant, and enzyme inhibitory assays were of the highest purity and were purchased from Sigma-Aldrich Co., (Germany). The Folin-Ciocalteu reagent was purchased from Carlo Erba Reagents (France), while potassium dihydrogen phosphate, dipotassium hydrogen phosphate, and sodium acetate were purchased from Merck (Germany), aluminium (III) chloride and sodium carbonate were purchased from the local distributer Semikem (B&H).

Total phenolic content, total flavonoid content, antioxidant activity, and photoprotective activity of the extracts were determined using an Agilent Cary 60 spectrophotometer. Enzyme inhibitory activity was assessed using a Thermo Fisher SkyHigh Microplate spectrophotometer. Identification and quantification of phenolic compounds were performed using an HPLC system (Agilent Technologies 1290 Infinity) equipped with a DAD detector (G4212).

2.2 Plant Material

Leaves from four *Olea europaea* L. cultivars – *Levantinka*, *Leccino*, *Istarska bjelica*, and *Oblica* – were collected in June 2024 from the AgroHerc Organic Agriculture orchard. The orchard, containing 5-year-old olive trees, is located in Gabela polje (43°05′05″N 17°40′05″E) near Čapljina (Fig. 1). Samples were dried at room temperature in a well-ventilated area for 10 days and stored in a cool, dark environment until analysis, which was performed at the Faculty of Science, University of Sarajevo.

2.3 Ultrasound extraction

Dried leaves were chopped into smaller pieces and subjected to ultrasound extraction for two hours at 30 °C. Ten grams of chopped leaves from each species were weighed into an Erlenmeyer flask and extracted with 150 ml of ethanol (96 %) in an ultrasonic cleaner (Sonic ultrasonic cleaner, frequency 20 kHz). After the extraction process, the resulting extract solutions were filtered and the solvent was evaporated to dryness (temperature of water bath was 40 °C) in a rotary evaporator. The dry extracts were stored at +4 °C until analysis.

2.4 Determination of total phenolic content

The total phenolic content was determined using the Folin-Ciocalteu method with minor modifications.[19] The prepared sample solution/standard solution (50 µl) was mixed with 2.5 ml of distilled water and 250 µl of diluted Folin-Ciocalteu reagent. After 3 min, 750 µl of 20 % Na_2CO_3 solution was added, and a volumetric flask (5 ml)

was filled to the mark. The prepared solution was left to stand for 45 min at room temperature, after which the absorbance was measured at 760 nm. Gallic acid was used as a standard, and ethanol was used as a blank. Results were expressed as milligrams of gallic acid equivalents *per* 100 g of dry material (mg GAE/100 g DM).

2.5 Determination of total flavonoid content

The total flavonoid content in investigated olive leaf extracts was determined using a slightly modified version of the colorimetric method with Al^{3+} described by *Chang et al*.20 The work solution was prepared by mixing 500 µl of the extract or standard solution with 500 µl of 2 % $AlCl_3$ solution. After standing at room temperature for 10 min, the absorbance was measured at 412 nm. A correction was made by mixing 500 µl of extract solution with ethanol, while a blank was prepared by mixing 500 µl of ethanol with 2 % $AlCl_3$. Quercetin was used as a standard. Results were expressed as milligrams of quercetin equivalents *per* 100 g of dry material (mg QUE/100 g DM).

2.6 Characterisation of phenolic compounds using HPLC-DAD analysis

An Agilent 1290 HPLC-DAD system was used to identify phenolic compounds in the ethanolic olive leaf extract. The stationary phase consisted of a 4.6 · 150 mm ROC C18 analytical column with 5 µm particles. The column temperature was maintained at 25 °C. The mobile phase flow rate was 1.0 ml min^{-1}. The mobile phases were 0.1 % formic acid (A) and methanol (B). Detection wavelengths were set at 280, 325, 340, and 370 nm. Identification of phenolic compounds was accomplished by comparing their retention times and UV spectra with standards from the database. The database was formed by analysing commercially purchased standards of phenolic compounds under the same conditions as the tested samples. The database contains over 30 phenolic compounds, including the most commonly found compounds in olive leaves such as oleuropein, hydroxytyrosol, luteolin-7-*O*-glucoside, and apigenin-7-*O*-glucoside. The results were expressed *per* 100 g of dry material.

2.7 Evaluation of antioxidant activity

2.7.1 DPPH assay

The antioxidant activity of olive leaf extracts was determined using the DPPH method.[21] Briefly, 100 µl of olive extract or standard was mixed with 1.0 ml of prepared DPPH solution (absorbance 0.7–0.9). The prepared sample was incubated at room temperature for 30 minutes in the dark. The ability of the sample to reduce DPPH radical was determined spectrophotometrically by measuring absorbance at 515 nm against a blank (ethanol). Results were determined as IC_{50} values expressed in mg/ml.

2.7.2 Ferric reducing antioxidant power (FRAP) test

The total antioxidant potential of the olive samples was determined using the FRAP assay with minor modifications as a measure of antioxidant power.[22] The FRAP reagent was prepared by mixing an acetate buffer (300 mM, pH 3.6), a solution of 10 mM TPTZ (2,4,6-*tris* (2-pyridyl)-s-triazine) in ethanol, and 20 mM $FeCl_3 \cdot 6H_2O$ in 20 mM HCl at 10 : 1 : 1 (by volume), and the reagent was warmed to 37 °C before use. For the spectrophotometric assay, 3.0 ml of FRAP reagent and 0.3 ml of the sample solution were mixed. The absorbance was measured after 10 min at 595 nm. The standard curve was prepared using different concentrations of ascorbic acid. The results were expressed in mg of ascorbic acid *per* g of dry material.

2.7.3 Ferrozine antioxidant assay

Chelation of Fe^{2+} by olive leaf extracts was evaluated according to the method by *Dinis et al*.[23] A volume of 25 µl of $FeCl_2$ solution (0.2 mM) was added to 0.2 ml of the extract solution. The reaction was initiated by adding 0.1 ml of ferrozine (5 mM), and the total volume was made up to 2 ml with ethanol. The mixture was incubated at room temperature for 10 min, and absorbance was measured at 562 nm. The results were expressed as milligrams of EDTA equivalents *per* 100 g of dry material.

2.8 Anti-Alzheimer effect (anti-cholinesterase activity)

Monitoring of the potential acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) inhibitory activity of the isolated extracts was performed spectrophotometrically using the method proposed by *Ellman et al*.[24] Briefly, the stock extract solutions were diluted in phosphate buffer (50 mM, pH 8). Then, 10 µl of extract solution was added to the wells, plus 50 µl of DTNB reagent, 90 µl of buffer, 50 µl of 0.44 U/ml AChE solution or 0.40 U/ml BuChE solution, and incubated for 10 min at 25 °C. Subsequently, 50 µl of acetylthiocholine iodide (ATCI)/butyrylthiocholine iodide (BTCI) was added, and the AChE/BChE inhibitory activity was determined by measuring the changes in the absorbance at 412 nm after 5 min at 25 °C. Blanks were prepared by substituting the enzymes with buffer, and buffer (instead of the extracts) was used as a control. The absorbance was measured at 412 nm. The percentage of inhibition was calculated according to Eq. (1).

$$\text{inhibitory activity}(\%) = \frac{1 - \text{absorbance of sample}}{\text{absorbance of control}} \cdot 100 \quad (1)$$

2.9 Photoprotective activity – SPF factor

To determine the sun protection factor (SPF), the dried extracts were diluted in ethanol to a concentration of 1 mg ml^{-1}. The absorbance of the extract solutions was measured in the wavelength range of 290–320 nm, at

5 nm increments, and three measurements were taken at each point. The readings were performed using a 1 cm quartz cell, with ethanol used as the blank. The SPF was calculated using Eq. (2), as described by *Mansur et al*.[25]

$$SPF = CF \cdot \sum_{290}^{320} EE(\lambda) \cdot I(\lambda) \cdot Abs(\lambda) \quad (2)$$

where λ is the wavelength, $EE(\lambda)$ is the erythemal effect spectrum, $I(\lambda)$ is the solar intensity spectrum, $Abs(\lambda)$ is the absorbance of the sunscreen product, and CF is the correction factor (equal to 10).

The values of $EE \cdot I$ presented in Table 1 are constants. They were determined by *Sayre et al*.[26]

Table 1 – Values of $EE \cdot I$

Wavelength, λ/nm	$EE \cdot I$ (normalised)
290	0.0150
295	0.0817
300	0.2874
305	0.3278
310	0.1864
315	0.0839
320	0.0180

3 Results and discussion

3.1 Phytochemical analysis

3.1.1 Total phenolic and flavonoid content

The results for total phenolic and total flavonoid content are presented in Table 2. The total phenolic content in the investigated olive leaf extracts ranged from 1392.15 mg GAE/100 g DM for the *Levantinka* cultivar to 2476.87 mg GAE/100 g DM for *Istarska bjelica*. Similarly, in the case of total flavonoid content, the extract from *Levantinka* (181.60 QE/100 g DM) had the lowest value, while the *Leccino* extract (265.30 QE/100 g DM) showed the highest amount. *Putnik et al*.[27] analysed ethanolic extract of *Olea* cv. *Oblica* leaves obtained by pressurised liquid extraction. Depending on the extraction conditions, the total phenolic content ranged from 41.13 to 62.99 mg GAE/g DM, while the total flavonoid content varied from 11.80 to 26.52 mg QE/g DM, which is significantly higher than the values obtained in this study.

Table 2 – Total phenolic and flavonoid content in different *Olea* cultivars

Sample	mg GAE/100 g DM	mg QUE/100 g DM
Levantinka	1392.15 ± 40.51	181.60 ± 7.73
Leccino	2136.13 ± 66.84	265.30 ± 10.69
Istarska bjelica	2476.87 ± 75.45	191.00 ± 5.16
Oblica	2251.05 ± 48.79	224.64 ± 8.28

3.1.2 Identification of phenolic compounds in the extracts by HPLC-DAD

Qualitative and quantitative analyses of the prepared extracts were performed using the HPLC-DAD technique (Fig. 2). The analysis confirmed the presence of five compounds. The obtained results are given in Table 3. Oleuropein and luteolin-7-*O*-glucoside were detected in all tested samples (Fig. 2), while luteolin was absent only in the extract of *Istarska bjelica*. Hydroxytyrosol was identified exclusively in the *Leccino* leaf extract, while quercetin was detected only in the *Oblica* leaf extract. The most abundant phenolic compound was oleuropein, with its content ranging from 673.32 to 2362.04 mg/100 DM.

Generalić-Mekinić et al.[28] investigated the oleuropein content in different extracts of *Oblica* and *Levantinka* cultivars. The ethanolic extracts contained the highest levels of oleuropein, with the *Oblica* extract having a higher amount compared to *Levantinka*. *Lukić et al*.[29] investigated four of the six cultivars from Croatia that were also included in our study. The qualitative and quantitative analysis confirmed the presence of 15 phenolic compounds. Oleuropein was the most abundant (2472.81–4497.35 mg/100 g DM), followed by luteolin-7-*O*-glucoside (323.39–485.70 mg/100 g DM). In another study, *Pasković et al*.[30] analysed the variation in phenolic composition across olive leaves depending on the cultivar, finding significant differences in the constituent concentrations. Among the cultivars investigated, *Istarska bjelica* showed the best potential for phytochemical cultivation.

Cukrov et al.[31] analysed the leaves of three Croatian native *Olea* cultivars, including *Oblica*. Oleuropein was detected as the predominant compound in all three investigated cultivars, though levels varied substantially (1685.24 to 5239.88 mg/100 g DW). The oleuropein content in the *Oblica* sample was 3068.24 mg/100 g DW, which is higher compared to our results. The phenolic leaves potential of *Istarska bjelica* and *Leccino* was investigated by *Polić-Pasković et al*.[12] The analysis confirmed different types of phenolic compounds. Oleuropein and luteolin-7-*O*-glucoside as the main compounds in both investigated samples were significantly more represented in the *Leccino* sample compared to *Istarska bjelica*.

Overall, all these previous studies, as well as our findings confirm that olive leaves are a rich source of oleuropein, with significant cultivar-dependent differences.[12,30–34] In addition to genotype, as one of the major factors of variability in olive leaf phenolic profile, some external factors, such as geographical location, stress, harvest period, extraction method also affect the qualitative and quantitative composition of *Olea* leaves.

3.2 Antioxidant activity

Given the different chemical constituents of olive leaf extracts, to obtain the most reliable data on the antioxidant activity of the extracts, three methods based on different mechanisms were used: DPPH, FRAP, and Ferrozine.

The ability to reach a 50 % reduction in DPPH radical was expressed as an IC_{50} value and ranged from 0.29 mg ml^{-1} for *Leccino* to 0.32 mg ml^{-1} for *Levantinka* and *Oblica* (Table 4).

All samples showed very similar activity. Besides the investigated extracts, a few phenolic compounds known as good antioxidants were used for comparison (gallic acid, quercetin, oleuropein, hydroxytyrosol, luteolin and luteolin-7-*O*-glucoside). Antioxidant activity for standards as IC_{50} ranged from 0.01 to 0.10 mg ml^{-1}, decreasing in the following order: gallic acid > quercetin > hydroxytyrosol > luteolin > luteolin-7-*O*-glucoside > oleuropein (Diagram 1). All standards showed significantly better activity than the investigated extracts. Gallic acid and quercetin

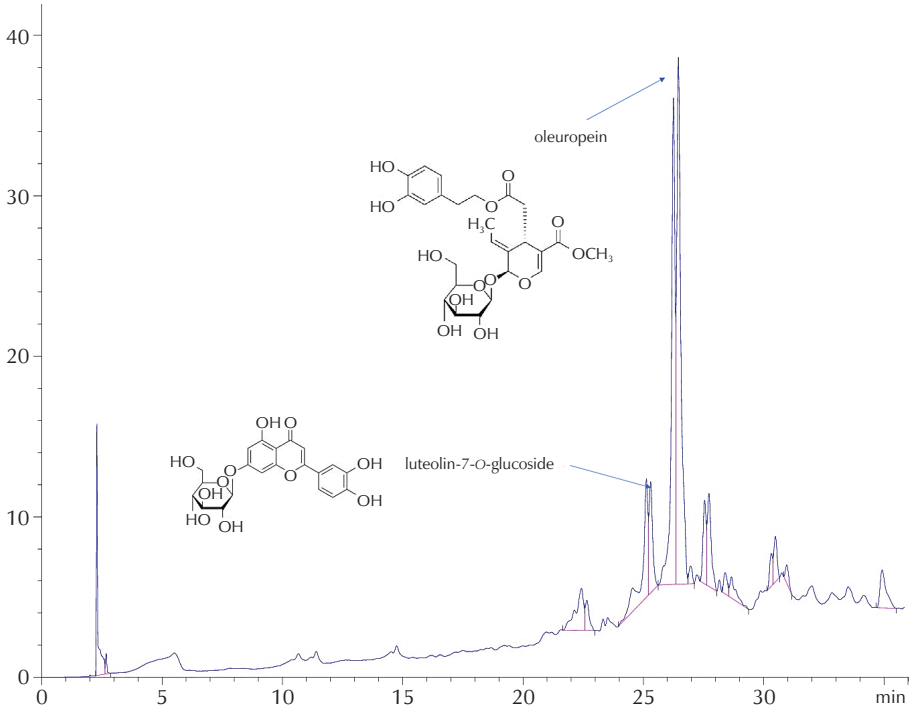

Fig. 2 – HPLC-DAD chromatogram at 280 nm of identified phenolic compounds in extract of *Istarska bjelica*

Table 3 – Concentration of identified phenolic compounds in investigated cultivars, expressed in mg 100 g^{-1} of dry material

Sample	Oleuropein	Hydroxytyrosol	Luteolin	Luteolin-7-*O*-glucoside	Quercetin
Levantinka	673.32 ± 9.83	nd*	13.00 ± 0.05	62.06 ± 0.57	nd*
Leccino	2342.77 ± 83.58	23.55 ± 1.39	7.59 ± 0.24	151.53 ± 3.00	nd*
Istarska bjelica	2362.04 ± 6.65	nd*	nd*	190.57 ± 2.27	nd*
Oblica	2281.21 ± 51.72	nd*	6.46 ± 0.06	113.72 ± 3.83	19.77 ± 0.30

* nd – not detected

Table 4 – Antioxidant activity of investigated extracts compared to their main phenolic compound

Sample	DPPH IC_{50} / mg ml^{-1}	FRAP / mg AAE/g DM	Ferrozine / mg EDTA/100 g DM
Levantinka	0.32 ± 0.01	10.67 ± 0.34	139.12 ± 3.88
Leccino	0.29 ± 0.01	20.77 ± 0.85	122.88 ± 2.03
Istarska bjelica	0.31 ± 0.01	23.62 ± 0.82	147.00 ± 2.58
Oblica	0.32 ± 0.01	21.95 ± 0.72	232.21 ± 9.70
Oleuropein	0.10 ± 0.00	329.08 ± 15.37	676.77 ± 6.97

were used as positive controls as well-known antioxidants. The number and position of hydroxyl groups (−OH) on the aromatic rings significantly influence antioxidant activity. Gallic acid has three −OH groups in *ortho*-position, enabling strong hydrogen donation and radical stabilisation *via* resonance, while quercetin has catechol structure in the B ring, allowing to donate hydrogen atoms to neutralise free radicals. The catechol system allows electron delocalisation, reducing the reactivity of the radical and preventing further damage.[35,36] The other standards are actually compounds that were identified in our samples. Some of the reasons for the higher activity of the standards compared to the tested samples may include the purity and effective concentration of the standards themselves, as opposed to those compounds in the extracts. Additionally, interfering components in the extracts may reduce the activity of these compounds through antagonistic or competitive effects.[37,38]

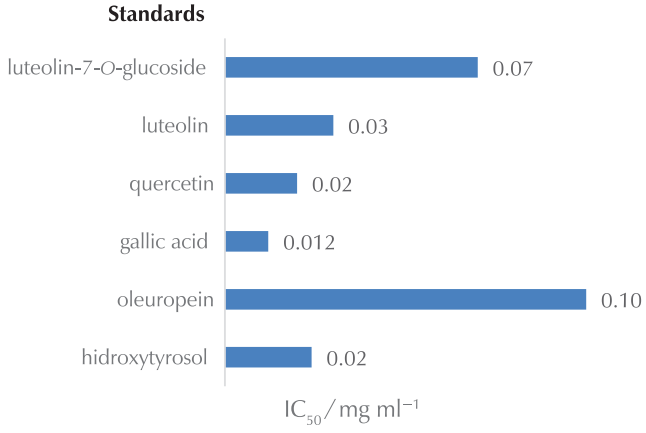

Diagram 1 – IC_{50} values of selected phenolic standards

Results for the FRAP method were expressed as milligrams of ascorbic acid equivalents per gram of dry material (Table 4). The extract of *Istarska bjelica* showed the highest ability to reduce FRAP reagent at 23.62 mg AAE/g DM, while the extract of *Levantinka* exhibited the lowest value at 10.67 mg AAE/g DM. The reduction power of *Leccino* (20.77 mg AAE/g DM) and *Oblica* (21.95 mg AAE/g DM) extracts was similar to *Istarska bjelica*. As the main compound in leaf extracts, the reduction power of oleuropein was analysed. The obtained result was 329 mg AAE/g. To obtain a more comprehensive assessment of the antioxidant activity of the investigated extracts, we evaluated their ability to chelate Fe^{2+}. The results were expressed as EDTA equivalents (Table 4). All extracts showed interaction with Fe^{2+} and chelation activity ranged from 122.88 mg EDTA/100 g DM (*Leccino*) to 232.21 mg EDTA/100 g DM (*Oblica*).

The *Oblica* extract exhibited almost twice higher chelation activity compared to the other three investigated extracts. Oleuropein showed a high affinity to chelate Fe^{2+} (676.77 mg EDTA/g).

In the study of *Paskovic et al.*,[30] the antioxidant activity of the same cultivars used in this study was investigated by DPPH and FRAP methods. The results obtained by DPPH assay were expressed as Trolox equivalents. *Istarska bjelica* extract exhibited the best activity at 339.77 mM TE-Q/g DW. The activities of the other three extracts were as follows (in decreasing order): *Levantinka* (325.66) > *Leccino* (320.47) > *Oblica* (286.93). Considering the FRAP assay results, *Istarska bjelica* had the best reduction ability in our study. The main difference between the results was for *Levantinka* and *Oblica*, which could be attributed to different sampling times.

Šimat et al.[33] studied the antioxidant activity of hydroethanolic extracts from six Mediterranean olive cultivars, including *Oblica* and *Levantinka*. The results showed that the analysed extracts exhibited similar activities based on the DPPH method. However, using the FRAP method, the *Oblica* extract displayed significantly higher activity than the *Levantinka* extract.

3.3 Enzyme-inhibitor activity

Alzheimer's disease (AD) is characterised by neuro-inflammation, enhanced production and accumulation of β-amyloid peptide, and elevated levels of the enzymes acetylcholinesterase (AChE) and butyrylcholinesterase (BChE).[39] Current literature suggests that these two enzymes play a pivotal role in the pathogenesis of Alzheimer's disease. Imbalances and changes in the AChE/BChE ratio result in a cholinergic deficit in the brain and a deficiency of the brain neurotransmitter acetylcholine. Today's pharmacotherapy focuses on drugs aimed at increasing acetylcholine levels by inhibiting cholinesterase.[39,40] Thus, general cholinesterase inhibitors that inhibit both AChE and BChE may provide therapeutic benefits for treating AD and other related dementias. The AChE and BChE were successfully inhibited by olive leaf extract rich in hydroxytyrosol and oleuropein.[41]

Four ethanolic extracts of olive leaves were evaluated for their activity as inhibitors of AChE and BChE. As shown in Table 5, extract of *Istarska bjelica* exhibited the best inhibitory activity for both enzymes (33.13 % for AChE) and (32.57 % for BChE). The extract of *Leccino* showed very similar activity for AChE inhibition (32.11 %). In terms of BChE inhibition, the *Oblica* leaf extract had a percent inhibition of 31.43 %. All tested extracts showed lower activity compared to the analysed standard donepezil.

Table 5 – Values of enzyme-inhibitory and photoprotective activity

Sample	AChE/%	BChE/%	SPF
Levantinka	27.51 ± 0.53	14.99 ± 0.43	16.87 ± 0.46
Leccino	32.11 ± 1.28	11.12 ± 0.42	16.25 ± 0.48
Istarska bjelica	33.13 ± 1.10	32.57 ± 1.58	15.57 ± 0.54
Oblica	26.58 ± 1.06	31.43 ± 0.68	16.74 ± 0.37
Donepezil	98.17 ± 1.54	73.91 ± 0.38	/

The mechanism of AChE/BuChE inhibition by the tested extracts was not the focus of this study. However, it is typically described in the literature as a mixed-type inhibition. Namely, the complexity of the extracts' composition prevents clear conclusions, as different components inhibit by different mechanisms.[42,43]

Romero-Marquez et al.[44] investigated the AChE inhibitory activity of olive leaf extracts from Spain, Portugal, Greece, and Italy. The results ranged from 17.81 % for Greek to 38.36 % for Spanish samples.

3.4 Photoprotective activity

Skin damage caused by excessive exposure to UVB and UVA radiation (such as sunburns, skin aging, and even tumorigenesis) has led to an increasing need for adequate protection. Sunscreen is one of the most important ways to protect the skin from UV radiation. Recently, there has been an increasing emphasis on the use of natural preparations due to the negative effects on human health and damage to the marine environment attributed to synthetic preparations. Plants, including olives, contain various bioactive compounds such as phenolic compounds that can absorb ultraviolet rays and are considered potential sources of sun protection.[45,46] The published research indicates a strong correlation between SPF (Sun Protection Factor) and phenolic compounds. The SPF value measures how effective a sun protection product is. Table 6 gives the values of UV protection depending on the SPF value in the range from 2 to 25.[47]

Table 6 – Percentage of UV protection depending on SPF value

SPF	Percentage of UV blocked / %
2	50
4	75
5	80
10	90
15	93

As far as we know, this is the first study of the photoprotective activity of olive leaf extracts of selected *Olea* cultivars. In this study, all investigated extracts had very similar SPF values ranging from 15.57 (*Istarska bjelica*) to 16.87 (*Levantinka*). According to Table 6, all tested extracts were found to block over 93 % of UV.

Asan-Ozusaglam et al.[48] tested the sun protection factor of olive leaf extracts of the *Ayvalık Yağlık* variety grown in Izmir and reported SPF values from 2.21 to 15.35. A study conducted by *Alkhami* and co-workers[49] showed the SPF activity of the four Syrian olive leaf cultivars and found that SPF values ranged from 14.48 to 29.96. The findings of our study, along with previous research, suggest that olive leaf extracts may serve as potential natural sources of sun protection.

4 Conclusion

The significance of this work lies in the fact that, for the first time, a qualitative and quantitative analysis of phenolic compounds and biological activity of leaf extracts of known *Olea* cultivars grown in Bosnia and Herzegovina has been performed. The results showed that the leaves, which are the most frequently unused by-product of olive cultivation, represent an excellent source of various phenolic compounds with significant biological potential. The *Istarska bjelica* variety showed the best potential. The results of this research provide valuable data for the use of olive leaves as a potentially inexpensive, renewable, and abundant source of bioactive compounds, with promising applications in food (dietary supplements), pharmaceutical (olive leaf extract capsules or tablets, gels, *etc*.), and cosmetics industries (creams, oils, serums, *etc*.). On the other hand, the data not only confirm the biological potential of olive leaves but also raise new research questions, including the need for the development of sustainable green extraction technologies, and a deeper understanding of the bioavailability and safety of the isolated compounds in real-world applications.

List of abbreviations
Popis kratica

AAE – ascorbic acid equivalents
AChE – acetylcholinesterase
AD – *Alzheimer's disease*
ATCI – acetylthiocholine iodide
BChE – butyrylcholinesterase
BTCI – butyrylthiocholine iodide
cv – cultivar
DM – dry material
DPPH – 1,1-diphenyl-2-picrylhydrazil
FRAP – ferric reducing antioxidant power
GAE – gallic acid equivalents
HPLC – high performance liquid chromatography
nd – not detected
QE – quercetin equivalents
SPF – sun protection factor

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SAŽETAK

Fenolni profil i biološki potencijal listova maslina organski uzgojenih sorti

Dušan Čulum,[a*] *Arijana Nuić*,[a] *Žarko Šimović* [b] i *Robert Vučina* [b]

Uzgoj maslina, osim plodova, stvara i znatne količine nusproizvoda poput lišća, koji često ostaju neiskorišteni. Iako je dobro poznato da sadrže zanimljive fenolne spojeve poput oleuropeina i flavonoida, s dokazanim prednostima za ljudsko zdravlje, taj nusproizvod i dalje je nedovoljno iskorišten.

U ovom radu analiziran je kemijski sastav i biološka aktivnost lišća sorti maslina *Levantinka*, *Leccino*, *Istarska bjelica* i *Oblica* uzgojenih u ekološkim agronomskim uvjetima sakupljenih tijekom lipnja 2024. godine. Ukupni sadržaj fenola kretao se od 1392,15 do 2476,87 mg EGK/100 g suhog materijala, dok je sadržaj ukupnih flavonoida varirao od 181,60 do 265,30 mg EK/100 g suhog materijala. Identificirano je pet fenolnih spojeva (oleuropein, hidroksitirozol, luteolin, luteolin-7-*O*-glukozid i kvercetin). Oleuropein je najzastupljeniji spoj, čiji je sadržaj varirao od 673,32 do 2362,04 mg/100 g suhog materijala. Ekstrakt *Leccina* pokazao je najbolju sposobnost reduciranja/ uklanjanja slobodnih radikala, dok je ekstrakt *Oblice* pokazao najbolju sposobnost kelatiranja prelaznih metala. Inhibicijska aktivnost enzima za acetilkolinesterazu (AChE) kretala se od 26,58 do 33,13 %, dok su rezultati za inhibiciju butirilkolinesteraze (BChE) varirali od 11,11 do 32,57 %. Fotoprotektivna aktivnost otopine ekstrakta određena je spektrofotometrijski, primjenjujući Mansurovu matematičku jednadžbu. Dobivene SPF vrijednosti bile su u rasponu od 15,57 do 16,87, što predstavlja blokadu više od 93 % UV zračenja. Rezultati ovog istraživanja pružaju korisne podatke za uporabu maslinovih listova kao potencijalno jeftinog, obnovljivog i obilnog izvora bioaktivnih spojeva.

Ključne riječi
Biološka aktivnost, oleuropein, lišće masline, fenolski spojevi, fotoprotektivna aktivnost, antioksidativna aktivnost, enzim inhibitorna aktivnost

[a] *Sveučilište u Sarajevu, Prirodno-matematički fakultet, Zmaja od Bosne 33-35, 71 000 Sarajevo, Bosna i Hercegovina*
[b] *AgroHerc Organic, Bišće polje b.b., 88 000 Mostar, Bosna i Hercegovina*

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