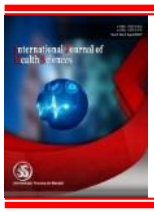

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A Study of the Effects of Variety and Season of Collection on the Amounts of Bioactive Compounds in Algerian Olive Leaves

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Algerian cultivars, bioactive compounds; DPPH scavenger activity; ferric reducing power; Olea europaea L.,

Abstract

This study explored the bioactive contents (including chlorophyll, carotenoid, phenolic and flavonoid compounds) of olive leaves (Olea europaea L.) from three Algerian cultivars (Aberkane, Aharoun, and Akerma) across four distinct seasonal periods (autumn, winter, spring, and summer). The investigation involved performing antioxidant potential tests. The findings indicated that all cultivars are rich in bioactive compounds and the Aberkane olive leaves exhibited the highest levels of chlorophyll a (2518.79 ± 19.32 µg/g), chlorophyll b (816.22 µg/g), carotenoid (120.22 ± 6.72 µg/g) phenolic (596.97 ± 29.07 mg GAE/g DE) and flavonoid (374.05 ± 12.54 mg EQ/g DE) compounds. Aberkane olive leaves presented also the best results of DPPH scavenger activity (IC50: 38.40 ± 1.75 µg/ml) and ferric reducing power (A0.5: 22.34 ± 1.98 µg/ml). In general, leaves collected during summer demonstrated the highest contents of bioactive compounds and the best antioxidant activities, compared to other seasons. Consequently, it can be deduced that both the cultivar type and the season of collection exert significant influences on the bioactive content and antioxidant potential of olive leaves.

Contents

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1 Introduction

Significant interest exists in edible plants containing antioxidants and phytochemicals that promote health, owing to their potential therapeutic properties. One notable example is the olive (Olea europaea L.), a member of the Oleaceae family which is typically found in Mediterranean basin countries. Beyond its economic significance, the olive tree possesses medicinal and nutritional value.

In Algeria, the agricultural and processing activities related to crops yield a significant quantity of food by-products, especially olive leaves which are marketed as olive leaves for tea or food supplements for pharmaceutical purposes. They serve as a reservoir of bioactive compounds that offer health-promoting advantages. Pharmacological investigations have unveiled the potential of olive leaf extract to induce hypoglycemic, antioxidant, hypotensive, and antimicrobial effects (Wainstein et al., 2012; Sarbishegi et al., 2017). Additionally, olive leaf extracts are among the food items acknowledged for their food preservation properties (Medina et al., 2008). The primary compounds identified in olive leaves belong to the categories of simple phenolics and flavonoids (Talhaoui et al., 2015).

In the process of metabolism, cells spontaneously produce reactive oxygen species (ROS), which are implicated in the development of various diseases (Halliwell, 1992). The phytochemical studies have demonstrated that bioactive components in olive leaves act as antioxidants to reduce the pro-oxidants or reactive species of pathologic significance (Somogyi et al., 2007).

Therefore, it is believed that the amount of antioxidants obtained from olive leaves varies for different olive cultivars and each variety, depending on several factors such as time of collection. Limited research has been conducted on antioxidant compounds in Algerian olive leaves. Thus, the study, presented in this paper, explores the impact of both olive variety and season of harvest on the contents of antioxidants in olive leaves from three Algerian cultivars (Aberkane, Aharoun and Akerma) grown in the north-east part of the country.

2 Materials and Methods

Sample collection

The samples investigated in this study were olive leaves of three olive varieties (Aberkane, Aharoun and Akerma) collected in 2022 during the concluding phases of autumn (November), winter (February), spring (April) and summer (August), representing the four seasons of the year. All cultivars were cultivated under identical agronomic and environmental conditions within the same olive groves situated on private property in Ain Soltane (Bordj Bou Arreridj -Algeria-) with the GPS coordinates of 36° 12' 99.70'' N and 4° 76'19.97'' E, where these varieties are among the most popular. Leaves were harvested from mature trees (≥ 5 years old). For each collection period, leaves were collected from 12 trees of each cultivar.

Preparation of extracts from olive leaf

By employing a magnetic stirrer and 150 ml of 70% ethanol, 10g of powdered olive leaves were subjected to extraction for 3 hours at room temperature and in the absence of light. Upon completion of the extraction process for various cultivars of Olea europaea L. leaves, the resulting extracts underwent filtration through microfilters to obtain a clear crude extract solution. Subsequently, the separation procedure was repeated, and the solvent was evaporated under vacuum conditions at 40°C using a rotary evaporator. All extraction procedures were carried out in triplicate. The resulting extracts were then frozen at -4°C for subsequent analysis (Carchi et al., 2021).

Phytochemical study
Chlorophyll pigments content

The determination of chlorophyll and carotenoid content in leaf samples follows the method outlined by Nagata & Yamashita (1992), with certain modifications. A total of 100 mg from each extract is combined with a 10 ml acetone-hexane mixture (4:6) and thoroughly vortexed to achieve uniform homogenization. Subsequently, the entire mixture is filtered through the Whatman n° 04 paper. The optical density of the resulting supernatant is then measured at 663 nm, 645 nm, 505 nm, and 453 nm simultaneously using a Shimadzu™ UV-VIS 1800 Spectrophotometer, USA. The chlorophyll content is calculated in milligrams per gram of leaf tissues. The quantities of chlorophyll 'a' (mg/100 mL) and chlorophyll 'b' (mg/100 mL) are estimated from these values using the following formulas:
Chlorophyll 'a' (mg/100 mL) = 0.991 x A663 - 0.0982 x A645.
Chlorophyll 'b' (mg/100 mL) = -0.462 x A663 + 1.59 x A645.

Total carotenoid content

The total carotenoid content of the samples was assessed following the method described by Sass-Kiss et al. (2005), with slight modifications. In summary, 10 ml of a mixed extraction solvent (hexane/acetone/ethanol, 2:1:1) was added to 5 g of dried plant material. After stirring for 30 minutes, the solution underwent centrifugation at 4500 rpm for 30 minutes, and the resulting supernatant liquid was transferred to another vial, retaining the upper phase. A second extraction was performed by adding 10 ml of hexane. The combination of the two hexane phases was utilized for the determination of total carotenoids through spectrophotometry at 420 nm. Concentrations of carotenoids were determined by referencing the calibration curve using β-carotene as the standard, and the results are expressed as µg/g of dry weight (µg/g DE).

Total phenolic contents

The determination of the total phenolic content in the ethanol extracts of Olea Europaea L. leaves was conducted using the Folin Ciocalteu reagent method, as outlined by Singleton & Rossi (1965). In this approach, 200 µl of the appropriately diluted sample or standard was added to 1 ml of Folin's reagent, which had been diluted 10 times. After a 4-minute interval, 800 µl of a 7.5% sodium carbonate solution was introduced. The mixture was allowed to react for two hours at room temperature in the absence of light. The obtained data were analyzed using the linear regression equation derived from the calibration graph endpoint: Y = 0.0060 x + 0.036, where Y represents absorption intensity, and X represents total phenolic compounds expressed as gallic acid equivalent (mg GAE/g DE). The R2 value for this equation is 0.9994. A Shimadzu™ UV-VIS 1800 Spectrophotometer, a double-beam UV-Vis spectrophotometer from the USA, was utilized to measure the absorbance of both the solution and the blank at 760 nm. To ensure accuracy, three measurements were taken.

Total flavonoid contents

The quantification of total flavonoids in the leaf extracts was conducted using the methodology devised by Jain et al. (2011). In this procedure, 1 ml of 2% AlCl3 was added to 1 ml of each extract or standard solution at varying concentrations. After allowing the reaction to proceed for 1 hour at room temperature, the absorbance at 430 nm was measured using a double-beam UV-vis spectrophotometer, such as the Shimadzu™ UV-VIS 1800 Spectrophotometer from the USA.

For the construction of the standard quercetin calibration curve, 1 ml of quercetin at different concentrations was employed. Triplicate samples were utilized for each analysis. The total flavonoid levels were quantified in milligrams of quercetin equivalent per gram of dry extract (mg QE/g DE) using the following equation derived from the calibration curve: y = 0.238 + 0.088, where y represents the absorbance, and x represents the quercetin content (µ.ml-1). The coefficient of determination (R2) for the calibration curve is 0.9901.

Antioxidant activity

DPPH free radical scavenging activity

Following the protocol detailed by Burits & Bucar (2000), the 2,2-Diphenyl-1-picrylhydrazyl (DPPH) scavenging assay was utilized to assess the free radical scavenging activity of the extracts. In brief, a DPPH stock solution was prepared by dissolving it in 100 ml of methanol and subsequently diluting it to achieve an absorbance of 0.91 ± 0.03 at 517 nm. The control, lacking the test sample, was supplemented with an equivalent volume (2 ml) of ethanol. To 2.5 ml of DPPH, 100 µl of each extract at various standards or concentrations (ascorbic acid) was added. The reduction in absorbance of the test mixture was monitored at 517 nm after 30 minutes. The assays were repeated three times. The antioxidant activity percentage (I%) for each sample is computed using the following equation:

%= [(Absorbance of control - Absorbance of the sample) / Absorbance of control] × 100.

Ferric reducing antioxidant power

Ferric Reducing Antioxidant Power analysis was conducted following the procedure delineated by Oyaïzu (1986). In this method, extracts (200 µL) were combined with phosphate buffer (500 µL, 2.0 M, pH 6.6) and potassium ferricyanide (2.5 mL, 1.0%), and the mixture was incubated at 50°C for 20 minutes. Subsequently, trichloroacetic acid (10%) in a volume of 2.5 mL was added to the mixture, followed by centrifugation at 650 rpm for 10 minutes. The upper layer of the solution (500 µL) was then blended with distilled water (2.5 mL) and ferric chloride (0.5 mL, 0.1%), and the absorbance was measured at 700 nm. An increase in absorbance of the reaction mixture indicated an elevation in reducing power.

Statistical analysis

Each experiment was replicated three times, and the results were expressed as means ± standard deviation. Statistical distinctions were evaluated utilizing either one-way ANOVA or Student's t-test. A simple correlation analysis between variables was also performed ($p < 0.05$) using the Tukey test. A p-value of 0.05 or lower was considered as evidence of a statistically significant difference.

3 Results and Discussions

Bioactive compounds are influenced by various intrinsic and extrinsic factors within the leaves. Each region and cultivar exhibit distinct characteristics, underscoring the necessity for this study. Plant pigments play a pivotal role in determining color, and influencing photosynthesis reactions (Sudhakar et al., 2016). Chlorophylls and carotenoids, the predominant pigments in plants, are recognized for their color properties and functional attributes (Schoefs, 2003).

They enable the absorption and conversion of light into chemical energy (Casida, 2009). Indeed, they act as antioxidants, particularly in combating the lipid peroxidation mechanism (Lanfer-Marquez et al., 2005). These pigments are also utilized in the food industry as additives and colorants. Foods with high chlorophyll content provide numerous health benefits (Indrasti et al., 2018).

There are two primary forms of chlorophyll pigments, namely chlorophyll a and chlorophyll b, distinguished by their chemical structure (Aramrueang et al., 2019) and biological functions. Chlorophyll a serves as the primary pigment in the photosynthetic system, while chlorophyll b acts as a secondary component that enhances light absorption and energy conversion (Trees et al., 2000). Olive leaf extracts showed highly significant differences in chlorophyll pigment content between varieties ($p<0.001$) and seasons ($p<0.001$).

The leaves of all studied cultivars presented the highest concentrations of both chlorophyll “a” and “b” in summer and the lowest values in winter except Aharoun cultivar. The leaves of the cultivar Aberkane showed the highest levels of chlorophyll “a” (2518.79 ± 19.32 µg/g) and “b” (816.22 µg/g), while the leaves of Akerma cultivar showed the lowest concentrations of chlorophyll “a” (650.15 ± 9.33 µg/g) and “b” (167.11± 8.18 µg/g µg/g) (Table 1).

The contents of chlorophyll in leaves may vary widely among different cultivars (Bahloul et al., 2014). Our results were higher than those described by Tarchoune et al. (2019), in Tunisian olive leaves. Furthermore, the retention of chlorophyll potentially increases with the leaves' prolonged lifespan (Brahmi et al., 2012).

Carotenoids play a crucial role in the plant photosynthesis process contributing to the yellow/orange coloration of cell membranes (Garcia-Vaquero et al., 2018). They exhibited physiological activities, notably functioning as antioxidants by scavenging free radicals (Domonkos et al., 2013). This chemical class functions as an antioxidant, providing protection to membranes against damage from free radicals and slowing down ageing processes (Bulda et al., 2008).

Table 1 displays the spectrophotometric measurement of total carotenoids based on the sample absorbance at 470 nm. The results indicated that extracts exhibited very similar quantities of total carotenoids.

Our studied olive leaf extracts showed no difference in carotenoid content between seasons, but a significant difference between cultivars ($p<0.05$). This indicates that variations in climatic conditions mainly temperature factors did not impact these compounds in the leaves throughout the year.

The ethanolic olive leaf extract of Aberkane cultivar represented the highest content of carotenoids throughout the year (120.22 ± 6.72 µg/g), while Akerma's extract recorded the lowest concentration (42. 60 ± 4.53 µg/g). These values are lower than those described by Benjeddou et al. (2019), who reported a total carotenoid concentration of 3000 µg/g.

Table 1
Pigment quantification of Algerian olive leaf extracts (Olea europaea L.) from three cultivars in different seasons. The results are reported as mean± SD (n=3)

	Chlorophyll a (µg/g)			
	Autumn	Winter	Spring	Summer
Aberkane	2224.11 ± 10.05 b	1918.12 ± 13.22 a	2491.20 ± 12.12 ab	2518.79 ± 16.21 b
Aharaoun	1947.75 ± 21.76 b	1601.50 ± 9.88 a	1924.35 ± 16.84 b	1980.85 ± 20.42 b
Akerma	893. 63 ± 6.76 b	657.15 ± 8.21 a	797.52 ± 9.46 b	800.77 ±6.39 ab
	Chlorophyll b (µg/g)			
	Autumn	Winter	Spring	Summer
Aberkane	789.19 ± 11.87 b	619.65 ± 9.52 a	806.73 ± 9.22 ab	817.22 ± 9.03 b
Aharaoun	329.33 ± 6.53 ab	268.90 ± 8.41 a	344.62 ± 10.05 b	313.46 ± 5.98 b
Akerma	220.47 ± 8.42 b	167.11 ± 4.04 a	190.68 ± 8.85 a	196.15 ± 7.44 a
	Carotenoids (µg/g)			
Aberkane	108.78 ± 5.85 a	98.55 ± 6.91 a	120.22 ± 6.72 a	113.52 ± 7.42 a
Aharaoun	69.76 ± 6.12 a	62.55 ± 3.82 a	65.94 ± 7.31 a	60.86 ± 6.31 a
Akerma	44.74 ± 3.73 a	42. 60 ± 4.53 a	47.62 ± 6.11 a	48.18 ± 5.42 a

The antioxidant capacity of olive leaves is primarily ascribed to the presence of phenolic compounds (Goulas et al., 2010), operating through various mechanisms such as scavenging reactive oxygen species (ROS), inhibiting lipid peroxidation, and chelating metal ions (Shahidi, 1997). The antioxidant effects of several phenolic compounds in olive leaves have been examined, and their efficacy has been linked to the characteristics of functional groups (Rahmanian et al., 2015). Moreover, some studies propose that phenolic compounds exhibit a synergistic effect on antioxidant capacity when combined, as observed in olive leaf extracts (Rietjens et al., 2007; Lee et al., 2010).

Phenolic compounds, whether assessed individually or in synergy, play a significant role in the antioxidant activity of olive leaves, as indicated by Wang et al. (2011), and the synergistic effect mentioned by Lee et al. (2010). Despite numerous scientific studies on the phenolic compounds and health benefits of olive oils, research on phenolic and flavonoid compounds contents of olive leaves remains limited, particularly in the context of Algerian olive cultivars. The total phenolics and total flavonoid contents of ethanolic extract leaves from three Algerian olive cultivars are presented in Figure (1). The total phenolic contents are expressed as gallic acid equivalent, while flavonoid contents as quercetin equivalent. Indeed, the analysis of

phenolic contents showed a statistical difference between different cultivars ($p<0.05$) and different harvesting dates ($p<0.05$).

The phenolic contents ranged between (500.74 ± 10.87 mg GAE/g DE) (winter) and 680.05 ± 18.11 mg GAE/g DE (summer) in Aberkane leaves, 364.90 ± 21.90 mg GAE/g DE (winter) and 596.97 ± 29.07 mg GAE/g DE (summer) in Aharoun leaves, 355.43 ± 23.77 mg GAE/g DE (winter) and 590.23 ± 20.87 mg GAE/g DE (summer) in Akerma leaves (Figure 1a). Regarding the cultivar factor, the Aberkane variety had the highest phenolic contents while Akerma had the lowest. Our results are higher than those of Sevim & Tuncay (2012), in Ayvalık and Memecik olive leaf extracts at different harvest times.

Regarding the impact of harvest time on quantities of phenolic compounds, it was observed that the highest polyphenol contents are observed in leaves harvested during summer in all ethanolic leaf extracts. These results are different from those of Romero et al. (2017), who observed the highest phenolic content during the cold season. Previously studies have demonstrated that flavonoids are the dominant phenolic sub-group and exhibit anti-inflammatory, antiallergic, antiviral, and anticarcinogenic properties (Middleton Jr, 1998).

The total flavonoid contents of our olive leaf extracts collected at the different harvest periods are presented in Figure 1b. The total flavonoids content of Aberkane, Aharoun and Akerma olive leaves changed between 200.74 ± 10.65 mg EQ/ g DE (winter) and 374.05 ± 12.54 mg EQ/ g DE (summer), 124.13 ± 6.90 mg EQ/ g DE (winter) and 283.96 ± 9.07 mg EQ/ g DE (summer), 155.43 ± 6.32 mg EQ/ g DE (winter) and 317.83 ± 4.87 mg EQ/ g DE (summer), respectively. The highest total flavonoid contents were determined in the Aberkane leaves harvested in summer. The flavonoid contents of studied olive leaves vary within cultivar ($p<0.05$) and are influenced by the timing of sampling/harvest ($p<0.05$).

The most significant transformations in plants took place during the summer season. These alterations, particularly the increase in levels of bioactive compounds such as phenols and flavonoids, can be linked to greater exposure to light/UV-B, rising temperatures (Brahmi et al., 2012), and decreased levels of rainfall (Arslan et al., 2013).

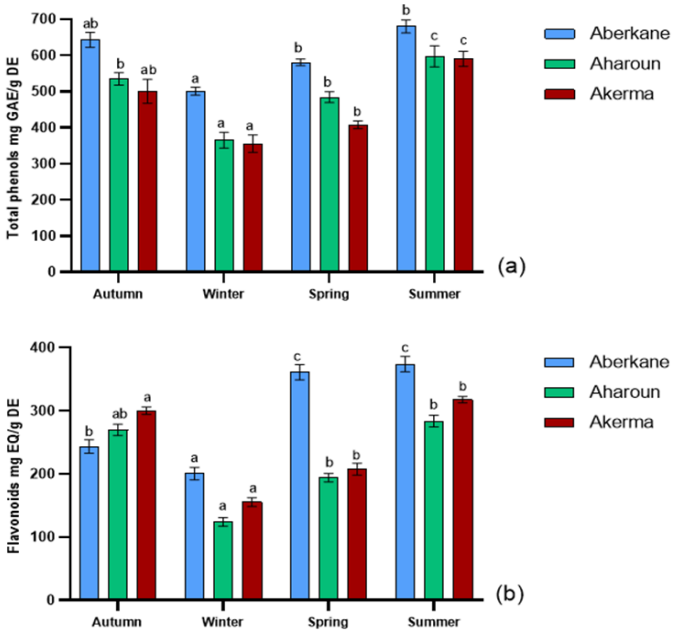

Figure 1. Total phenols (a), total flavonoids (b), of Algerian olive leaf extracts (Olea europaea L.) from three cultivars in different seasons. The results are reported per 1 g of extract and are presented as mean± SD (n=3). Columns marked with different letters are statistically different ($p < 0.05$). DE: dry extract, GAE: Gallic acid equivalents, CE: Catechin equivalent.

The antioxidant properties of olive leaf extracts were examined through DPPH and FRAP methods. To assess the ability of various samples to scavenge free radicals, the DPPH assay is widely recognized as a reliable method. This assay relies on the capability of sample antioxidants to neutralize stable free DPPH radicals (Chinnici et al., 2004). In the FRAP assay, the antioxidant compounds present in a sample donate electrons to

the ferric ions, converting them to ferrous ions. This electron transfer reflects the sample's ability to neutralize free radicals and oxidative species, which are associated with various diseases and aging processes. Both cultivar and harvest season significantly ($p<0.05$) affect the scavenging activity and ferric-reducing antioxidant power (FRAP).

According to our results, both DPPH scavenging activity (Figure 2 a) and FRAP (Figure 2 b) values followed this order: Akerma > Aharoun > Aberkane. The values of IC50 (scavenging activity assay) are ranging between 38.40 ± 1.75 and 82.98 ± 2.81 µg/ml, while those of A0.5 (FRAP test) are ranging between 22.34 ± 1.98 µg/ml and 53.22 ± 2.07. In general, winter was characterized by the highest both A0.5 and IC50 values, while summer was characterized by the lowest values. Olive leaf extracts in the study of Khaliq et al. (2015), showed values of scavenging activity (IC50) higher than those presented in the present work indicating fewer antioxidant activities. These results follow those published in literature by different researchers, indicating that the rich profile of phenolic compounds is the main responsible for the free radical scavenging properties of Olea europaea L. (Moudache et al., 2016). The high DPPH radical scavenging activity of these cultivars suggests their use in diseases arising from free radical attack. Similarly, the values of A0.5 in this work are higher than those of Hayes et al. (2011).

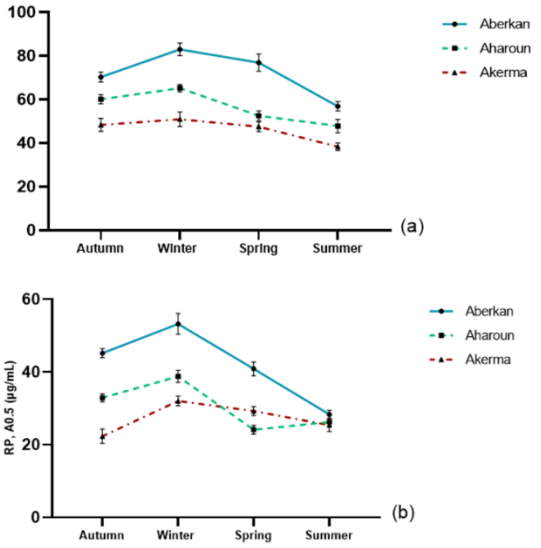

Figure 2. Free radical scavenging activity by the DPPH assay(a), Reducing power (b), of ethanolic extracts (Olea europaea L.) from three Algerian olive leaves cultivars in comparison with gallic acid. Values are expressed as mean± SD (n=3). Columns marked with different letters are statistically different ($p < 0.05$).

Generally, multiple researchers have proposed a positive correlation between elevated concentrations of bioactive compounds such as phenols and flavonoids in extracts and their antioxidant activity, suggesting that higher levels of these compounds are associated with increased antioxidant potential (Mokrani & Madani, 2016). In our current study, we employed Tukey test correlation to assess the relationship between secondary metabolites and antioxidant properties. As shown in Table 2, the total phenol contents demonstrated a robust and significant correlation with the IC50 of DPPH scavenging assay values, exhibiting a correlation coefficient of $r = 0.9790$. Conversely, a moderate correlation was observed with the FRAP value, with a correlation coefficient of $r = 0.7665$. The association between phenolic contents and reducing activity, as identified through the FRAP assay, has been previously documented by Papoti et al. (2018).

Furthermore, a strong correlation was identified between flavonoids and the IC50 of DPPH and FRAP values, with correlation coefficients of $r = 0.7089$ and $r = 0.7111$, respectively. In contrast, chlorophyll a, chlorophyll b and total carotenoids values exhibited low correlations with the DPPH scavenging assay, with correlation coefficients of $r = 0.3559$ and $r = 0.3442$ and $r = 0.3442$, $r = 0.2008$, respectively. These results suggest that the synergism between phenolic compounds and flavonoids primarily accounts for the antioxidant activity found in the extracts. Numerous authors have substantiated a significant correlation

between phenolic and flavonoid contents and the antioxidant activity of various extracts (Gullon et al., 2018; Talhaoui et al., 2015).

Table 2
Correlation between different studied variables

	Chlorophyll A	Chlorophyll B	Total Carotenoids	Phenolics	Flavonoids	DPPH	FRAP
Chlorophyll a	–	0.9660	0.6558	*	*	0.3559	0.4400
Chlorophyll b	*	–	0.8791	NS	*	0.3442	0.1334
Phenolics	0.3446	0.3500	0.2784	–	0.6553	0.9790	0.7665
Flavonoids	0.5222	0.3562	0.3998	*	–	0.7089	0.7111
Total Carotenoids	*	*	–	NS	*	0.2008	0.3175
DPPH	*	0.1332	NS	*	*	–	0.9973
FRAP	0.1223	0.1543	NS	*	*	*	–

*Significant correlation by the Tukey test considering 5% significance. NS – Not significant

4 Conclusion

The timing of harvest and the type of olive plant exerts a significant influence on secondary metabolite contents and antioxidant activities in olive leaves. Depending on the harvest time, the highest concentrations of phenols, flavonoids and pigments were identified in Aberkane olive leaves collected in summer. Generally, the contents of bioactive compounds and antioxidant activity values were relatively low in winter and higher in leaves collected in summer. The study demonstrates the strong correlation between phenols and flavonoids with DPPH scavenging and FRAP assays. Additionally, studies will be undertaken to identify specific compounds, aiming to clarify these antioxidant mechanisms. Moreover, conducting further in vivo studies will be crucial to assess the antioxidant capacity of olive leaf extract.

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References

Aramrueang, N., Asavasanti, S., & Khanunthong A. (2019). Leafy Vegetables. In Integrated Processing Technologies for Food and Agricultural By-Products; Pan, Z., Zhang, R., Zicari, S., Eds.; Elsevier Inc.: Amsterdam, The Netherlands, 245–272.

Arslan, D., Karabekir, Y., & Schreiner, M. (2013). Variations of phenolic compounds, fatty acids and some qualitative characteristics of Sarıulak olive oil as induced by growing area. *Food Research International*, *54*(2), 1897-1906. https://doi.org/10.1016/j.foodres.2013.06.016

Bahloul, N., Kechaou, N., & Mihoubi, N. B. (2014). Comparative investigation of minerals, chlorophylls contents, fatty acid composition and thermal profiles of olive leaves (Olea europeae L.) as by-product. *Grasas y Aceites*, *65*(3), e035-e035.

Benjeddou, H., Ahmed, C. B., & Rouina, B. B. (2019). Influence of antioxidative enzymes, phytohormones and pigments in alternate bearing of three olive cultivars. *Scientia Horticulturae*, *253*, 17-23. https://doi.org/10.1016/j.scienta.2019.04.036

Brahmi, F., Mechri, B., Dabbou, S., Dhibi, M., & Hammami, M. (2012). The efficacy of phenolics compounds with different polarities as antioxidants from olive leaves depending on seasonal variations. *Industrial Crops and Products*, *38*, 146-152. https://doi.org/10.1016/j.indcrop.2012.01.023

Bulda, O. V., Rassadina, V. V., Alekseichuk, H. N., & Laman, N. A. (2008). Spectrophotometric measurement of carotenes, xanthophylls, and chlorophylls in extracts from plant seeds. *Russian Journal of Plant Physiology*, *55*, 544-551.'

Burits, M., & Bucar, F. (2000). Antioxidant activity of Nigella sativa essential oil. *Phytotherapy research*, *14*(5), 323-328.

Carchi, J. A. Y. ., Catagua, T. C. M. ., Rivera, D. G. B. ., Mera, V. B. ., & Rosario, M. del . (2021). From beginner to expert, experience of the rotating nursing intern in pre-professional practice. *International Journal of Health Sciences*, *5*(2), 111–117. https://doi.org/10.29332/ijhs.v5n2.1291

Casida, J. E. (2009). Pest toxicology: the primary mechanisms of pesticide action. *Chemical research in toxicology*, *22*(4), 609-619.

Chinnici, F., Bendini, A., Gaiani, A., & Riponi, C. (2004). Radical scavenging activities of peels and pulps from cv. Golden Delicious apples as related to their phenolic composition. *Journal of Agricultural and food chemistry*, *52*(15), 4684-4689.

Domonkos, I., Kis, M., Gombos, Z., & Ughy, B. (2013). Carotenoids, versatile components of oxygenic photosynthesis. *Progress in lipid research*, *52*(4), 539-561. https://doi.org/10.1016/j.plipres.2013.07.001

Garcia-Vaquero, M., & Rajauria, G. (2018). Analytical techniques for phytochemical estimation in fruit juices. In *Fruit Juices* (pp. 669-692). Academic Press. https://doi.org/10.1016/B978-0-12-802230-6.00033-3

Goulas, V., Papoti, V. T., Exarchou, V., Tsimidou, M. Z., & Gerothanassis, I. P. (2010). Contribution of flavonoids to the overall radical scavenging activity of olive (Olea europaea L.) leaf polar extracts. *Journal of Agricultural and Food Chemistry*, *58*(6), 3303-3308.

Gullon, B., Eibes, G., Moreira, M. T., Herrera, R., Labidi, J., & Gullon, P. (2018). Yerba mate waste: A sustainable resource of antioxidant compounds. *Industrial crops and products*, *113*, 398-405. https://doi.org/10.1016/j.indcrop.2018.01.064

Halliwel, B. (1992). Free radicals, antioxidants and human disease. Where are we now?. *J Lab Clin Med*, *119*, 598-620.

Hayes, J. E., Allen, P., Brunton, N., O'grady, M. N., & Kerry, J. P. (2011). Phenolic composition and in vitro antioxidant capacity of four commercial phytochemical products: Olive leaf extract (Olea europaea L.), lutein, sesamol and ellagic acid. *Food Chemistry*, *126*(3), 948-955. https://doi.org/10.1016/j.foodchem.2010.11.092

Indrasti, D., Andarwulan, N., Purnomo, E. H., & Wulandari, N. U. R. (2018). Stability of chlorophyll as natural colorant: A review for suji (Dracaena Angustifolia Roxb.) leaves' case. *Current Research in Nutrition and Food Science Journal*, *6*(3), 609-625.

Jain, D. P., Pancholi, S. S., & Patel, R. (2011). Synergistic antioxidant activity of green tea with some herbs. *Journal of advanced pharmaceutical technology & research*, *2*(3), 177.

Khaliq, A., Sabir, S. M., Ahmad, S. D., Boligon, A. A., Athayde, M. L., Jabbar, A., ... & Khan, A. (2015). Antioxidant activities and phenolic composition of Olive (Olea europaea) leaves. *J Appl Bot Food Qual*, *88*, 16-21.

Lanfer-Marquez, U. M., Barros, R. M., & Sinnecker, P. (2005). Antioxidant activity of chlorophylls and their derivatives. *Food research international*, *38*(8-9), 885-891. https://doi.org/10.1016/j.foodres.2005.02.012

Lee, O. H., & Lee, B. Y. (2010). Antioxidant and antimicrobial activities of individual and combined phenolics in Olea europaea leaf extract. *Bioresource technology*, *101*(10), 3751-3754. https://doi.org/10.1016/j.biortech.2009.12.052

Medina, E., Romero, C., Brenes, M., García, P., de Castro, A., & García, A. (2008). Profile of anti-lactic acid bacteria compounds during the storage of olives which are not treated with alkali. *European Food Research and Technology*, *228*, 133-138.

Middleton Jr, E. (1998). Effect of plant flavonoids on immune and inflammatory cell function. *Flavonoids in the living system*, 175-182.

Mokrani, A., & Madani, K. (2016). Effect of solvent, time and temperature on the extraction of phenolic compounds and antioxidant capacity of peach (Prunus persica L.) fruit. *Separation and Purification Technology*, *162*, 68-76. https://doi.org/10.1016/j.seppur.2016.01.043

Moudache, M., Colon, M., Nerín, C., & Zaidi, F. (2016). Phenolic content and antioxidant activity of olive by-products and antioxidant film containing olive leaf extract. *Food Chemistry*, *212*, 521-527. https://doi.org/10.1016/j.foodchem.2016.06.001

Nagata, M., & Yamashita, I. (1992). Simple method for simultaneous determination of chlorophyll and carotenoids in tomato fruit. *Nippon shokuhin kogyo gakkaishi*, *39*(10), 925-928.

Oyaizu, M. (1986). Studies on products of browning reaction antioxidative activities of products of browning reaction prepared from glucosamine. *The Japanese journal of nutrition and dietetics*, *44*(6), 307-315.

Papoti, V. T., Papageorgiou, M., Dervisi, K., Alexopoulos, E., Apostolidis, K., & Petridis, D. (2018). Screening olive leaves from unexploited traditional Greek cultivars for their phenolic antioxidant dynamic. *Foods*, *7*(12), 197.

Rahmanian, N., Jafari, S. M., & Wani, T. A. (2015). Bioactive profile, dehydration, extraction and application of the bioactive components of olive leaves. *Trends in Food Science & Technology*, *42*(2), 150-172. https://doi.org/10.1016/j.tifs.2014.12.009

Rietjens, S. J., Bast, A., & Haenen, G. R. (2007). New insights into controversies on the antioxidant potential of the olive oil antioxidant hydroxytyrosol. *Journal of agricultural and food chemistry*, *55*(18), 7609-7614.

Romero, C., Medina, E., Mateo, M. A., & Brenes, M. (2017). Quantification of bioactive compounds in Picual and Arbequina olive leaves and fruit. *Journal of the Science of Food and Agriculture*, *97*(6), 1725-1732.

Sarbishegi, M., Gorgich, E. A. C., & Khajavi, O. (2017). Olive leaves extract improved sperm quality and antioxidant status in the testis of rat exposed to rotenone. *Nephro-Urology Monthly*, *9*(3).

Sass-Kiss, A., Kiss, J., Milotay, P., Kerek, M. M., & Toth-Markus, M. (2005). Differences in anthocyanin and carotenoid content of fruits and vegetables. *Food Research International*, *38*(8-9), 1023-1029. https://doi.org/10.1016/j.foodres.2005.03.014

Schoefs, B. (2003). Chlorophyll and carotenoid analysis in food products. A practical case-by-case view. *TrAC Trends in Analytical Chemistry*, *22*(6), 335-339. https://doi.org/10.1016/S0165-9936(03)00602-2

Sevim, D., & Tuncay, Ö. (2012). Ayvalik ve Memecik zeytin Çeşitlerinin yaprağı ve meyvelerinin toplam fenolik madde miktarı ve antioksidan Aktiviteleri. *Gıda*, *37*(4), 219-226.

Shahidi, F. (Ed.). (1997). *Natural antioxidants: chemistry, health effects, and applications*. The American Oil Chemists Society.

Singleton, V. L., & Rossi, J. A. (1965). Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. *American journal of Enology and Viticulture*, *16*(3), 144-158.

Somogyi, A., Rosta, K., Pusztai, P., Tulassay, Z., & Nagy, G. (2007). Antioxidant measurements. *Physiological measurement*, *28*(4), R41.

Sudhakar, P., Latha, P., & Reddy, P. V. (2016). *Phenotyping crop plants for physiological and biochemical traits*. Academic Press.

Talhaoui, N., Taamalli, A., Gómez-Caravaca, A. M., Fernández-Gutiérrez, A., & Segura-Carretero, A. (2015). Phenolic compounds in olive leaves: Analytical determination, biotic and abiotic influence, and health benefits. *Food Research International*, *77*, 92-108. https://doi.org/10.1016/j.foodres.2015.09.011

Tarchoune, I., Sgherri, C., Eddouzi, J., Zinnai, A., Quartacci, M. F., & Zarrouk, M. (2019). Olive leaf addition increases olive oil nutraceutical properties. *Molecules*, *24*(3), 545.

Trees, C. C., Clark, D. K., Bidigare, R. R., Ondrusek, M. E., & Mueller, J. L. (2000). Accessory pigments versus chlorophyll a concentrations within the euphotic zone: A ubiquitous relationship. *Limnology and Oceanography*, *45*(5), 1130-1143.

Wainstein, J., Ganz, T., Boaz, M., Bar Dayan, Y., Dolev, E., Kerem, Z., & Madar, Z. (2012). Olive leaf extract as a hypoglycemic agent in both human diabetic subjects and in rats. *Journal of medicinal food*, *15*(7), 605-610.

Wang, X., Li, C., Liu, Y., Li, H., & Di, D. (2011). Efficient method for screening and identification of radical scavengers in the leaves of Olea europaea L. *Biomedical Chromatography*, *25*(3), 373-380.

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