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## Bioactive compounds and antioxidant activity of leaves from olive trees grown in Paraná, Brazil









**Abstract** – The objective of this work was to evaluate the bromatological composition, bioactive compounds, antioxidant activities, and color of the leaves of olive cultivars Arbosana, Arbequina, Frantoio, Manzanilla, and Koroneiki in northwestern Paraná, Brazil. Leaves of each cultivar were collected for the experiment, which was carried out in a completely randomized design. The dry matter (DM) of olive leaves contains mostly carbohydrates (56.15 to 59.42 g 100 g<sup>-1</sup> DM) and fibers (17.37 to 19.73 g 100 g<sup>-1</sup> DM) in its bromatological composition. The total polyphenol content ranges from 13.27 to 22.81 mg GAE g<sup>-1</sup>, with cultivar Manzanilla standing out, and the flavonoid content from 6.50 to 7.65 mg QE g<sup>-1</sup>. Manzanilla shows the highest antioxidant activity of 93.56 and 78.15% in the DPPH and ABTS assays, respectively. When compared with the other cultivars, Manzanilla and Koroneiki have leaves with a higher green intensity and total chlorophyll content of 0.958 and 0.833 mg 100 mL<sup>-1</sup>, respectively. In the ABTS assay, there is a correlation both between total polyphenol content and antioxidant activity and between chlorophyll content and antioxidant activity. Olive leaves have characteristics that allow their application as an additive or ingredient for the development of food products with satisfactory antioxidant activity.

**Index terms:** *Olea europaea*, Koroneiki, Manzanilla, phenolic compounds.

### Compostos bioativos e atividade antioxidante de folhas de oliveiras cultivadas no Paraná, Brasil

**Resumo** – O objetivo deste trabalho foi avaliar a composição bromatológica, os compostos bioativos, as atividades antioxidantes e a cor das folhas das cultivares de oliveira Arbosana, Arbequina, Frantoio, Manzanilla e Koroneiki no noroeste do Paraná, Brasil. Folhas de cada cultivar foram coletadas para o experimento, realizado em delineamento inteiramente casualizado. A matéria seca (MS) das folhas de oliveiras contém majoritariamente carboidratos (56,15 a 59,42 g 100 g<sup>-1</sup> de MS) e fibras (17,37 a 19,73 g 100 g<sup>-1</sup> de MS) em sua composição bromatológica. O teor de polifenóis totais varia de 13,27 a 22,81 mg EAG g<sup>-1</sup>, com destaque para a cultivar Manzanilla, e o conteúdo de flavonoides de 6,50 a 7,65 mg EQ g<sup>-1</sup>. Manzanilla apresenta a maior atividade antioxidante de 93,56 e 78,15% nos ensaios DPPH e ABTS, respectivamente. Em comparação às demais cultivares, Manzanilla e Koroneiki apresentam folhas com maior intensidade de verde e teor de clorofila total de 0,958 e 0,833 mg 100 mL<sup>-1</sup>, respectivamente. No ensaio ABTS, há correlação tanto entre o teor de polifenóis totais e a atividade antioxidante quanto entre o teor de clorofila e a atividade antioxidante. As folhas de oliveira apresentam características que permitem sua aplicação como aditivo ou ingrediente para o desenvolvimento de produtos alimentícios com atividade antioxidante satisfatória.

**Termos para indexação:** *Olea europaea*, Koroneiki, Manzanilla, compostos fenólicos.

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## Introduction

Olive (*Olea europaea* L.) is a fruit tree of the botanical family Oleaceae, typical of the Mediterranean, but also found in many regions of the world such as the Southern and Southeastern regions of Brazil (Cavalheiro et al., 2015; Rosa et al., 2021).

The two main products of this plant are olive oil and table olives, with an estimated global production of about 2.66 million tons of table olives and 3.01 million tons of olive oil in 2020/2021 (IOC, 2022). Spain is presently the world's largest olive oil producer, and cultivars Picual, Royal, and Arbequina are the main ones exploited commercially (Lama-Muñoz et al., 2020). In Brazil, Arbequina is the most popular cultivar (Martiny et al., 2020).

The olive oil industry generates several by-products including olive pomace, waste water and olive leaves, represented by a mixture of leaves and branches obtained from olive tree pruning, harvesting, and cleaning (Özcan & Matthäus, 2017; Souilem et al., 2017). The leaves constitute around 25% of the dry weight of the total pruning residues (Espeso et al., 2021) and are either used in animal feed and biomass production (Brahmi et al., 2012; Orak et al., 2019) or burned with the excess branches gathered from pruning (Özcan & Matthäus, 2017), causing an environmental problem.

Currently, the extraction of bioactive compounds from olive leaves has gained interest due to their therapeutic effects. Olive leaves are rich in phenolic compounds that exhibit a wide range of biological activities, including antioxidant, antimicrobial, antihypertensive, anti-inflammatory, and cardioprotective capacities, which have beneficial effects on human health, lowering cholesterol and protecting against carcinogenesis, diabetes, and atherosclerosis, for example (Nunes et al., 2016; Özcan & Matthäus, 2017; Khemakhem et al., 2018; Orak et al., 2019). Among the phenolic compounds present in olive leaves, the most common is oleuropein, but hydroxytyrosol, verbascoside, apigenin-7-glucoside, and luteolin-7-glucoside are found in olive leaf extracts (Özcan & Matthäus, 2017).

Therefore, carrying out researches to find new uses for olive by-products, mainly due to the presence of bioactive compounds, is important not only to the economy, but also to the environment and human health (Nunes et al., 2016; Rubel et al., 2021). The phenolic

compounds extracted from olive leaves are generally used in the production of medicine, cosmetics, and pharmaceutical products (Khemakhem et al., 2018; Orak et al., 2019). Recently, these compounds have also been applied in the food industry to improve nutritional profile, increase chemical stability against lipid oxidation, and stabilize foams and emulsions (Espeso et al., 2021), as well as to preserve foods and extend the shelf-life of edible oils, meat products, and milk derivatives (Souilem et al., 2017; Orak et al., 2019; Rubel et al., 2021).

In Bangladesh, Rubel et al. (2021), for example, found that the antioxidant and antimicrobial properties of olive leaves extended the shelf life of mutton meatball, whereas, in Spain, Pérez-Gálvez et al., (2020) concluded that the leaves of this species can be used as a natural pigment for food coloring.

In Brazil, Martiny et al. (2020) used olive leaves as a bioactive agent in packaging to extend lamb meat shelf life. Specifically in the states of Rio Grande do Sul and Santa Catarina, the studies were carried out using fruit-bearing trees (Cavalheiro et al., 2015; Antunes et al., 2021; Martiny et al., 2021; Rosa et al., 2021), considering the effects of edaphoclimatic conditions and olive tree genotype on the chemical composition of the leaves (Orak et al., 2019; Özcan & Matthäus, 2017). The hypothesis of the present study is that the leaves of non-bearing olive trees have chemical composition, bioactive compounds, antioxidant activity, and antimicrobial properties that are similar or superior to those of yielding trees.

The objective of this work was to evaluate the bromatological composition, bioactive compounds, antioxidant activities, and color of the leaves of olive cultivars Arbequina, Arbosana, Frantoio, Manzanilla and Koroneiki in northwestern Paraná, Brazil.

## Materials and Methods

The evaluated leaves were collected from non-bearing trees of the five studied olive cultivars (Arbosana, Arbequina, Frantoio, Manzanilla, and Koroneiki) in February 2021, in Unidade de Difusão Tecnológica of Cocamar, located in the northwest of the state of Paraná, Brazil (23°35'45"S, 52°03'54"W, at 408 m altitude). The experiment was carried out in a completely randomized design. For each cultivar, about 2 kg of leaves were collected from different parts of

four trees with an average age of 7 years. Of the total samples, three trees of each cultivar were considered true samples and analyzed in three replications.

Olive leaves were dried at 55°C for 24 hours until constant mass was reached; it was then crushed, sieved at 60 mesh, and stored in vials protected from light, under refrigeration at 4°C for 3–5 days. The olive leaves powder was analyzed for the content of carbohydrates, protein, lipids, fiber, ashes, and moisture (Horwitz, 2005).

The extraction of phenolic compounds from olive leaves powder was carried out with 100% methanol in a proportion of 1:1000 (m/v), in a vortex mixer for one minute, followed by homogenization for 10 min and final centrifugation (3,000 rpm/15 min). The supernatant was used for analyses. Total polyphenols content (TPC) was determined by Folin-Ciocalteu method (Singleton & Rossi, 1965). An extract was mixed with Folin-Ciocalteu reagent and sodium carbonate and incubated in dark for 30 min. The reading was performed by spectrophotometer model IL-226-NM (Kasuki, Japan) at 725 nm and compared to a standard curve of gallic acid. Results were expressed by mg gallic acid equivalent (GAE) per g.

Total flavonoids content (TFC) was determined following Buriol et al. (2009). An extract was mixed with aluminum chloride and methanol and left to rest in dark for 30 min. The reading was carried out in a spectrophotometer at 425 nm and the absorbance was compared to a standard curve of quercetin. Results were expressed in mg quercetin equivalent (QE) per g.

The antioxidant activities of olive leaves powder were performed with 2,2-Diphenyl-1-Picrylhydrazyl (DPPH) free radical scavenging and with 2,2'-azino-bis (3-ethylbenzothiazoline) 6-sulfonic acid (ABTS) free radical scavenging in percentage. DPPH solution (2.85 ml; 60 µM) was mixed with an aliquot of extract (150 µl) and incubated (30 min) in the dark; and the absorbance was measured at 515 nm according to Li et al. (2009). ABTS<sup>+</sup> solution (1,960 µl) was mixed with an aliquot (40 µl) of supernatant according to Re et al. (1999) and the absorbance was measured after 6 min by a spectrophotometer at 734 nm.

The color was determined in olive leaves powder in three distinct points of the samples using a CR400 colorimeter (Konica Minolta, China) by a CIELAB color scale. The CIELAB color space is a color space defined by the International Commission on

Illumination in 1976 and expresses color as three values: L\* for perceptual luminosity (100 ¼ white; 0 ¼ black), a\* for red and green (+red; -green) and b\* for yellow and blue (+yellow; -blue). Chlorophyll A, chlorophyll B, and β-carotene were determined in olive leaves powder by pigments extraction with acetone-hexane in 4:6 ratio, mixed with 1 g of sample. Absorbance was measured at 663, 645, 505, and 459 nm by spectrophotometer (Nagata & Yamashita, 1992). Total chlorophyll was calculated by the sum of chlorophyll A and chlorophyll B.

The results were submitted to analysis of variance using statistical program SPSS (v.15.0) (IBM SPSS Statistics, SPSS Inc., Chicago, USA) from Windows. The results were expressed as mean ± standard deviation. When significant differences were found, a Tukey test was performed at 5% probability. Principal component analysis (PCA) was performed to find relationships between different parameters: Arbosana (AS), Arbequina (AB), Frantoio (F), Manzanilla (M), Koroneiki (K), total polyphenol content (TPC), total flavonoid content (TFC), antioxidant activity (DPPH and ABTS assay), chlorophyll A (AC), chlorophyll B (BC), total chlorophyll (TC), carotenoids, and color (L\*, a\*, b\*) to detect possible clusters within variables. Pearson's correlation analysis was performed for all the cultivars and variables TPC, TFC, DPPH, ABTS, TC, and carotenoids.

## Results and Discussion

Olive leaves stand out for their carbohydrate content and fibers (Table 1). The main carbohydrates found in olive leaves are mannitol, glucose, oligosaccharides, and fibers including cellulose, hemicellulose, and lignin (Souilem et al., 2017; Khemakhem et al., 2018). There is not difference between olive cultivars for protein, fiber, and carbohydrates content.

The lipid content varied between cultivars: the Arbequina had the lowest content, and the Manzanilla had the highest content. The fatty acids found in olive leaves are oleic and linolenic acid (Nunes et al., 2016). Other studies were carried out regarding olive leaves bromatological composition and the results reveal higher levels of lipids than those found in this work (Cavalheiro et al., 2015; Antunes et al., 2021).

The Frantoio and Koroneiki cultivars had the highest ash content. Some minerals like copper, zinc,

and selenium can support antioxidant activity of the plants. The main minerals present in olive leaves are potassium, manganese, magnesium, iron, and copper (Nunes et al., 2016; Souilem et al., 2017). Antunes et al. (2021) studied the same cultivars that were grown in this work in the state of Rio Grande do Sul, Brazil, in 2019. The ash content was lower than that of this study and varied from 3.00% to 4.38%, in which the highest one was of the Koroneiki cultivar.

The chemical composition of plants is one of the parameters used to measure their nutritional value, and several factors may influence the chemical composition of dried olive leaves such as olive variety, climate conditions, tree age and wood proportion (Cavalheiro et al., 2015).

Phenolic compounds are plant secondary metabolites known for their diverse biological activities (Rajha et al., 2022). Olive leaves contain a large variety of phenolic derivatives, and consist of simple phenols, flavonoids (flavones, flavanones, flavonols, 3-flavanols), and secoiridoids (Özcan & Matthäus, 2017). The total polyphenol content of olive

leaves (dry matter) ranges from 13.27 to 22.81 mg GAE g<sup>-1</sup>, and Manzanilla cultivar showed the highest value (Table 2). These values are above those found by Antunes et al. (2021), who studied the same cultivars as those of this work, in the state of Rio Grande do Sul, Brazil, with TPC in olive leaves ranging from 6.51 to 10.88 mg EAG g<sup>-1</sup>, and the lowest contents were of the Manzanilla and Arbequina cultivars.

A lot of factors can influence the TPC in olive leaves such as date of collection, drying conditions, cultivation zone, extraction procedure, storage conditions, climatic conditions, bromatological composition (Özcan & Matthäus, 2017; Orak et al., 2019). About the extraction method, the solvent used influences the extraction of bioactive compounds. Brahmi et al. (2012) state that the total polyphenol and flavonoid contents are higher in methanol extract than in hexane, chloroform, and ethyl acetate. Eco-friendly extraction methods are suitable for the extraction of bioactive compounds from olive leaves (Rosa et al., 2021). Martiny et al. (2021) evaluated the total polyphenol content of Arbequina cultivar leaves

**Table 1.** Bromatological composition, on a dry matter basis, of the leaves of olive (*Olea europaea*) tree cultivars grown in the state of Paraná, Brazil<sup>(1)</sup>.

Chemical composition	Cultivars				
	Arbosana	Arbequina	Frantoio	Manzanilla	Koroneiki
Moisture (%)	5.32±0.17AB	5.38±0.23AB	5.04±0.37AB	5.66±0.22A	4.76±0.23B
Protein (g 100 g <sup>-1</sup> DM)	11.16±0.59A	11.82±0.76A	10.52±1.52A	10.64±0.40A	11.76±0.20A
Lipids (g 100 g <sup>-1</sup> DM)	1.88±0.07B	1.59±0.04C	1.92±0.13B	2.42±0.01A	1.86±0.11B
Fiber (g 100 g <sup>-1</sup> DM)	17.37±2.61A	18.00±0.50A	19.73±1.48A	17.23±1.03A	18.84±0.08A
Carbohydrates (g 100 g <sup>-1</sup> DM)	59.42±1.36A	58.08±0.66A	56.15±2.27A	59.03±0.73A	56.58±0.59A
Ashes (g 100 g <sup>-1</sup> DM)	4.83±0.61B	5.10±0.45B	6.62±0.06A	5.00±0.60B	6.18±0.26A

<sup>(1)</sup>Means followed by equal letters, in the same line, do not differ by the Tukey test at 5% probability. Results are expressed as mean±standard deviation.

**Table 2.** Bioactive compounds and antioxidant activity, on a dry matter basis, of the leaves of olive (*Olea europaea*) tree cultivars grown in the state of Paraná, Brazil<sup>(1)</sup>.

Analysis	Cultivars				
	Arbosana	Arbequina	Frantoio	Manzanilla	Koroneiki
Bioactive compounds <sup>(2)</sup>					
TPC (mg GAE g <sup>-1</sup> )	14.82±0.57B	14.57±1.63B	13.27±1.30B	22.81±3.61A	17.20±1.48B
TFC (mg QE g <sup>-1</sup> )	7.23±0.95A	7.22±0.12A	6.50±0.05A	7.13±0.36A	7.65±0.07A
Antioxidant activity <sup>(3)</sup>					
DPPH (%)	89.56±2.83AB	91.38±2.77AB	82.90±6.96B	93.56±0.48A	86.80±3.31AB
ABTS (%)	57.43±2.23B	62.27±3.00B	57.66±2.11B	78.15±7.56A	68.06±3.78AB

<sup>(1)</sup>Means followed by equal letters, in the same line, do not differ by the Tukey test at 5% probability. <sup>(2)</sup>TPC, total polyphenol content; and TFC, total flavonoid content. <sup>(3)</sup>DPPH, 2,2-Diphenyl-1-picrylhydrazyl assay; and ABTS, 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) assay. Results are expressed as mean±standard deviation.

grown in the state of Rio Grande do Sul, Brazil, using microwave-assisted extraction and the results showed higher TPC values than those found in this study.

The most important factors that differentiate the profile of phenolic compounds and the antioxidant activity of olive leaves is the genotype of olive tree (Özcan & Matthäus, 2017; Orak et al., 2019). Cavalheiro et al. (2015) studied five varieties of olive trees (Ascolano, Arbosana, Negrinha do Freixó, Koroneiki, and Grappolo) and found differences in chemical composition between the cultivars. Lama-Muñoz et al. (2020) also found differences in phenolic compound content of olive leaves of six cultivars grown in Spain.

Flavonoids constitute an important class of polyphenols. The most common flavonoids present in olive leaves are apigenin, kaempferol, and luteolin (Nunes et al., 2016). Total flavonoid content showed no statistical differences between cultivars. Although the Manzanilla cultivar stood out for its high content of total polyphenols in relation to the other cultivars studied, there was no difference between the cultivars for the total flavonoid content (Table 2).

The antioxidant activity of olive leaves powder from five cultivars determined by DPPH radical scavenging showed that extracts from leaves of Manzanilla cultivar had the greatest scavenging free radicals, which were 10.66% higher than those of Frantoio cultivar, which showed the lowest scavenging power (Table 2). The ABTS radical scavenging method showed greater antioxidant activity in Manzanilla cultivar when compared to other cultivars.

Antioxidant compounds are important for preserving food and protecting organisms against damage caused by free radicals. Several reports have

shown that olive leaves have antioxidant activity due mainly to oleuropein and hydroxytyrosol, which are strong free radical scavengers and perform metal-chelating activities (Khemakhem et al., 2018; Rubel et al., 2021). The presence of hydroxyl groups in oleuropein chemical structure, particularly the 1,2-dihydroxybenzene moiety, could donate hydrogen to prevent oxidation.

The olive leaves (dry matter) of the cultivars studied showed no difference in luminosity. The scale a\* showed greenish tendency for all the cultivars and Koroneiki cultivar exhibited the highest value. The scale b\* showed yellow tendency and Manzanilla had the greatest value (Table 3). Although the Manzanilla cultivar has a greater yellow color, the results show no difference in the analysis of  $\beta$ -carotene between cultivars.

Chlorophylls and carotenoids are the most abundant pigments in nature. Depending on their structure, they can be presented in colors: greenish-blue (chlorophyll A) or greenish-yellow (chlorophyll B) (Sharma et al., 2021). Chlorophylls and carotenoids extracted from plants are used in food products as natural pigments due to their color and physicochemical properties (De Mejia et al., 2020; Sharma et al., 2021).

In the present study, all the cultivars exhibited higher chlorophyll A content in the leaves compared with chlorophyll B content (Table 3). The Koroneiki, Manzanilla, and Arbequina cultivars presented higher chlorophyll A levels in relation to other cultivars. The total chlorophyll content ranged from 0.501 to 0.958 mg 100 mL<sup>-1</sup> and Manzanilla cultivar exhibited higher value than those of other cultivars. The total chlorophyll content can vary according to several factors, such as

**Table 3.** Color and pigments, on a dry matter basis, of the leaves of olive (*Olea europaea*) trees cultivars grown in the state of Paraná, Brazil<sup>(1)</sup>.

Analysis	Cultivars				
	Arbosana	Arbequina	Frantoio	Manzanilla	Koroneiki
L*( <sup>2</sup> )	52.51±1.46A	53.77±1.18A	52.93±0.24A	54.90±0.11A	55.40±2.11A
a*( <sup>3</sup> )	-4.4±0.20C	-5.47±0.13B	-4.65±0.05C	-5.76±0.04AB	-5.92±0.11A
b*( <sup>4</sup> )	14.87±0.90AB	15.05±0.46AB	14.08±0.22B	16.07±0.89A	15.20±0.29AB
$\beta$ -carotene	0.299±0.11A	0.313±0.01A	0.356±0.02A	0.256±0.02A	0.360±0.02A
Chlorophyll A	0.387±0.03C	0.604±0.09AB	0.551±0.04B	0.728±0.04A	0.718±0.01A
Chlorophyll B	0.113±0.07A	0.142±0.04A	0.129±0.03A	0.230±0.01A	0.115±0.01A
Total chlorophyll	0.501±1.02C	0.746±0.13B	0.680±0.08BC	0.958±0.04A	0.833±0.01AB

<sup>(1)</sup>Means followed by equal letters, in the same line, do not differ by the Tukey test at 5% probability. <sup>(2)</sup>L\*, 100 ¼ white; 0 ¼ black. <sup>(3)</sup>a\*, +red; -green. <sup>(4)</sup>b\*, +yellow; -blue. Pigments are expressed in mg 100 mL<sup>-1</sup>. Results are expressed as mean±standard deviation.

water, temperature, photoperiod, and the period when the leaves were harvested (Chen et al., 2011). The lack of water in the leaves both influences the synthesis of chlorophyll and accelerates the decomposition of chlorophyll, accelerating the yellowing of the leaves (Li et al., 2018). However, no relationship between leaf moisture and chlorophyll and  $\beta$ -carotene content was observed in this work.

Carotenoids give brilliant yellow and red colors to many fruits, vegetables, roots, flowers, and autumn leaves. Carotenoids impart yellow, orange, and red colors to foods and include:  $\beta$ -carotene,  $\alpha$ -carotene,  $\beta$ -cryptoxanthin, lycopene, lutein and zeaxanthin (Rodríguez-Amaya, 2019; De Mejia et al., 2020). There was no difference for  $\beta$ -carotene between cultivars (Table 3). The content of carotenoids and chlorophylls is influenced by leaf age (Brahmi et al., 2012). For the carotenoids to be evidenced, the plant needs to age, for the chlorophylls to degrade and consequently change color. These results show that due to their high chlorophyll content, olive leaves have potential use as natural food dye and research has been carried out in this regard (De Mejia et al., 2020; Pérez-Gálvez et al., 2020).

Principal Component Analysis (PCA) was used to graphically present the relationship between the variables – TPC, TFC, DPPH and ABTS assay, chlorophyll A, chlorophyll B, total chlorophyll,  $\beta$ -carotene, color – and the cultivars. PCA explained 85.89% of variables in two axes, P1 (65.49%) and P2 (20.40%). Arbequina, Manzanilla, and Koroneiki cultivars were found in the quadrants on the right, while Arbosana (AS) and Frantoio (F) cultivars appeared in the left quadrants (Figure 1).

Regarding the variables, total polyphenol content (TPC), total flavonoids content (TFC), DPPH, chlorophyll B and color  $b^*$  were found in the upper right quadrant, close to the Arbequina and Manzanilla cultivars, showing a strong relationship between them. The variables ABTS, chlorophyll A, total chlorophyll, and luminosity were found in the lower right quadrant, close to the Koroneiki cultivar. Color  $a^*$  and Arbosana cultivar were shown in the upper left quadrant while carotenoids and Frantoio cultivar were in the lower left quadrant, indicating relationship between them.

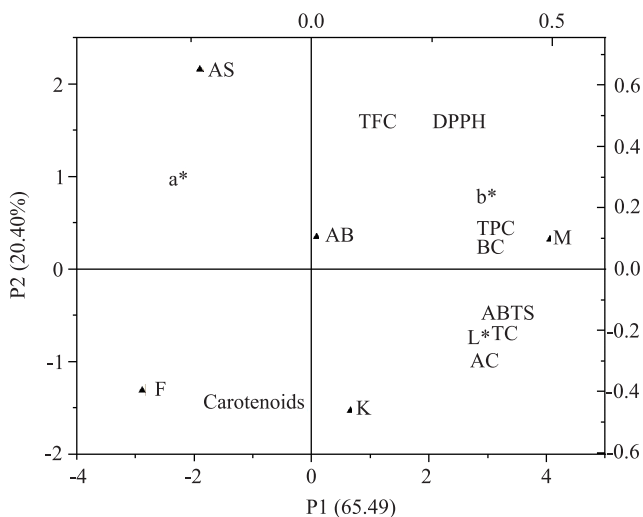
The data show the strong relationship between olive leaves secondary compounds, such as TPC and TFC, and the high antioxidant activity exhibited by

Manzanilla, Arbequina, and Koroneiki cultivars. There was a relationship between chlorophyll B and the Arbequina and Manzanilla cultivars; the content of chlorophyll A and total chlorophyll with the Koroneiki cultivar; and  $\beta$ -carotene with the Frantoio cultivar.

There was a positive correlation ( $r = 0.913$ ) between the total polyphenols content of the cultivars and their antioxidant activity by ABTS method. Olive leaves also exhibited a positive correlation ( $r = 0.904$ ) between total chlorophyll content and antioxidant activity by ABTS method (Table 4).

Although carotenoids and chlorophylls are pigments that are able to decrease the oxidation of other molecules (Pérez-Gálvez et al., 2020), there was not positive correlation between the antioxidant activity by the DPPH method and the  $\beta$ -carotene ( $r = -0.899$ ).

Olive leaves are considered a cheap source of phenolic compounds with high antioxidant capacity that has beneficial health properties (Özcan & Matthäus, 2017). The demand for olive leaves extract has increased for use in foodstuff, food additives, and functional food materials for human and animal feed.



**Figure 1.** Principal component analysis (PCA) of the leaves of the Arbosana (AS), Arbequina (AB), Frantoio (F), Manzanilla (M), and Koroneiki (K) olive (*Olea europaea*) trees cultivars. P1 and P2, first two principal components; TPC, total polyphenol content; TFC, total flavonoid content; DPPH and ABTS, assays to determine antioxidant activity; AC, chlorophyll A; BC, chlorophyll B; TC, total chlorophyll; and L\*,  $a^*$ , and  $b^*$ , colors.

**Table 4.** Pearson's correlation coefficient (r) between different antioxidant capacity assays (ABTS and DPPH) and total polyphenol content (TPC), total flavonoid content (TFC), total chlorophyll (TC), and  $\beta$ -carotene, on a dry matter basis, of the leaves of olive (*Olea europaea*) tree cultivars grown in the state of Paraná, Brazil.

Parameters	Pearson's correlation coefficient	
	ABTS (%)	DPPH (%)
TPC (mg GAE g <sup>-1</sup> )	0.913*	0.644
TFC (mg QE g <sup>-1</sup> )	0.549	0.050
TC (mg 100 mL <sup>-1</sup> )	0.904*	0.445
$\beta$ -carotene (mg 100 mL <sup>-1</sup> )	-0.484	-0.899*

\*Significant by Pearson's correlation test at 5% probability.

## Conclusions

1. Carbohydrates and fiber are major compounds in the dry matter of olive (*Olea europaea*) leaves from Arbosana, Arbequina, Frantoio, Manzanilla and Koroneiki cultivars; and there is not difference between cultivars for protein, fiber, and carbohydrates content.

2. Manzanilla cultivar stands out for its high content of total polyphenols and antioxidant activity.

3. Manzanilla and Koroneiki cultivar show greater green intensity and higher total chlorophyll content when compared to the other cultivars.

4. There is a correlation both between the total polyphenol content and antioxidant activity and between the chlorophyll content and the antioxidant activity by the ABTS assay.

5. Olive leaves have characteristics that allow their application as an additive or ingredient for the development of food products with satisfactory antioxidant activity.

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