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Extraction and Characterization of the Phenolic Compounds from Leaves of *Olea europaea* L. via PLE

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Oleuropein is the predominant phenolic compound in olive leaves. It presents high antioxidant and anti-inflammatory activities and has antimicrobial action. The objective of this study was to extract the phenolic compounds from dried olive leaves using pressurized ethanol in a continuous flow and characterize them. The flow rate (0.5 to 1.0 mL min-1), temperature (30 to 60 °C) and pressure (10 to 20 MPa) were the operational parameters. The results showed that the temperature was a significant factor in the yield of the extracts, with higher yields at the highest temperature. The oleuropein content in the extracts ranged from 80.5 to 82.9 mg g-1, and the extract presented different classes of compounds. The total phenolic components were in the range of 10.5 to 12.8 mg GAE g-1 of extract. The best condition to obtain an extract with the highest yield and total phenolic compounds, and suitable oleuropein content and antioxidant capacity was 60 °C, 10 MPa and 0.5 mL min-1.

* 1. Introduction

The olive tree (*Olea europaea* L.) is a fruit tree used for ornamental purposes and to produce olives. The production of the fruits is the main objective of its cultivation, aiming to obtain the olive oil as the final product. However, the olive leaves have essential components for human health and high polyphenol content (Erbay and Icier, 2010).

Oleuropein and its derivative, the hydroxytyrosol, are the predominant phenolic compounds in the olive leaves (Erbay and Icier, 2010, Solarte-Toro et al., 2018). Oleuropein is a bitter-tasting glycoside which accounts for 73% of the total leaf phenolic constituents (Pereira et al., 2007). It has high antioxidant and anti-inflammatory power (Al-Azzawie and Alhamdani, 2006), antimicrobial (Cicerale et al., 2012), antiviral (Lee-Huang et al., 2007), antitumor (Goulas et al., 2009), anticancer (Kimura and Sumiyoshi, 2009), and neuroprotective (Omar, 2010a) actions, beneficial effects for cardiovascular diseases (Bulotta et al., 2014), and other beneficial effects on human health.

Pressurized liquid extraction (PLE) employs GRAS (Generally Recognized as Safe) liquid solvents at high pressures and operating temperature below the boiling point to achieve fast and efficient extraction (Albarelli et al., 2016). PLE generally has a higher percentage of mass yield compared to conventional extraction techniques for the extraction of bioactive products with high polarity (Setyaningsih et al., 2016). Adjustments of the process parameters (temperature, pressure and solvent flow) may promote greater selectivity and increase the yield of the extracted compounds. These characteristics make PLE a clean alternative technology for the extraction of biocomposites of interest to the food, pharmaceutical and cosmetic industries.

Therefore, it is possible to obtain oleuropein by employing several new techniques with promising results like ultrasound (Cabrera-Bañegil et al., 2017) and extraction using homogenization with dimethyl sulfoxide (DMSO) and solid-phase extraction (Crawford et al., 2018). However, the PLE with pressurized ethanol and in a continuous flow has not been sufficiently explored. Thus, the main purpose of this study was to extract and characterize the phenolic compounds from olive leaves using pressurized ethanol in a continuous flow. The operating conditions evaluated were the temperature, pressure, and flow and the extracts were assessed for the contents of oleuropein, total phenolic compounds, and antioxidant activities. Furthermore, the phenolic compounds profile was also determined.

* 1. Materials and Methods

## 2.1 Raw material

The raw material used was from commercial cultivation of olive trees located in the mountain range of Mantiqueira, São Paulo (22º00’48,6” S 46º37’59,4” W), Brazil. The olive varieties used were Arbequina, Koroneiki, and Arbosana. The leaves were separated manually from the branches and dried in a greenhouse with forced air circulation (Lab Store, New Ethics, model: 400/4ND, power: 1580 W) at 35 ºC for approximately 36 h (until constant weight) The solvent used for extraction was absolute ethyl alcohol 99.5 GL (Nuclear, São Paulo, Brazil) without previous treatment. Moisture, ash, lipid, protein, crude fiber, and carbohydrate contents were determined according to the Association of Official Analytical Chemists guidelines. All analyzes were performed in triplicates.

## 2.2 Extraction with pressurized fluid

The extractions were conducted using a 2³ factorial design with triplicates at the central point. Table 1 shows the factors and levels used in the factorial design for extraction using pressurized ethanol.

*Table 1: Factors and levels of factorial design 23 with triplicates at the central point for the extraction from olive leaves using pressurized ethanol*

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Factors** | **Units** | **Levels** | | |
| **(-1)** | **(0)** | **(1)** |
| Temperature | °C | 30 | 45 | 60 |
| Pressure | MPa | 10 | 15 | 20 |
| Flow rate | mL min-1 | 0.5 | 0.75 | 1.0 |

Initially, 4 g of the dried and ground leaves were added to the extractor vessel. Then, the extraction system was filled with ethanol under the conditions of temperature, pressure and flow rate proposed in Table 1. The kinetic extraction was of 300 min for each condition. The obtained ethanolic extracts were evaporated in a circulating air oven at 55 ºC until constant weight. The mass yield (%) of the dry extracts was determined using an analytical balance (Denver Instrument, model: APX-200).

**2.3 Analysis of oleuropein content in the extracts**

For the analysis of the oleuropein content, extractions were made for all the experimental conditions described in Table 1. The ethanol was evaporated, and the extracts were analyzed. Analyzes were performed on High-Performance Liquid Chromatography (HPLC) using a C-18 column. The conditions for analysis were: mobile phase: methanol: water (50:50) using methanol with 1.0 μg μL-1 concentration, flow rate: 1 mL min-1, wavelength: 280 nm, oven temperature: 30 °C and injected volume: 5 μL. The oleuropein content was obtained using a five-point analytical curve of oleuropein (0.125–0.75 mg. mL-1). The analytical curve was linear (R2 = 0.985). The analysis was conducted in triplicate.

**2.4 Determination of total phenolic components content and DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging assay**

The Folin-Ciocalteu method was used to determine the total phenolic content present in the samples (Georgé et al., 2005). The content of phenolic compounds was expressed in milligrams of gallic acid equivalents per gram of extract (mg GAE g-1 de extract). The antioxidant activity of the extracts was performed using the free radical scavenging method DPPH, according to the methodology proposed by Liyana-Pathirana and Shahidi, (2005). The concentration of the sample with a 50% reduction in DPPH (IC50) was calculated from the graph equation, consisting in the percentage of antioxidant activity versus concentration in μg mL-1.

**2.5 Characterization of the extracts by HPLC–DAD–MSn Analysis**

The extract of olive leaves obtained under conditions of 60 °C, 10 MPa and flow rate of 0.5 mL.min-1, which was the condition with the best result in the factorial design,was suspended in a methanol/formic acid mixture (99.5: 0.5 (v / v)) (8 mg of extract mL-1) and centrifuged at 3000 g for 5 min at 4 °C. The supernatant was filtered through 0.22 μm membranes (Millipore), and 5 μL was injected into the HPLC-DAD-MSn apparatus.

A Shimadzu HPLC apparatus equipped with quaternary pumps (LC-20AD), a degasser (DGU-20A3R), and an injection valve (SIL-20A HT) with a 20 μL loop, connected in series to a DAD detector (SPD-M20A) and a mass spectrometer with an ion trap analyzer and an electrospray ionization (ESI) source (model AmaZon Speed, Bruker Daltonics) was used to assess the phenolic compounds. The phenolic compounds were separated on a C18 Synergi HydroRP column (4 μm, 250 mm × 4.6 mm, Phenomenex) at a flow rate of 0.7 mL.min-1 and a column temperature of 30 °C, using a mobile phase consisting of mixtures of water/formic acid [99.5:0.5 (v/v)] (solvent A) and methanol/formic acid [99.5:0.5 (v/v)] (solvent B) in an isocratic gradient from 40:60 (v/v) A/B for 30 min. The column eluate was split to allow only around 0.15 mL.min-1 to enter the ESI interface. The UV–VIS spectra were obtained between 200 and 600 nm, and the chromatograms were processed at 280 nm. The mass spectra were acquired with a scan range from m/z 100 to 800. The MS parameters were set as follows: ESI source operating in negative ion mode; dry gas (N2) temperature of 310 °C; flow rate of 8 L min-1 and nebulizer gas with 30 psi. MS2 and MS3 were set in manual mode applying fragmentation energy of 1.6 V. The phenolic compounds were identified based on the following information: elution order and retention time in the reversed phase column, UV–VIS and MS spectra features as compared to standards analyzed under the same conditions, and data available in the literature.

* 1. Results and Discussion

**3.1 Physicochemical characterization of the raw material**

Table 2 shows the moisture, ash, lipid, protein, crude fiber, and carbohydrate contents of the dried olive leaves obtained in the present study and those found in the literature.

Table 2: Physicochemical characterization of the olive leaves used in the experiments (in dry mass).

|  |  |  |
| --- | --- | --- |
| **Component (%)** | **Present study** | **Literature\*** |
| Moisture | 5.8 ± 0.05 | 4.8 |
| Ash | 5.6 ± 0.2 | 11.9 |
| Lipids | 9.0 ± 0.7 | 3.7 |
| Protein | 13.0 ± 0.5 | 10.8 |
| Crude fiber | 3.4 ± 0.3 | 14.5 |
| Carbohydrate | 63.1 | 59.0 |

The physicochemical characterization of the olive leaves demonstrates the similarity between the raw material used in this study and the one used in other studies available in the literature\* (Cavalheiro et al., 2014; Coppa et al., 2017). The moisture content directly influences the extraction process by limiting solvent penetration into the matrix pores and reducing mass transfer. Thus, reduced values of moisture content are essential. Therefore, it is advised to use a dry material in the extraction with pressurized liquid (Moret and Conte, 2014).

The olive leaves from cultivars such as Arbequina that are cultivated in the south of Brazil have an interesting chemical composition compared to varieties cultivated in other countries, due to their high protein content. The contents of ash (4.4%), proteins (12.2%) and lipids (8.1%) obtained by (Cavalheiro et al., 2014) when evaluating olive leaves produced in Brazil, in the region of Caçapava do Sul, RS, were similar to the results obtained in this study.

**3.2 Extraction with pressurized liquid**

The experimental design defined to obtain the kinetic extraction curves is shown in Table 3. It is observed that the highest extraction occurred up to approximately 70 min, after this period, the diffusion is predominant. The values of mass percentage yields ranged from 7.2 to 15.1% and are compatible with the yield values obtained by several authors. The temperature is the operational parameter with the highest positive effect in the extraction, providing a higher yield of the extract (14.5 to 15.1%) in the experiments with higher temperatures (60 oC), independently of the pressure and solvent flow rate. In this condition, it can be demonstrated that it is possible to use the lowest solvent flow rate and pressure aiming solvent and energy saving. This can be explained by the fact that temperature is a parameter of significant influence in a PLE process, being able to improve the extraction efficiency by disturbing the dipole and hydrogen bond interactions between the analyte and the matrix, decreasing the activation energy required for the desorption, and decreasing the surface tension and viscosity of the solvent (Moreno et al., 2007).

In fact, the statistical analysis confirmed the significant influence of the temperature (p ≤ 0.05). The pressure did not present a statistically significant effect (p > 0.05). Generally, high pressure values allow the solvent to remain in the liquid form, even at temperatures above the boiling point and facilitate solvent penetration into the sample pores.

In the case of the present study, the temperatures used (30-60 oC) were below the boiling point of the ethanol (78 oC), with no significant influence of the pressure on the yield. The flow range used was not statistically significant to change the mass percentage yield (p > 0.05). The flow is directly related to mass transfer mechanisms and should be evaluated whenever possible (Moret and Conte, 2014). The significant effect of the curvature indicates that the quadratic terms of the independent parameters are essential. However, the linear regression model applied for the independent variables (Eq. 1) resulted in a linear correlation coefficient of 0.9959 and variance of 97.98% for the variable yield of olive leaf extract.

(1)

Where is the extract yield (%), and T is the temperature (°C).

Oleuropein yield values in the extracts were similar for all extraction conditions conducted (Table 3). Therefore, the effects of temperature, pressure, and ethanol flow were not statistically significant for the oleuropein content in the extracts (p> 0.05).

*Table 3: Yield of the extracts and oleuropein, phenolic compounds contents and antioxidant activity of olive leaf extracts obtained in the extraction with pressurized ethanol according to experimental design.*

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| Exp | T  (ºC) | P  (MPa) | F  (mL min-1) | YG  (w/w) % | YOLE  (mg g-1 extract) | Total phenolics (mg GAE g-1 extract) | IC50  (μg mL-1) |
| 1 | 30 | 10 | 0.5 | 7.9 | 86.6 | 10.5 ± 0.4 c | 36.1 ± 2.6 ab |
| 2 | 30 | 20 | 0.5 | 9.7 | 86.8 | 11.7 ± 0.4 abc | 36.3 ± 1.6 ab |
| 3 | 30 | 10 | 1.0 | 7.2 | 85.2 | 11.3 ± 0.4 abc | 32.2 ± 8.2 b |
| 4 | 30 | 20 | 1.0 | 7.7 | 86.9 | 11.2 ± 0.3 bc | 29.3 ± 2.6 b |
| 5 | 45 | 15 | 0.75 | 9.7 ± 0.5 | 85.5 ± 0.2 | 11.8 ± 0.4 abc | 46.4 ± 1.3 a |
| 6 | 60 | 10 | 0.5 | 15.1 | 89.2 | 12.8 ± 0.5a | 42.4 ± 2.2 a |
| 7 | 60 | 20 | 0.5 | 14.9 | 84.9 | 11.5 ± 0.4 abc | 43.5 ± 0.7 a |
| 8 | 60 | 10 | 1.0 | 14.7 | 84.6 | 12.5 ± 0.6 a | 42.2 ± 0.4 a |
| 9 | 60 | 20 | 1.0 | 14.5 | 80.5 | 12.1 ± 0.2 ab | 43.5 ± 0.6 a |

T is temperature, P is pressure, F is flow rate, YG is extract yield and YOLE is oleuropein yield. IC50: Concentration of extract required to decrease the concentration of DPPH by 50%. Different letters in the same column differ from each other by the Tukey test at 5% significance.

**3.4 Determination of total phenolic components and antioxidant activity**

Table 3 shows the total phenolic component contents and the antioxidant activity of the extracts. The values of the total phenolic components obtained ranged from 10.5 ± 0.4 to 12.8 ± 0.5 mg GAE g-1 of extract. The highest total phenolic compounds contents (11.5-12.8 mg GAE g-1 of extract) were observed in the extracts obtained using the highest temperature (60 oC). The increase in the pressure and flow rate did not result in a significant impact (p > 0.05) on the total phenolic content. Therefore, to obtain an extract with higher total phenolic compounds, it is recommended the experiment 6 (60 oC, 10 MPa and 0.5 mL min-1).It is observed that there is a variation of the results found in the literature regarding the phenolics in leaves of O. *europaea*, with some of them close to the results found in this study. The variations may occur according to the cultivar, harvest season, climate and olive growing conditions (Coppa et al., 2017).

The antioxidant capacity results, expressed as IC50, are shown in Table 3. The values obtained demonstrate the good antioxidant capacity of the extracts, varying from 29.3 to 45.2 μg mL-1. The highest antioxidant capacity was observed in the extracts obtained at the lowest temperature (30 oC) (p ≤ 0.05), independently of the pressure and flow rates (p > 0.05). Therefore, the antioxidant activity of the extracts obtained was not correlated with the total phenolic content of the olive leaf extracts. Probably, the antioxidant behavior of the extracts is defined by the interactions between the various constituents and not only by the oleuropein content (Mylonaki et al., 2008). From the statistical analysis, it was verified that the temperature is the most critical factor in the extraction of these components, with prominence for antioxidant activity. In the operational condition of extraction of 30 ºC it was possible to obtain the highest antioxidant activity in the extracts (29.3 to 36.3 μg mL-1). The linear regression model (Eq. 3) obtained by the statistical analysis of the process variables presented a linear regression coefficient of 0.9739 and a model adjustment of 86.95%.

(3)

where DPPH IC50 is the antioxidant activity of extracts (μg mL-1), and T is the temperature.

The use of olive leaf extract rich in oleuropein and other phenolic compounds with antioxidant activity is promising for the enrichment of olive oil, for example, increasing its polyphenol composition and its oxidative stability (Jimenez et al., 2011; Coppa et al., 2017).

**3.3 Characterization of the Extract by HPLC–DAD–MSn Analysis**

The HPLC–DAD chromatogram processed at 280 nm shows the separation of 18 peaks, corresponding to 21 compounds from olive leaf extracts (Figure 1). Considering that a description of phenolic identification from olive leaves had already been reported by Peralbo-Molina et al. (2012), Quirantes-Piné et al. (2013), Li et al. (2014) and Kelebek et al. (2017), some aspects are discussed below.

The phenolic compounds identified in the olive leaf extracts belong to different classes such as iridoid precursors, secoiridoids and derivatives, flavonoids and phenolic acids. Irinoids are compounds from a broad group of monoterpenes as well as glucoside derivatives. The secoiridoid compounds are derived from iridoids by the opening of the pentacyclic ring.



*Figure 1: Chromatogram obtained by HPLC–DAD of the phenolic compounds from olive leaves extracted at 60°C, 10 MPa, 0.5 mL min-1.*

Peak 2 was proposed as being ferulic acid. The mass spectrum showed the deprotonated molecule [M–H]− at *m*/*z* 193, and the MS2 spectrum showed a base peak at *m*/*z* 134. The fragment ion at *m*/*z* 134 was a result of the loss of the ethoxyl and hydroxyl groups (Peralbo-Molina et al., 2012). Peak 3 was tentatively identified as an oleoside-derivative, based on the [M-H]- at *m*/*z* 571, and the MS2 mass fragment of *m*/*z* 389, suggesting the presence of an oleoside residue in the molecule. Peak 4a was tentatively assigned as oleoside or secologanoside, based on the [M-H]- at *m*/*z* 389, since both compounds present the same fragmentation pattern and it is not possible to distinguish between them only by MSn analyses (Peralbo-Molina et al., 2012; Quirantes-Piné et al., 2013). Peak 5 was tentatively identified as oleoside methyl ester (Peralbo-Molina et al., 2012; Quirantes-Piné et al., 2013). Peak 4b was tentatively identified as verbascoside due to the [M-H]- at *m*/*z* 623 and consecutive losses of 162 u (*m/z* 461) and 146 u (*m/z* 315) observed in the MS2 and MS3 spectra, respectively. Peralbo-Molina et al. (2012) firstly attributed the fragment at *m/z* 461 to the loss of one glucose moiety ([M-H-162]-); however, Li et al. (2014) found out by LC-QqTOF analysis that this product ion was a result of neutral loss of a caffeoyl rather than a hexose moiety. The product ion at *m/z* 315 ([M-H-162-146]-) in the MS3 spectrum corresponded to the loss of one rhamnose moiety from the *m/z* 461 MS2 fragment ion. Peak 7 was identified as oleuropein. It presented the same retention time, MS spectrum, and MS2 and MS3 fragmentation patterns as authentic oleuropein standard. Peak 9 was tentatively identified as an oleuropein isomer since it presented the same spectroscopic characteristics than oleuropein (peak 7). Peak 10 was assigned as being ligstroside. The mass spectrum showed the [M – H]− at *m*/*z* 523 and the MS2 spectrum showed a base peak at *m*/*z* 361 [M–H–162]−, corresponding to the loss of one glucose moiety (Quirantes-Piné et al., 2013). Among the flavonoids tentatively identified in the olive extracts, there is luteolin (peak 18), luteolin-7-*O*-rutinoside (peak 6a), luteolin hexoside isomers (peaks 6b, 8 and 11), rutin (peak 6c) and quercetin (peak 15). Identification of these compounds was based on their mass spectra and fragmentation patterns, mainly neutral loss of sugar moieties or typical aglycone fragmentation, compared to the literature data (Kelebek et al., 2017; Peralbo-Molina et al., 2012; Quirantes-Piné et al., 2013).

* 1. Conclusion

The operational conditions evaluated in this study allow to evaluate the extraction of active compounds from the olive leaf, mainly oleuropein, for milder temperatures, high pressures and continuous flow concerning the information available in the literature regarding PLE extraction.

The temperature was the most active operational parameter to obtain an extract of olive leaves by PLE in a continuous flow. In the 60 ºC condition, the highest yields (14.5 to 15.1%) were obtained. The pressure and flow rate did not have significant influence; therefore, lower flow (0.5 mL min-1) and pressure (10 MPa) levels can be used in the extraction to promote energy and solvent savings. The phenolic compounds profile identified in the olive leaf extracts belong to different classes such as iridoid precursors, secoiridoids and derivatives, flavonoids and phenolic acids. The parameters of temperature, pressure and solvent flow did not influence the content of oleuropein under the experimental conditions of this study. The antioxidant activity was higher in extracts obtained at a temperature of 30 ºC, the flow rate of 0.5 mL min-1 and pressure of 10 MPa.

The extraction with pressurized ethanol has low toxicity to the environment and is promising to obtain oleuropein and other phenolic compounds from olive leaves. The best condition to obtain an extract with the highest yield and total phenolic compounds and suitable oleuropein and antioxidant capacity would be 60 oC, 10 MPa, and 0.5 mL min-1.

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References

Al-Azzawie, H.F., Alhamdani, M.S.S., 2006. Hypoglycemic and antioxidant effect of oleuropein in alloxan-diabetic rabbits. Life Sci. 78, 1371–1377.

Albarelli J., Vardanega R., Wilkins N., Santos D., Marechal F., Meireles M., 2016, Economical analysis of a pressurized fluid-based process applied for phytochemicals recovery in a sustainable biorefinery concept for brazilian ginseng roots, Chemical Engineering Transactions, 50, 355-360

Bulotta, S., Celano, M., Lepore, S.M., Montalcini, T., Pujia, A., Russo, D., 2014. Beneficial effects of the olive oil phenolic components oleuropein and hydroxytyrosol: focus on protection against cardiovascular and metabolic diseases. J. Transl. Med. 12, 219.

Cavalheiro, C.V., Rosso, V.D., Paulus, E., Cichoski, A.J., Wagner, R., Cristiano, R. de M., Barin, J.S., 2014. Chemical composition of olive leaves (Olea europaea L.) from the region of Caçapava do Sul, RS, Brazil. Ciência Rural 44, 1874–1879.

Cicerale, S., Lucas, L., Keast, R., 2012. Antimicrobial, antioxidant and anti-inflammatory phenolic activities in extra virgin olive oil. Curr. Opin. Biotechnol. 23, 129–135.

Coppa, C.F.S.C., Cebin, S., Rosim, R.E., Augusto, C., Oliveira, F. De, Elisabete, C., Gonçalves, C.B., 2017. Extraction of oleuropein from olive leaves using a hydroalcoholic solvent. Brazilian J. Food Technol.

Erbay, Z., Icier, F., 2010. The importance and potential uses of olive leaves. Food Rev. Int. 26, 319–334.

Georgé, S., Brat, P., Alter, P., Amiot, M.J., 2005. Rapid determination of polyphenols and vitamin C in plant-derived products. J. Agric. Food Chem. 53, 1370–1373.

Goulas, V., Exarchou, V., Troganis, A.N., Psomiadou, E., Fotsis, T., Briasoulis, E., Gerothanassis, I.P., 2009. Phytochemicals in olive-leaf extracts and their antiproliferative activity against cancer and endothelial cells. Mol. Nutr. Food Res. 53, 600–608.

Jimenez, P., Masson, L., Barriga, A., Chávez, J., Robert, P., 2011. Oxidative stability of oils containing olive leaf extracts obtained by pressure, supercritical and solvent-extraction. Eur. J. Lipid Sci. Technol. 113, 497–505.

Kelebek, H., Selli, S., Kola, O., 2017. Quantitative determination of phenolic compounds using LC-DAD-ESI-MS/MS in cv. Ayvalik olive oils as affected by harvest time. J. Food Meas. Charact. 11, 226–235.

Kimura, Y., Sumiyoshi, M., 2009. Olive leaf extract and its main component oleuropein prevent chronic ultraviolet B radiation-induced skin damage and carcinogenesis in hairless mice. J. Nutr. 139, 2079–86.

Lee-Huang, S., Huang, P.L., Zhang, D., Lee, J.W., Bao, J., Sun, Y., Chang, Y.T., Zhang, J., Huang, P.L., 2007. Discovery of small-molecule HIV-1 fusion and integrase inhibitors oleuropein and hydroxytyrosol: Part I. Integrase inhibition. Biochem. Biophys. Res. Commun. 354, 872–878.

Lee, O., Lee, B., 2010. Bioresource Technology Antioxidant and antimicrobial activities of individual and combined phenolics in Olea europaea leaf extract. Bioresour. Technol. 101, 3751–3754.

Li, C., Liu, Y., Abdulla, R., Aisa, H.A., Suo, Y., 2014. Determination of Phenylethanoid Glycosides in Lagotis brevituba Maxim. by High-Performance Liquid Chromatography-Electrospray Ionization Tandem Mass Spectrometry. Anal. Lett. 47, 1862–1873.

Liyana-Pathirana, C.M., Shahidi, F., 2005. Antioxidant activity of commercial soft and hard wheat (Triticum aestivum L.) as affected by gastric pH conditions. J. Agric. Food Chem. 53, 2433–2440.

Moreno, E., Reza, J., Trejo, A., 2007. Extraction of polycyclic aromatic hydrocarbons from soil using water under subcritical conditions. Polycycl. Aromat. Compd. 27, 239–260.

Moret, S., Conte, L.S., 2014. Pressurized liquid extraction (PLE), in: Moret, S., Purcato, G., Conte, L.S. (Eds.), Il Campione per l’analisi Chimica. Springer-Verlag Mailand, pp. 177–214.

Mylonaki, S., Kiassos, E., Makris, D.P., Kefalas, P., 2008. Optimisation of the extraction of olive (Olea europaea) leaf phenolics using water/ethanol-based solvent systems and response surface methodology. Anal. Bioanal. Chem. 392, 977–985.

Omar, S.H., 2010. Cardioprotective and neuroprotective roles of oleuropein in olive. Saudi Pharm. J. 18, 111–121.

Peralbo-Molina, Á., Priego-Capote, F., Luque De Castro, M.D., 2012. Tentative identification of phenolic compounds in olive pomace extracts using liquid chromatography-tandem mass spectrometry with a quadrupole- quadrupole-time-of-flight mass detector. J. Agric. Food Chem. 60, 11542–11550.

Pereira, A.P., Ferreira, I.C.F.R., Marcelino, F., Valentão, P., Andrade, P.B., Seabra, R., Estevinho, L., Bento, A., Pereira, J.A., 2007. Phenolic compounds and antimicrobial activity of olive (Olea europaea L. Cv. Cobrançosa) leaves. Molecules 12, 1153–1162.

Quirantes-Piné, R., Lozano-Sánchez, J., Herrero, M., Ibáñez, E., Segura-Carretero, A., Fernández-Gutiérrez, A., 2013. HPLC-ESI-QTOF-MS as a powerful analytical tool for characterising phenolic compounds in olive-leaf extracts. Phytochem. Anal. 24, 213–223. https://doi.org/10.1002/pca.2401

Setyaningsih, W., Hidayah, N., Saputro, I. E., Palma, M., Barroso, C. G., 2016. Profile of phenolic compounds in Indonesian rice (*Oryza sativa*) varieties throughout post-harvest practices. Journal of Food Composition and Analysis, vol. 54 pp. 55-62.

Solarte-Toro J.C., Romero-Garcia J.M., Lopez-Linares J.C., Ramos E.R., Castro E., Alzate C.A.C., 2018, Simulation approach through the biorefinery concept of the antioxidants, lignin and ethanol production using olive leaves as raw material , Chemical Engineering Transactions, 70, 925-930.