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Evaluation of the neuroprotective and antidiabetic potential of phenol-rich extracts from virgin olive oils by *in vitro* assays

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ABSTRACT: In this work, phenol-rich extracts from 'Cornicabra' and 'Picual' virgin-olive oils (EVOOs) were examined, for the first time, to establish their capacity to inhibit key enzymes involved in Alzheimer´s disease (AD) (acetylcholinesterase (AChE), butyrylcholinesterase (BuChE) and 5-lipoxygenase (LOX)), major depressive disorder (MDD) and Parkinson´s disease (PD) (monoamine oxidases: *h*MAO-A and *h*MAO-B respectively), and *diabetes mellitus* (DM) (α-glucosidase and α-amylase). 'Cornicabra' displayed the best inhibitory activity against all enzymes, when compared to 'Picual': BuChE (IC_{50} = 156 ± 4 and 308 ± 33 mg mL^{-1}), LOX (IC_{50} = 26 ± 0.5 and 37 ± 3 mg mL^{-1}), *h*MAO-A (IC_{50} = 20 ± 2 and 37 ± 0.2 mg mL^{-1}), *h*MAO-B (IC_{50} = 131 ± 7 and 215 ± 13 mg mL^{-1}) and α-glucosidase (IC_{50} = 154 ± 17 and 251 ± 31 mg mL^{-1}), respectively. The behaviour observed can be associated with the higher content of secoiridoids, lignans and phenolic acids in 'Cornicabra' EVOO.

***Keywords*:** Extra-virgin olive oil; Picual cv.; Cornicabra cv.; Phenols; Neuroprotection; *Diabetes mellitus*.

1. Introduction

Management of the devastating central nervous system (CNS) disorders, including Alzheimer´s (AD), Parkinson´s Diseases (PD) and Major Depressive Disorder (MDD), is one of the most defiant medical challenges (Olesen et al., 2012).. Based on the cholinergic hypothesis, high levels of acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE) enzymes lead to lower amounts of the neurotransmitter acetylcholine (ACh). Bearing this in mind, restoring the levels of this neurotransmitter *via* inhibition of these enzymes is actually the most useful therapeutic approach to treat AD and other forms of dementia (Kulshreshtha & Piplani, 2016). Additionally, 5-lipoxygenase (5-LOX) has an important role in inflammation, catalyzing the conversion of arachidonic acid into different leukotrienes. Leukotrienes are considered as potent inflammatory mediators and are involved in diseases linked to oxidative stress and inflammation (Dzoyem & Eloff, 2015; Leyen et al., 2008). Considering this, LOX inhibitors could effectively reduce the inflammation associated with neurodegenerative disorders (Czapski, Czubowicz, Strosznajder, & Strosznajder, 2016). Monoamine oxidase isoforms (*h*MAO-A and *h*MAO-B) catalyze the oxidative deamination of a variety of neurotransmitters. *h*MAO-A inhibitors are used as antidepressant agents for MDD with a central serotonin and noradrenaline deficiency. Similarly, *h*MAO-B inhibitors are applied in PD therapy, where a central dopamine deficiency is responsible for the characteristic motor deficits (Finberg & Rabey, 2016; Jung, Roy, & Choi, 2017).

α-Glucosidase and α-amylase are key enzymes in the management of the *diabetes mellitus* due to the hydrolysis of starch by α-amylase, and the absorption of glucose in the small intestine by α-glucosidase, leading to a fast increase of blood glucose levels. Inhibitors of these enzymes slowdown carbohydrate digestion blunting the postprandial

plasma glucose rise (Etxeberria, de la Garza, Campion, Martinez, & Milagro, 2012; Figueiredo-González, Grosso, Valentão, & Andrade, 2016).

The prevention of pathologies by diet management is an important public health challenge (Reboredo-Rodríguez et al., 2017). In fact, the Mediterranean diet, characterized by a high intake of exogenous dietary phenolics as a consequence of a high intake of virgin olive oil (VOO), fruit, nuts, vegetables, and cereals, is associated with a lower incidence of several diseases (Casas et al., 2014; Hervert-Hernández, García, Rosado, & Goñi, 2011; Mayneris-Perxachs et al., 2014). VOO, the main fat of the Mediterranean diet, is *per se* considered as a functional food mainly due to phenolic compounds present in its compostion. Hydroxytyrosol and tyrosol and their secoiridoid derivatives are the most representative phenols in VOOs. In addition, lignans (pinoresinol and acetoxypinoresinol, among others), flavones (luteolin and apigenin) and simple phenolic acids (*p*-coumaric acid and vanillic acid) can also be found in a minor extent (Reboredo-Rodríguez et al., 2014).

Some of these phenolic compounds could be an acceptable resource of enzyme inhibitors, being already addressed to exhibit several remarkable biological activities, including the capacity to inhibit ChEs (Conforti et al., 2010; El-Hassan et al., 2003; Szwajgier & Borowiec, 2012; Tang et al., 2016), 5-LOX (Bekir, Mars, Souchard, & Bouajila, 2013; Lee, Kim, Kim, Lee, & Kang, 2010; Voss, Sepulveda-Boza, & Zilliken, 1992), *h*-MAO (Bandaruk Mukai, Kawamura, Nemoto, & Terao, 2012; Chaurasiya, Ibrahim, Muhammad, Walker, & Tekwani, 2014), and/or α-glucosidase and α-amylase (Hadrich, Bouallagui, Junkyu, Isoda, & Sayadi, 2015; Tadera, Minami, Takamatsu, & Matsuoka, 2006).

Taken this background into account, as far as we know, this is the first time that the neuroprotective and antidiabetic potential of phenol-rich extracts from monovarietal extra VOOs (obtained from 'Cornicabra' and 'Picual' varieties, two of the most representative Spanish cultivars) was evaluated using cell-free models as easy tools and a fast approach for initial screening. Given the fact that chemical profile of EVOOs can have a great effect in their biological activity, a systematic phenolic characterization of the studied oils was undertaken by LC-ESI-IT-MS.

2. Materials and methods

2.1. Chemicals and standards

Phenolic compounds analysis. All the solvents were of analytical or LC-MS grade purity (depending on if they were used for the extraction or chromatographic analysis). Methanol, acetic acid and *n*-hexane were supplied from Panreac (Barcelona, Spain); meanwhile acetonitrile was purchased from Lab-Scan (Dublin, Ireland). Doubly deionized water (conductivity value of 18.2 MΩ) was produced using a Milli-Q system (Millipore, Bedford, MA, USA) located in the laboratory.

Commercial standards of hydroxytyrosol, tyrosol, luteolin, apigenin, *p*-coumaric, ferulic acid, and quinic acid were purchased from Sigma-Aldrich (St.Louis, MO, USA) and (+)-pinoresinol was acquired from Arbo Nova (Turku, Finland). The pure standard of oleuropein was purchased from Extrasynthese (Lyon, France).

Firstly, a stock solution (at a concentration of 500 mg L^{-1} for each standard) was prepared by dissolving the appropriate amount of the compounds in methanol. Then a series of working solutions of these analytes were freshly prepared by diluting the mixed standard solution with methanol, at appropriate ratios to yield the needed

concentrations within the range 0.5–250 mg L^{-1}. The proper amount of 3,4-dihydroxyphenylacetic acid (DOPAC), purchased from Sigma-Aldrich (St. Louis, MO, USA), was dissolved in methanol to prepare an internal standard (IS) solution with a concentration of 500 mg L^{-1}. All solutions were stored in darkflasks at -20°C. Stability controls assured their appropriate conservation for, at least, 6 months.

In vitro enzyme inhibition assays. Acarbose, galantamine, α-glucosidase (=maltase from *Saccharomyces cerevisiae*), α-amylase (from porcine pancreas), 4-nitrophenyl α-D-glucopyranoside (PNP-G), dinitrosalicylic acid, soluble starch, AChE (from electric eel), acetylthiocholine iodide (ATCI), BuChE (from equine serum), *S*-butyrylthiocholine iodide (BTCI), bovineserum albumin (BSA), 5,5-dithiobis(2-nitrobenzoic acid) (DTNB), Trizma®hydrochloride (Tris–HCl), N-Methyl-N-propargyl-3-(2,4-dichlorophenoxy)propylamine hydrochloride or clorgyline, kynuramine dihydrobromide crystalline, monoamine oxidase A and B (human recombinant, expressed in baculovirus infected BTI insect cells), lipoxydase (from glycine max) and linoleic acid, were obtained from Sigma-Aldrich (St. Louis, MO, USA). Quercetin (99.0%) was obtained from Extrasynthèse (Genay, France). Ethanol was acquired to Chem-Lab NV (Zedelgem, Belgium). Potassium sodium tartrate tetrahydrate, trisodium phosphate, potassium dihydrogen phosphate, sodium hydroxide and sodium chloride were obtained from Merck (Darmstadt, Germany). Magnesium chloride hexahydrate ($MgCl_2 \cdot 6H_2O$) was purchased from Fluka (Steinheim, Germany).

2.2. Virgin olive oil samples

Two different monovarietal extra-virgin olive oil (EVOOs) samples were purchased at local delicatessen shop in Galicia (North-West of Spain). The origin and the crop season

were specified in the labels of the bottles. Both cultivars 'Picual' and 'Cornicabra' were produced in Extremadura and Castilla-La Mancha, respectively (Middle-South of Spain) in the crop season 2016/2017. Once in the laboratory, three different bottles of 500 mL from each variety were pooled and homogenized to obtain a final representative sample prior to analysis. The samples were stored during 48 hours in amber glass bottles without headspace at -20ºC in darkness until analysis.

2.3. Phenolic compounds analysis

2.3.1. Extraction of phenolic compounds from VOOs

Phenolic compounds were extracted, by duplicate, from VOOs according to the methodology proposed by Bajoub, Fernández-Gutierrez, & Carrasco-Pancorbo (2016). Briefly, phenolic compounds were extracted by shaking the olive oil sample (2 g with the addition of 25 µL of the IS solution (amount of IS which was evaporated under N_2)) with *n*-hexane (1 mL) and acetonitrile (6 mL) for 3 min in a vortex; the mixture was then centrifuged at 3500 rpm for 6 min at room temperature. The acetonitrile fraction was separated and the residue was extracted with acetonitrile once again (2 mL) and centrifuged under the same conditions. The combined acetonitrile extracts were evaporated under reduced pressure at 37°C (rotary evaporator, Büchi R-210). The obtained residue was reconstituted in 1 mL of acetonitrile:water (50:50, v/v) and filtered through 0.22 µm membrane (nylon) filter. These extracts were previously evaporated until dryness and re-dissolved in the corresponding buffers according to Section 2.4., before being used in *in vitro* enzyme inhibition assays.

2.3.2 Antioxidant capacity of phenolic extracts

The antioxidant activity was assessed by the DPPH method (Gorinstein et al., 2003) with some modifications. An 80 mg L^{-1} solution of the DPPH radical in methanol which showed an absorbance of approximately 1.4 at 515 nm was prepared. A hydromethanolic extract (50 µL) obtained from the olive oil by the IOC method was previously diluted with a hydroalcoholic solution (550 µL) of ethanol 70% (v/v). The diluted extract was added to 400 µL DPPH solution. The mixture was vigorously stirred for a few seconds and kept in the dark for 15 minutes. Absorbance was measured at 517 nm against methanol. Olive oil antioxidants scavenge the DPPH cation radical, resulting in decolorization of its purple solution. Trolox was used as standard and the results were expressed as µmol Trolox equivalents/kg oil.

2.3.3. LC-MS analysis of phenolic extracts

Phenolic extracts were analyzed using an Agilent 1260-LC system (Agilent Technologies, Waldbronn, Germany) equipped with a diode-array detector (DAD), which was coupled to a Bruker Daltonic Esquire 2000TM ion trap mass spectrometer (Bruker Daltonik, Bremen, Germany) by an electrospray ionization (ESI) interface. The same LC system was also coupled to a micrOTOF-Q II^{TM} mass spectrometer (Bruker Daltonics) by means of an ESI source. Several injections of each extract were made using high resolution-MS to corroborate the identity of the compounds under study. LC-ESI-IT-MS was employed to carry out the quantification experiments in full scan mode.

Chromatographic data acquisition and examination of DAD signals was performed by using ChemStation B.04.03 software (Agilent Technologies). Bruker mass spectrometers were controlled using the software Esquire Control and the resulting files were treated with the software Data Analysis 4.0 (Bruker).

A Zorbax C18 analytical column (4.6 × 150 mm, 1.8 μm particle size) protected by a guard cartridge of the same packing was used. Column oven temperature was set at 25ºC. The mobile phases were 0.5% acetic acid in water (A) and acetonitrile (B). A flow rate of 0.8 mL min^{-1} was used and an injection volume of 10 μL of the phenolic extracts and/or standards was selected. The established elution gradient was the following: 0 to 10 min, 5-30% B; 10 to 12 min, 30-33% B; 12 to 17 min, 33-38% B; 17 to 20 min, 38-50% B; 20 to 23 min, 50-95% B. Finally, the B content was decreased to the initial conditions (5%) in 2 min and the column re-equilibrated before the subsequent injection for 2.5 min.

The Q-TOF MS system was operating in negative and positive mode (to increase the information achieved about the EVOO samples) within the range of 50-1200 m/z, at a scan speed of 240 ms. A drying gas (N_2) temperature of 300ºC and a flow of 9.0 L min^{-1} were selected as optimum. The capillary voltage was set at 4500 V and the end plate offset at -500 V. Internal calibration was performed using sodium formate clusters. The ion trap mass analyzer was used in negative ion mode. The capillary voltage was set at +3200 V and the MS detector was programmed to perform scans with the *m/z* range 50–800. The following parameters of ESI-MS were used: drying gas temperature, 300°C; drying gas flow, 9 L min^{-1}; and nebulizing gas pressure, 30 psi.

The identification of the phenolic compounds present in the analyzed samples was based on the use of pure standards (when available), retention time data, extracted ion chromatograms, accurate MS signals and comparing the MS/MS spectra with previously published results (Bajoub et al., 2016). **Table 1** summarizes the main phenolic compounds identified, including their name, acronym, molecular formula, *m/z* ESI-IT MS signal considered for their quantification, as well as their retention time.

Additionally a table in supplementary material includes LC-ESI-Q-TOF MS data (accurate MS signals, molecular formula, mSigma error, and fragmentation patterns). Calibration curve of every available pure standard was constructed using different concentrations of the standard mixture solution and plotting the peak areas *versus* the concentrations, obtaining correlation coefficients (r^2) above 0.999. When a pure standard was not available, the quantification was made using the calibration curve of a similar (or structurally related) compound: hydroxytyrosol was used for oleuropein aglycon and related compounds; tyrosol was used for ligstrosides aglycon and related compounds; lignans were quantified in terms of pinoresinol; luteolin was used for diosmetin; ferulic acid was used for vanillic acid and vanillin; and finally, oleuropein was used for all elenolic acid-derivatives. The results were expressed in mg per kg of olive oil, as mean ± standard deviation for two independent injections (of two different extracts; n = 4).

2.4. In vitro enzyme inhibition

2.4.1. Neuroprotective activity

Cholinesterases. Inhibition of AChE and BuChE was assessed according to a previously described methodology (Vinholes et al., 2011). Briefly, the extract dissolved in buffer A (50 mM Tris-HCl, pH 8) or just buffer A (negative control) was added to each well, together with ATCI or BTCI. Then, DTNB and buffer B (50 mM Tris-HCl, with 0.1% BSA, pH 8) were added. The absorbance was measured at 405 nm in a Multiskan Ascent plate reader (Thermo Electron Corporation). The rates of reactions were calculated after addition of AChE (0.44 U mL^{-1} in buffer B) or of BuChE (0.1 U mL^{-1} in buffer B). Galantamine was used as positive control. The concentration of the extracts

varied from 125 to 1000 µg of dry extract mL^{-1} for AChE and from 62.5 to 1000 µg of dry extract mL^{-1} for BuChE.

Lipoxygenase. The inhibitory effect on 5-LOX was assessed in 96-well plates, using a previously documented procedure (Pereira, Taveira, Valentão, Sousa, & Andrade, 2015). Briefly, 20 µL of extracts, 200 µl of phosphate buffer (pH 9) and 20 µL of LOX (100 U) were added to each well. After 5 min pre-incubation at room temperature, the reaction was started by addition of 20 µL of linoleic acid (4.18 mM in ethanol). The reaction time was 3 min. The absorbance was measured at 234 nm in a Synergy™ HT plate reader (Biotek Instruments, Winooski, USA) operated by Gen5 Software. Quercetin was used as positive control. Quercetin was used as positive control. The concentration of the extracts varied from 6 to 384 µg of dry extract mL^{-1}.

Monoamine oxidases. Inhibition of *h*MAO-A and *h*MAO-B was assessed according to a methodology that has been described before by Bernardo, Ferreres, Gil-Izquierdo, Valentão, & Andrade, 2017. Concisely, a mixture of kynuramine and the extract dissolved in phosphate buffer (pH 7.4) or just buffer (negative control) was incubated for 10 min, at 37ºC. The reaction was initiated by adding of *h*-MAOs (17 U mL^{-1}) and the mixture was further incubated at 37ºC for 70 min. Afterwards, the reaction was stopped by addition of NaOH 2 N and the absorbance was measured at 314 nm using a spectrophotometer (Heλios α, Unicam, Cambridge, United Kingdom). Clorgyline was tested as positive control. The concentration of the extracts varied from 4 to 65 µg of dry extract mL^{-1} for *h*MAO-A and from 14 to 260 µg of dry extract mL^{-1} for *h*MAO-B.

2.4.2. Antidiabetic activity

α-Glucosidase. α-Glucosidase inhibitory activity was assessed by a previously reported procedure (Vinholes et al., 2011). Briefly, each well contained PNP-G, phosphate buffer and extract or buffer (negative control). The reaction was initiated by adding the enzyme solution (0.28 U mL^{-1}). The plates were incubated at 37ºC for 10 min. The rate of release of 4-nitrophenol from PNP-G at 405 nm was measured in a Multiskan Ascent plate reader (Thermo Electron Corporation) from 0 to 10 min. Acarbose was the positive control. The concentration of the extracts varied from 31 to 1000 µg of dry extract mL^{-1}.

α-Amylase. α-Amylase inhibitory activity was assessed following the procedure described by Figueiredo-González et al., (2016). In short, a mixture of starch and the extract or buffer (negative control) was incubated for 10 min, at 25ºC. The reaction was initiated by adding porcine pancreatic α-amylase (15 U mL^{-1}) and the mixture was further incubated at 25ºC for 10 min. Afterwards, the reaction was stopped with DNS and the tubes were incubated at 100ºC for 5 min. The mixture was cooled to room temperature and the absorbance was measured at 540 nm using a spectrophotometer (Heλios α, Unicam, Cambridge, United Kingdom). Acarbose was tested as positive control. The concentration of the extracts varied from 120 to 800 µg of dry extract mL^{-1}.

2.5. Statistical analysis

Quantification of compounds from oils was achieved from four determinations and results are shown as mean (±SD). The IC_{50} values were calculated from three independent assays, each of them performed in triplicate, and all results are presented as mean values. Values obtained were compared using unpaired *t*-test (GraphPad Prism 6

Software, Inc., San Diego, CA, USA). Differences at $p < 0.05$ were considered statistically significant.

3. Results and discussion

3.1. Antioxidant capacity and phenolic profiling of the studied EVOO extracts

It is well-known that olive cultivars (varieties) considerably influence the composition of their resulting EVOOs, especially with regard to the composition in terms of bioactive phenolic compounds (Reboredo-Rodríguez et al., 2016). Moreover, EVOO quality is strictly related to several specific activities of the hydrophilic phenols, including their antioxidant power and other properties that might affect its sensory and healthy characteristics.

The antioxidant capacity of the studied oils was assessed by determining the hydrophilic phenolic compounds ability to scavenge free DPPH radical. High antioxidant capacities were registered for both EVOOs (1877 ± 116 and 1895.5 ± 66 µM Trolox/kg in 'Cornicabra' and 'Picual', respectively) due to this parameter is directly related to the phenolic content (Samaniego Sanchez et al., 2007). In fact, antioxidant capacity of other olive oils such as Arbequina and Leccino varieties (440 and 1300 µM Trolox/kg, respectively) was low possibly due to the lower phenolic content (Laddomada et al., 2013; Tovar, Romero, Alegre, Girona, & Motilva, 2002).

The phenolic profile of monovarietal 'Cornicabra' and 'Picual' EVOOs, determined by LC-ESI-IT-MS, is displayed in **Figure 1**. A total of 30 phenolic compounds belonging to several chemical families (such as phenolic acids, flavonoids, lignans, simple phenols

or secoiridoids) characterized the complex and heterogeneous profile pattern of the target oils. The quantitative results (mg analyte kg^{-1} EVOO), specified in **Table 2**, varied from low amounts of phenolic acids to high concentrations of secoiridoids derivatives. The coming paragraphs include a comprehensive description of the results for each chemical class.

Phenolic acids. The studied EVOOs contained hydroxybenzoic acids (vanillic acid (Van)), hydroxycinnamic acids (*p*-coumaric acid (*p*-Cou)) and vanillin (Val) at very low concentration; values were lower than 0.2 mg kg^{-1}. Even though they are found at relatively low levels, phenolic acids have been associated with colour and sensory qualities of foods and they are also used as potential markers of geographical origin and olive cultivars (Bendini et al., 2007). The main difference between both olive oils was the high level of *p*-Cou in 'Cornicabra' oil in comparison with 'Picual' oil.

Flavonoids. This chemical category includes flavones, flavonols, flavanones, flavanols, anthocyans and the derived glucosides. As far as flavones are concerned, 'Cornicabra' and 'Picual' oils provided similar levels of luteolin (Lut), apigenin (Apig) and diosmetin (Dios) (around 1 mg kg^{-1} EVOO), being Lut (0.67 ± 0.05 - 0.71 ± 0.03 mg kg^{-1} EVOO) the most abundant compound of this group.

Lignans. Syringaresinol (Syr), pinoresinol (Pin) and acetoxypinoresinol (Ac-Pin) were the identified compounds belonging to this group. In both EVOOs, Pin content (around 1 mg kg^{-1} VOO) was higher than the concentration of the rest of the analytes of this category. One particular feature of 'Cornicabra' oils is the high levels of Ac-Pin (and lignans, in general) when compared with other oils; this was observed in the current study, as 'Cornicabra' oil showed 0.45 ± <0.01 mg kg^{-1} of Ac-Pin and 'Picual' oil had a

concentration level of 0.08 ± <0.01 mg kg^{-1}. The same has been observed by other authors (Bajoub et al., 2017; Brenes et al., 2000).

Simple phenols. Hydroxytyrosol (Hyt), tyrosol (Ty) followed by hydroxytyrosol acetate (Hyt-Ac) and oxidized hydroxytyrosol (O-Hyt) constituted the second group in terms of concentration levels. The overall content of simple phenols in 'Picual' oil was up 5-fold higher than in 'Cornicabra'. These differences were attributed to Hyt (9.7 ± 0.3 mg kg^{-1} in 'Picual' *vs* 0.96 ± 0.03 mg kg^{-1} in 'Cornicabra') and Ty (5.6 ± 0.2 mg kg^{-1} in 'Picual' *versus* 2.1 ± 0.1 mg kg^{-1} in 'Cornicabra') levels. Regarding to Hyt-Ac, it is important to note that it was just found in 'Picual' EVOO.

Secoiridoids. They constitute a specific group of compounds in *Oleaceae* plants. Oleuropein is by far the most abundant compound present in olive fruits; however, during the crushing and malaxation processes, hydrolysis of the glycosidic bond occurs and the aglycons pass into the olive oil. In the studied EVOOs, secoiridoids comprised 70-79% of the total phenolic compounds. A total of 12 secoiridoids derivatives such as oleuropein aglycon-related compounds (Hy-D-Ol-Agl, DOA, 10 Hy-Ol-Agl, Ol Agl, 2 isomers of Ol Agl and methyl Ol Agl) and ligstroside aglycon-related analytes (D-Lig Agl and 4 isomers of Lig Agl) were finally identified in the studied EVOOs. Oleuropein and ligstroside aglycons-derivatives were tentatively quantified using other standards (since they are not available as commercially pure ones). So, Hyt was used as reference to quantify oleuropein derivatives (Hyt molecule is, indeed, part of their structure) and Ty was used for ligstroside derivatives (Ty moiety is part of their structure). There were remarkable differences between these EVOOs in terms of some secoiridoids, such as: DOA (also known as 3,4-DHPEA-EDA: 165 ± 18 mg kg^{-1} in 'Cornicabra' and 92 ± 4 mg kg^{-1} in 'Picual'), D-Lig Agl (known also as *p*-HPEA-EDA or oleocanthal: 369 ± 31

mg kg^{-1} in 'Cornicabra' and 84 ± 0.4 mg kg^{-1} in 'Picual'), Ol Agl main peak (3,4-DHPEA-EA: 56 ± 5 mg kg^{-1} in 'Cornicabra' and 91 ± 10 mg kg^{-1} in 'Picual'), and Lig Agl main peak (*p*-HPEA-EA: 181 ± 10 mg kg^{-1} in 'Cornicabra' and 154 ± 1 mg kg^{-1} in 'Picual'). Great differences between both olive oils were also observed when the total amount of secoiridoids is considered; the total concentration level was quite higher in 'Cornicabra' (865 ± 62 mg kg^{-1} EVOO) than 'Picual' (506 ± 23 mg kg^{-1} EVOO).

VOO is considered as a functional food as stated by the European Food Safety Authority (EFSA) which approved a health claim on olive oil polyphenols (Commission Regulation (EU) 432/2012, 2012). This health claim established literally that *olive oil polyphenols contribute to the protection of blood lipids from oxidative stress and also stated that the claim may be used only for olive oil containing at least 5 mg of HTyr and its derivatives (e.g. oleuropein complex and Tyr) per 20 g of olive oil; in order to bear the claim information shall be given to the consumer that the beneficial effect is obtained with a daily intake of 20 g of olive oil. due to its content in healthy compounds*. Simple phenols and secoiridoids in 'Cornicabra' and 'Picual' EVOOs were established (868 and 524 mg kg^{-1}, respectively) to verify the EFSA requirement. The contribution of HTyr and its derivatives per 20 g of 'Cornicabra' and 'Picual' EVOOs, were 7.4 and 10.5, respectively. These values were 3.5 and 2 times higher than the minimum established. As a conclusion, these EVOOs could present the specific health claim on the oil label.***Non-phenolic (but related) compounds***. The olive oil elaboration process also causes partial modification of oleuropein and ligstroside compounds, which gives rise to several elenolic acid (EA) derivatives. EA cannot be considered as a phenolic compound, but secoiridoids can be defined as a hydroxyethyl group attached to the C4 carbon of a phenol group; in other words, EA is part of the chemical structure of the secoiridoids. A total of 4 non-phenolic compounds derived from elenolic acid (D-Ald-D

EA, Desoxy-EA, Hy-EA and EA) were determined in both EVOOs considering oleuropein as an appropriate standard for their quantification. Similar levels of EA -the most abundant compound of these derivatives- were observed for 'Cornicabra' and 'Picual' samples, with 209 ± 38 and 190 ± 22 mg kg^{-1}, respectively. Quinic acid was a minor non-phenolic compound which was just found in 'Cornicabra' oil.

3.2. Biological activities

3.2.1. Neuroprotective potential

The challenging search for natural inhibitors capable of hindering key enzymes associated with neurodegenerative disorders is increasing. Currently, improving the cholinergic deficit by restoring the levels of acetylcholine through the inhibition of the two major forms of cholinesterases (BuChE and AChE) is the main treatment for the symptoms of AD and other forms of dementia (Menichine et al., 2009). In the present work, the ability to inhibit AD-related enzymes by phenol-rich extracts obtained from 'Cornicabra' and 'Picual' oils was evaluated.

As can be observed in **Figure 2**, 'Cornicabra', 'Picual' extracts inhibited BuChE in a dose-dependent manner. As a measure of the inhibitory potency of the tested extracts, IC_{50} and IC_{25} values were calculated and displayed in **Table 3**. Throughout the text, both IC_{25} and IC_{50} values are expressed as mg of EVOO mL^{-1}. However, when they were compared to the positive control of the corresponding assay, IC values were converted to µg of dry extract mL^{-1} of the buffer. The IC_{50} values found for both EVOOs against BuChE ranged from 156 ± 4 to 308 ± 33 mg of EVOO mL^{-1}, 'Cornicabra' oil being more effective that 'Picual' oil.. Both EVOOs showed lower inhibitory activities against BuChE than the reference drug galantamine (IC_{50} = 6.8 ± 0.5 µg mL^{-1}).

Regarding AChE, only 'Cornicabra' extract was able to inhibit this enzyme in a dose-dependent manner (**Figure 2**), although to a lesser extent than against BuChE. 'Cornicabra'´s activity against AChE was lower than the action exerted by the reference drug galantamine (IC_{50} = 2.2 ± 0.3 µg mL^{-1}) (**Table 3**). Due to solubility issues, it was not possible to calculate the IC_{50} value for AChE inhibition (IC_{25} = 503 ± 36 mg of EVOO mL^{-1}).

Among the analyzed oils, 'Cornicabra' oil was the most potent against both enzymes, revealing stronger anticholinergic effects. This dual ChE inhibition can effectively increase the efficacy of the treatment, and broaden its application to other disorders affecting the CNS (Giacobini, 2004). On the contrary, 'Picual' oil was selective BuChE inhibitor. This selectivity showed by some natural products may be positive, because they could also prevent the formation of new β-amyloid plaques (associated to BuChE enzyme), thus limiting AD progression (Taveira et al., 2014).

Our results are not in agreement with those recently published by Collado-González et al., (2017), who did not observed inhibitory activity against either AChE or BuChE for hexane-based extracts obtained from 'Cornicabra' and 'Picual' oils. These authors suggested that the lack of cholinesterase activity might be due to the lack of flavonoids and other phenolic compounds from hexane extracts after being removed by a solid phase extraction (SPE) clean-up technique.

Some phenolic compounds found in EVOOs have already demonstrated potential for cholinesterase inhibition. Szwajgier and Borowiec (2012) evaluated the anticholinesterase activity of several phenolic acids; they concluded that *p*-Cou had the largest share, even though it was present at a significantly lower concentration in the tested samples. In another interesting work, Conforti et al., (2010) reported that Lut

isolated from plants exhibited a promising activity against AChE and BuChE. Among lignans isolated from plants, Syr showed an inhibitory effect against AChE (El-Hassan et al., 2003) and Pin also displayed selective inhibitory effects on BuChE, but not against AChE (Tang et al., 2016). As can be seen in **Table 2**, *p*-Cou and Syr contents were significantly higher in 'Cornicabra' (0.19 ± <0.01 and 0.21 ± 0.02 mg kg^{-1}, respectively) than in 'Picual' (0.09 ± <0.01 and 0.11 ± 0.01 mg kg^{-1}, respectively) olive oils. At this point, it is important to note that the biological activity of any extract reflects not only the action of those molecules present at the highest levels, but also the possible synergistic/antagonistic interactions of all their compounds, regardless of their concentration.

Neuroinflammation is also closely related to the pathogenesis of AD and PD (Czapski et al., 2016; Dzoyem & Eloff, 2015). In this work, the anti-inflammatory activity of oil samples by 5-LOX inhibition has been also evaluated. To the best of our knowledge, no studies on 5-LOX inhibitory activity of vegetable-obtained oils have been reported up to date. 'Cornicabra' and 'Picual' oils were both able to inhibit this enzyme, although the effect was lower than the one showed by the positive control (quercetin, IC_{50} = 3.2 ± 0.2 µg mL^{-1}). 'Cornicabra' and 'Picual' inhibited 5-LOX in a dose manner dependent (**Figure 2**), with an IC_{50} of 26 ± 0.5 and 37 ± 3 mg of EVOO mL^{-1}, respectively (**Table 3**).. Bekir et al., (2013) suggested that the inhibition of 5-LOX is more important when the matrix is rich in phenolic compounds. In fact, the greatest inhibition was exerted by 'Cornicabra' oil, which contains higher amounts of total phenolics (1100 ± 50 mg kg^{-1}) than 'Picual' oil (722 ± 17 mg kg^{-1}).

New and emerging research has reported that *p*-HPEA-EDA (369 ± 31 and 84 ± 0.4 mg kg^{-1} in 'Cornicabra' and in 'Picual', respectively) and Hyt (0.96 ± 0.03 mg kg^{-1} in

'Cornicabra' and 9.7 ± 0.3 mg kg^{-1} in 'Picual') can display anti-inflammatory potential by inhibiting LOX and, consequently, they could act directly upon neuroinflammation associated with neurodegenerative disorders (Parkinson & Keast, 2014). In addition, phenolic compounds, such as *p*-Cou and Lut already showed LOX-inhibitory activity (Lee et al., 2010; Voss et al., 1992). Our hypothesis is that there can be a certain synergistic effect between Hyt and/or *p*-HPEA-EDA and other olive oil phenols, which might be responsible for the observed activity.

The inhibitors *of h*MAO-A are nowadays used as anti-depressant drugs, whereas *h*MAO-B inhibitors can be used to treat PD (Jung et al., 2017). This is the first time that the effect of any olive oil is checked against *h*MAO enzymes. Data from the present study provide evidence of the concentration-dependent inhibitory effect of the tested extracts from 'Cornicabra' and 'Picual' oils on *h*MAO-A and on *h*MAO-B activities (**Figure 2**). Although the observed activity, for both EVOOs and against both *h*MAO, was significantly lower than that of clorgyline (*h*MAO-A, IC_{50} = 0.035 ± 0.005 µg mL^{-1} and *h*MAO-B, IC_{50} = 23 ± 0.3 µg mL^{-1}), IC_{50} values were calculated (**Table 3**). 'Cornicabra' oil displayed IC_{50} values of 20 ± 2 and 131 ± 7 mg of EVOO mL^{-1} for *h*MAO-A and *h*MAO-B, respectively, while 'Picual' oil showed IC_{50} values of 37 ± 0.2 and 215 ± 13 mg of EVOO mL^{-1} for the same enzymes.. Several studies have determined that some natural flavonoids are potent inhibitors of *h*-MAO enzymes. For example, Apig and Lut significantly inhibited *h*-MAO (Bandaruk et al., 2012; Chaurasiya et al., 2014). Apig (0.19 mg kg^{-1} EVOO) and Lut (0.67 ± 0.05 - 0.71 ± 0.03 mg kg^{-1} EVOO) contents were similar in both EVOOs and, consequently, the activity observed may also result from synergism and/or antagonism phenomena that occur among the several phenolic constituents. It should be noted that our extracts displayed higher inhibitory activity towards *h*MAO than towards the cholinesterase enzymes.

Since they were obtained using recombinant enzymes with high homology levels with the human ones, the results presented might be of great interest in the search for compounds to treat PD and MDD.

3.2.2. Antidiabetic potential

Natural inhibitors of α-glucosidase and α-amylase, important enzymes involved in the management of DM, can be an effective therapeutic approach for reducing hyperglycemia (Etxeberria et al., 2012; Hadrich et al., 2015). As far as we know, this is the first report evaluating the functional role of phenol-rich extracts from EVOOs in the prevention of DM. To the best of our knowledge, just one work reported that phenol-rich EVOO extracts from 'Frantoio', 'Ortice' and 'Ortolana' produced in Campania (Italy) showed antidiabetic activity, through inhibition of both enzymes (Loizzo, Di Lecce, Boselli, Menichini, & Frega, 2011). In addition, others phytoprostanes-rich EVOO extracts from 'Cornicabra' and 'Picual' varieties also were reported as possessors of strong hipoglycemic activity (Collado-González et al., 2017).

A dose-response behavior for α-glucosidase inhibition was observed for all the selected oils (**Figure 3**). It is very interesting to notice that rich-phenol extracts from EVOOs were stronger inhibitors of α-glucosidase than acarbose, used as positive control (IC_{50} = 356 ± 21 μg mL^{-1}). Data suggest a high efficacy of their active compounds for antidiabetic therapy. 'Cornicabra' and 'Picual' oils exhibited IC_{50} values of 246 ± 27 and 291 ± 37 μg of dry extract mL^{-1}, respectively (**Table 3**). Loizzo et al., (2011) had found IC_{50} values ranging between 184 and 776 μg of dry extract mL^{-1} for phenol-rich EVOO extracts produced in Italy. On the contrary, Collado-Gonzalez et al., (2017) found lower IC_{50} values with phytoprostanes-rich VOO extracts from 'Cornicabra' and 'Picual'

varieties (1.23 and 0.44 µg of dry extract mL^{-1}, respectively). This fact could be explained by the use of different extraction procedures, which affects the chemical composition of these extracts and, consequently, their biological activity.

With respect to α-amylase inhibition, as illustrated in **Table 3**, only the IC_{25} value for 'Cornicabra' was calculated (481 ± 21 mg of EVOO mL^{-1}). The strong inhibition of α-glucosidase and mild inhibitory activity against α-amylase seems to be positive in therapy for postprandial hyperglycemia, with lower side effects (Etxeberria et al., 2012). Our results are not in accordance with those published by Loizzo et al., (2011) who reported 50% inhibitory activity for phenol-rich EVOO extracts produced in Italy. Similarly, Collado-González et al., (2017) also reported higher activities for phytoprostanes-rich extracts from EVOOs produced in Spain (IC_{50} = 1.4-2.8 µg mL^{-1}). This fact could be ascribed to hexane-based extraction, which surely influenced the chemical composition of these extracts.

Given the fact that recent studies have also shown polyphenols from plant extracts to be effective inhibitors of α-amylase and α-glucosidase (Figueiredo-González et al., 2016; Tadera et al., 2006), data reported herein could be explained by the polyphenolic content of the tested extracts: 'Cornicabra' showed higher secoiridoid content (865 ± 62 mg kg^{-1}) *vs* 'Picual', which exhibited a concentration level of 506 ± 23 mg kg^{-1}, and consequently, 'Cornicabra' oil displayed the highest inhibitory effect on both antidiabetic enzymes. Loizzo et al., (2011) pointed out the positive effect of the secoiridoids on antidiabetic activity. In fact, they reported that 'Frantoio' from Futani (Italy) exhibited the highest phenolic and secoiridoid content and the major inhibitory activity against both enzymes.

Other compounds present in olive oil, such as Ol and Hyt, have stood out for their protective action against diabetes by *in vitro* and *in vivo* experimental studies (this represents a very interesting subject recently reviewed by Bulotta et al., (2016). Hadrich et al., (2015) observed that Hyt was a more potent inhibitor of α-glucosidase than acarbose. Nevertheless, the same was not observed for α-amylase. Anyway, the biological activity exhibited by our oils logically reflect not only the action of the molecules found at very high levels, but also the response of minor molecules, namely Lut and Apig, which could modulate the activity of the major constituents; a similar hypothesis has been previously proposed (Collado-González et al., 2017; Tadera et al., 2006). Moreover, synergistic interactions between phenolic compounds and/or multivariate interactions with other nonphenolic compounds, such as carotenoids and/or monounsaturated fatty acids, could be also relevant (Collado-González et al., 2017).

4. Conclusions

This is the first report evaluating the neuroprotective and antidiabetic potential ability of phenol-rich extracts from 'Cornicabra' and 'Picual' EVOOs. Phenolic bioactive compounds could act as multi-target ligands to inhibit multiple enzymes involved in Alzheimer's, Parkinson's diseases, major depressive disorder and *diabetes mellitus*.

Both phenol-rich extracts from EVOOs were able to inhibit simultaneously BuChE, LOX, *h*MAO-A and *h*MAO-B, in a dose-dependent manner, and could be considered as interesting natural products to be used as part of the strategies to reduce disorders affecting the CNS, including AD, MDD and PD. 'Cornicabra' oil extract was also able to inhibit AChE enzyme. This dual ChE inhibition can effectively increase the efficacy of the treatment of several disorders affecting the CNS.

As a promising result, it is worth highlighting that phenol-rich extracts from EVOOs could be considered as an effective complementary therapy for postprandial hyperglycemia due to the fact that they presented stronger inhibitions of α-glucosidase than the commercial inhibitor (acarbose).

It is important to note that, in general, 'Cornicabra' oil extracts exhibited a stronger inhibition of the studied enzymes than 'Picual' oil extracts; this fact can be linked to its higher contents in secoiridoids (865 *vs.* 506 mg kg^{-1}), lignans (1.7 *vs.* 1.1 mg kg^{-1}) and phenolic acids (0.48 *vs.* 0.20 mg kg^{-1}).

Although CNS inhibition activities were lower than those of the positive controls, our results suggest that the extracts obtained from 'Cornicabra' and 'Picual' EVOOs could represent valuable strategies to prevent and/or reduce events underlying CNS and hyperglycemia related-disorders. To confirm these preliminary results, *in vivo* assays

are needed to elucidate the molecular pathways and intracellular targets responsible for olive oil phenol's neuroprotective and antidiabetic effects.

Conflict of interest

The authors declare no conflict of interest.

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Figure legends

Figure 1. Overlapped base peak chromatograms (BPCs) obtained using the optimum LC-ESI-IT MS conditions for Cornicabra and Picual EVOO samples. The peak numbers are the same as those used in Table 1.

Figure. 2. Inhibition of acetylcholinesterase (AChE), butyrylcholinesterase (BuChE), 5-lipoxygenase (5-LOX), monoamine oxidase A (*h*MAO-A) and monoamine oxidase B (*h*MAO-B) by phenol-rich extracts from 'Cornicabra' and 'Picual' EVOOs. Results are expressed by mean ± SEM of three experiments, each performed in triplicate.

Figure. 3. α-Glucosidase and α-amylase inhibition by phenol-rich extracts from Cornicabra and Picual EVOOs. Results show mean ± SEM of three experiments, performed in triplicate.

Table 1. Main phenolic compounds (or related analytes) identified in the studied EVOOs samples by using LC-ESI-IT-MS.

Peak compound		Acronym	Molecular Formula	m/z	Retention Time (min)
Phenolic acids and others					
5	Vanillic acid	Van	$C_8H_8O_4$	167	9.8
7	*p*-Coumaric acid	*p*-Cou	$C_9H_8O_3$	163	11.8
8	Vanillin	Val	$C_8H_8O_3$	151	11.9
Flavonoids					
16	Luteolin	Lut	$C_{15}H_{10}O_6$	285	16.8
22	Apigenin	Apig	$C_{15}H_{10}O_5$	269	19.8
23	Diosmetin	Dios	$C_{16}H_{12}O_6$	299	20.4
Lignans					
15	Syringaresinol	Syr	$C_{22}H_{25}O_8$	417	16.4
17	Pinoresinol	Pin	$C_{20}H_{22}O_6$	357	17.5
18	Acetoxypinoresinol	Ac-Pin	$C_{22}H_{24}O_8$	415	18.2
Simple phenols					
2	Oxidized hydroxytyrosol	O-Hyt	$C_8H_8O_3$	151	3.2
3	Hydroxytyrosol or 3,4-dihydroxyphenylethanol	Hyt	$C_8H_{10}O_3$	153	6.9
4	Tyrosol or *p*-hydroxyphenylethanol	Ty	$C_8H_{10}O_2$	137	8.7
11	Hydroxytyrosol acetate	Hyt-Ac / 3,4-DHPEA-AC	$C_{10}H_{12}O_4$	195	13.3
Secoiridoids					
	Oleuropein derivatives				
13	Hydroxy decarboxymethyl oleuropein aglycone	Hy-D-Ol-Agl	$C_{17}H_{20}O_7$	335	14.5
14	Dialdehydic form of decarboxymethyl oleuropein aglycone or Dialdehydic form of decarboxymethyl elenolic acid linked to hydroxytyrosol	DOA / 3,4-DHPEA-EDA	$C_{17}H_{20}O_6$	319	15.3
19	10-hydroxy oleuropein aglycone	10 Hy-Ol-Agl	$C_{19}H_{22}O_9$	393	18.2
25	Oleuropein aglycone (main peak)	Ol Agl / 3,4 DHPEA-EA	$C_{19}H_{22}O_8$	377	21.8
20	Oleuropein aglycone (Isomer 1)	Ol Agl (Is1) / 3,4 DHPEA-EA (Is1)	$C_{19}H_{22}O_8$	377	18.5
27	Oleuropein aglycone (Isomer 2)	Ol Agl (Is2) / 3,4 DHPEA-EA (Is2)	$C_{19}H_{22}O_8$	377	22.4
28	Methyl oleuropein aglycone	Methyl Ol Agl	$C_{20}H_{24}O_8$	391	23.5; 23.7
	Ligstroside derivatives				
21	Dialdehydic form of decarboxymehtyl ligstroside aglycone or Dialdehydic form of decarboxymethyl elenolic acid linked to tyrosol or Oleocanthal	D-Lig Agl / *p*-HPEA-EDA	$C_{17}H_{20}O_5$	303	18.6
29	Ligstroside aglycone (main peak)	Lig Agl / *p*-HPEA-EA	$C_{19}H_{22}O_7$	361	23.7
24	Ligstroside aglycone (Isomer 1)	Lig Agl (Is1) / *p*-HPEA-EA (Is1)	$C_{19}H_{22}O_7$	361	21.6
26	Ligstroside aglycone (Isomer 2)	Lig Agl (Is2) / *p*-HPEA-EA (Is2)	$C_{19}H_{22}O_7$	361	22.1
30	Ligstroside aglycone (Isomer 3)	Lig Agl (Is3) / *p*-HPEA-EA (Is3)	$C_{19}H_{22}O_7$	361	23.9
Non phenolic but structurally-related compounds					
1	Quinic acid		$C_7H_{12}O_6$	191	2.0
	Elenolic acid derivatives				
6	Decarboxymethylated form of elenolic acid or Dialdehydic form of decarboxymethyl of elenolic acid	D-Ald-D EA	$C_9H_{12}O_4$	183	10.6
9	Desoxy elenolic acid	Desoxy-EA	$C_{11}H_{14}O_5$	225	12.3
10	Hydroxy elenolic acid or hydroxylated form of elenolic acid	Hy-EA	$C_{11}H_{14}O_7$	257	12.7
12	Elenolic acid	EA	$C_{11}H_{14}O_6$	241	14.3

Table 2. Concentration levels of the phenolic compounds found in the target extra EVOOs expressed as mean ± standard deviation (mg kg^{-1}, n=4).

Phenolic compounds			**Cornicabra**	**Picual**
Phenolic acids and others		Van	0.04 ± < 0.01 [a]	0.05 ± < 0.01 [a]
		p-Cou	0.19 ± < 0.01 [a]	0.09 ± < 0.01 [b]
		Val	0.09 ± 0.01 [a]	0.06 ± < 0.01 [b]
		Sub-total	*0.31 ± 0.01* [a]	*0.20 ± <0.01* [b]
Flavonoids		Lut	0.71 ± 0.03 [a]	0.67 ± 0.05 [a]
		Apig	0.19 ± 0.01 [a]	0.19 ± 0.02 [a]
		Diosmetin	0.09 ± 0.01 [a]	0.12 ± < 0.01 [b]
		Sub-total	*0.99 ± 0.03* [a]	*0.98 ± 0.06* [a]
Lignans		Syr	0.21 ± 0.02 [a]	0.11 ± 0.01 [b]
		Pin	1.0 ± < 0.1 [a]	0.90 ± 0.03 [a]
		Ac-Pin	0.45 ± < 0.01 [a]	0.08 ± < 0.01 [b]
		Sub-total	*1.7 ± 0.09* [a]	*1.1 ± 0.03* [b]
Simple phenols		O-Hyt	0.09 ± < 0.01 [a]	0.20 ± 0.01 [b]
		Hyt	0.96 ± 0.03 [a]	9.7 ± 0.3 [b]
		Ty	2.1 ± 0.1 [a]	5.6 ± 0.2 [b]
		Hyt-Ac	nd	2.3 ± 0.01
		Sub-total	*3.2 ± 0.01* [a]	*18 ± 0.10* [b]
Secoiridoids	*Oleuropein derivatives*	Hy-D-Ol Agl	1.3 ± 0.1 [a]	9.7 ± 0.6 [b]
		DOA	165 ± 18 [a]	92 ± 4 [b]
		10 Hy-Ol Agl	nd	4.8 ± 0.3
		Ol Agl (IS1)	9.1 ± 0.6 [a]	7.2 ± 0.5 [b]
		Ol Agl	56 ± 5 [a]	91 ± 10 [b]
		Ol Agl (IS 2)	12 ± 1 [a]	9.0 ± 0.1 [b]
		Methyl Ol Agl	nd	2.7 ± 0.1
	Ligstroside derivatives	D-Lig Agl	369 ± 31 [a]	84 ± 0.4 [b]
		Lig Agl (IS 1)	42 ± 3 [a]	29 ± 2 [b]
		Lig Agl (IS 2)	7.6 ± 0.3 [a]	6.3 ± 0.03 [b]
		Lig Agl	181 ± 10 [a]	154 ± 1 [b]
		Lig Agl (IS 3)	23 ± 3 [a]	16 ± 1 [b]
		Sub-total	*865 ± 62* [a]	*506 ± 23* [b]
Non-phenolic compounds but structurally-related compounds				
	Elenolic acid derivatives	D-Ald-D EA	nd	0.67 ± 0.06
		Desoxy-EA	20 ± 1 [a]	1.8 ± 0.1 [b]
		Hy-EA	0.12 ± 0.01 [a]	3.4 ± 0.1 [b]
		EA	209 ± 38 [a]	190 ± 22 [b]
		Sub-total	*229 ± 26* [a]	*196 ± 22* [b]
		Quinic acid	0.16 ± 0.01	nd
Total			**1100** ± 50 [a]	**722** ± 17 [b]

nd: not detected.
Different letters in the same row indicate significant differences (p <0.05).

Table 3. Effect of the extracts (from Cornicabra and Picual EVOOs) and positive controls against the enzymes involved in neurodegenerative and diabetes disorders.

	Oils							
	EVOO				Positive Controls [3]			
	Cornicabra		Picual		Galantamine	Quercetin	Clorgyline	Acarbose
	µg dry extract mL^{-1}	mg EVOO mL^{-1}	µg dry extract mL^{-1}	mg EVOO mL^{-1}				
Neuroprotection								
BuChE [2]	249 ± 6	156 ± 4 [a]	357 ± 38	308 ± 33 [b]	6.8 ± 0.5			
AChE [1]	805 ± 58	503 ± 36	–	–	2.1 ± 0.3			
LOX [2]	42 ± 1	26 ± 0.5 [a]	43 ± 4	37 ± 3 [b]		3.2 ± 0.2		
*h*MAO-A [2]	32 ± 4	20 ± 2 [a]	43 ± 0.3	37 ± 0.2 [b]			0.035 ± 0.005	
*h*MAO-B [2]	209 ± 11	131 ± 7 [a]	249 ± 15	215 ± 13 [b]			23 ± 0.3	
Diabetes								
α-Glucosidase [2]	246 ± 27	154 ± 17 [a]	291 ± 37	251 ± 31 [b]				356 ± 21
α-Amylase [1]	770 ± 34	481 ± 21	–	–				1.4 ± 0.06

[1] IC_{25} values for the olive oils.

[2] IC_{50} values for the olive oils.

[3] IC_{50} values expressed as µg mL^{-1} for positive controls.

Different letters indicate significant differences (p <0.05) between values expressed as mg EVOO mL^{-1} for the oils.

Highlights

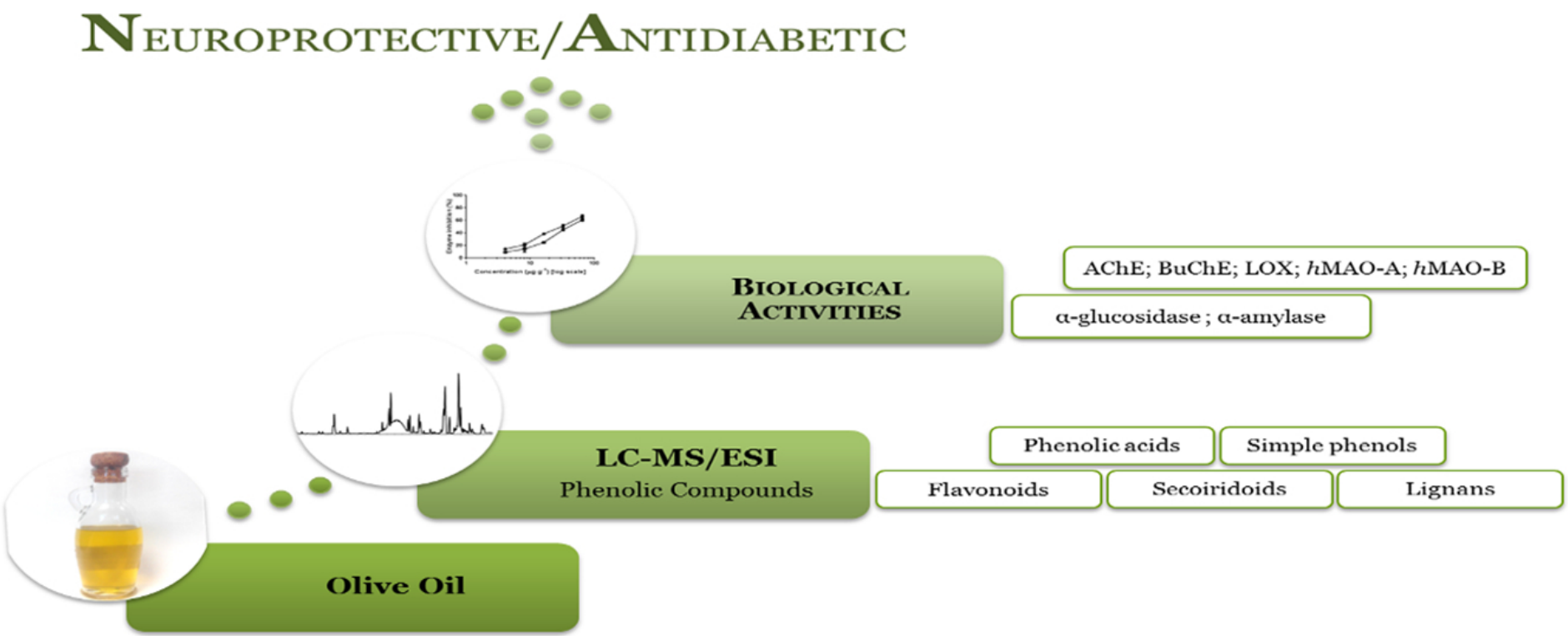
1) 'Cornicabra' extracts showed higher phenolic concentration than 'Picual' extracts.

2) 'Cornicabra' and 'Picual' EVOOs could present the EFSA health claim on the oil label.

3) Phenol-rich extracts from EVOO could be involved CNS and hyperglycemia disorders.

4) VOO extracts were stronger inhibitors of α-glucosidase than commercial inhibitor.

5) 'Cornicabra' exhibited stronger inhibition of target enzymes than 'Picual' extracts.



Graphics Abstract

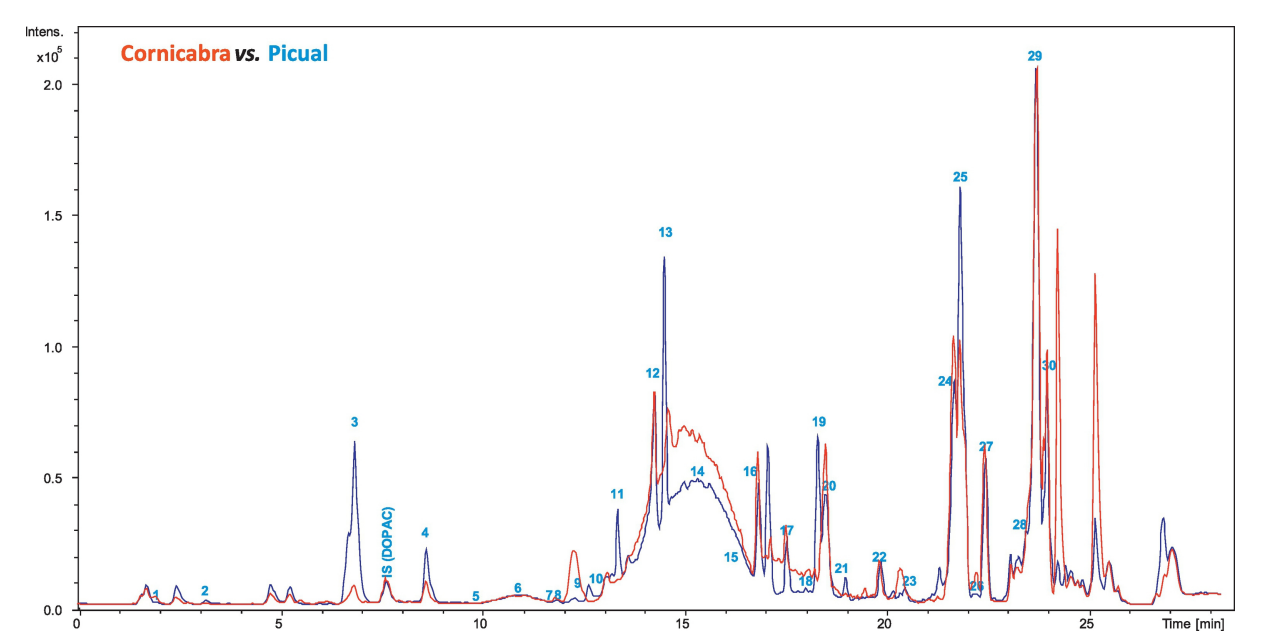

Intens.
x10^5
Cornicabra vs. Picual
2.0
1.5
1.0
0.5
0.0
0
5
10
15
20
25
Time [min]
1
2
3
IS (DOPAC)
4
5
6
7
8
9
10
11
12
13
14
15
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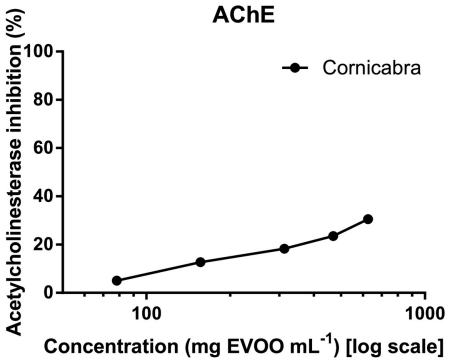
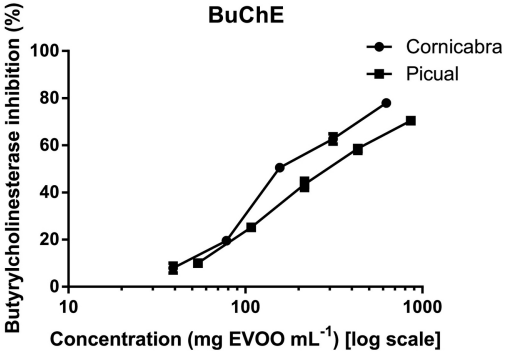
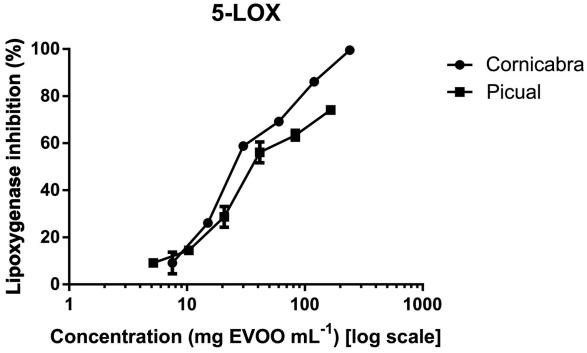
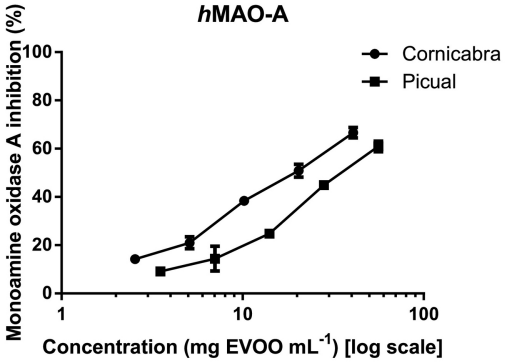
Figure 1


AChE
Acetylcholinesterase inhibition (%)
Cornicabra
Concentration (mg EVOO mL-1) [log scale]


BuChE
Butyrylcholinesterase inhibition (%)
Cornicabra
Picual
Concentration (mg EVOO mL-1) [log scale]


5-LOX
Lipoxygenase inhibition (%)
Cornicabra
Picual
Concentration (mg EVOO mL-1) [log scale]


hMAO-A
Monoamine oxidase A inhibition (%)
Cornicabra
Picual
Concentration (mg EVOO mL-1) [log scale]

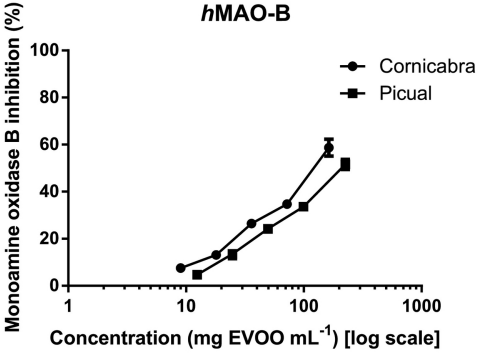
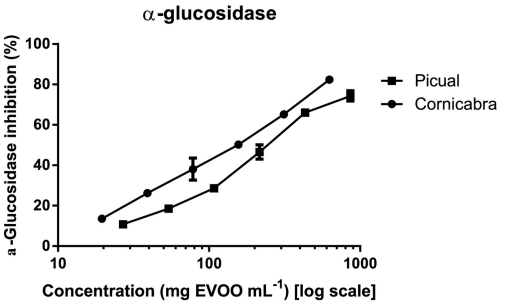

hMAO-B
Monoamine oxidase B inhibition (%)
Cornicabra
Picual
Concentration (mg EVOO mL-1) [log scale]

Figure 2


α-glucosidase
α-Glucosidase inhibition (%)
100
80
60
40
20
0
10
100
1000
Concentration (mg EVOO mL-1) [log scale]
Picual
Cornicabra

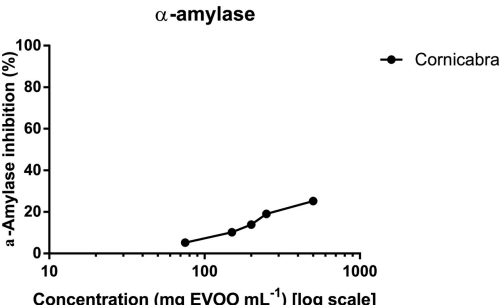

α-amylase
α-Amylase inhibition (%)
100
80
60
40
20
0
10
100
1000
Concentration (mg EVOO mL-1) [log scale]
Cornicabra

Figure 3