HPLC-ESI-QTOF-MS as a powerful analytical tool for characterizing phenolic compounds in olive leaf extracts

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Keywords:	olive leaves, phenolic compounds, high-performance liquid chromatography, electrospray ionization, quadrupole time of flight

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HPLC-ESI-QTOF-MS as a powerful analytical tool for characterizing phenolic compounds in olive leaf extracts

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Keywords: olive leaves; phenolic compounds; high-performance liquid chromatography; electrospray ionization; quadrupole time of flight.

SHORT ABSTRACT

HPLC-ESI-QTOF was used to characterize the phenolic compounds from two olive leaves extracts obtained by pressurized liquid extraction using water and ethanol as extracting solvents. The information provided by QTOF mass spectrometer enabled the in-depth characterization of both olive leaf extracts, allowing the tentative identification of 31 different phenolic compounds in these extracts including secoiridoids, simple phenols, flavonoids, cinnamic-acid derivatives and benzoic acids. A new compound in olive leaves, lucidumoside C, was also identified.

ABSTRACT

Introduction – *Olea europaea* L. leaves may be considered a cheap, easily available natural source of phenolic compounds. In a previous study we evaluated the possibility of obtaining bioactive phenolic compounds from olive leaves by pressurized liquid extraction (PLE) for their use as natural antioxidants. The alimentary use of these kinds of extract makes a comprehensive knowledge of their composition essential.

Objective – To undertake a comprehensive characterization of two olive leaf extracts obtained by PLE using high-performance liquid chromatography coupled to electrospray ionization-quadrupole time of flight mass spectrometry (HPLC-ESI-QTOF-MS).

Methodology – Olive leaves were extracted by PLE using ethanol and water as extraction solvents at 150°C and 200°C respectively. Separation was carried out in a HPLC system equipped with a C18 column working in a gradient elution program coupled to ESI-QTOF-MS operating in negative ion mode.

Results – This analytical platform was able to detect 48 compounds and tentatively identify 31 different phenolic ones in these extracts, including secoiridoids, simple phenols, flavonoids, cinnamic-acid derivatives and benzoic acids. Lucidumoside C, a new compound in olive leaves, was also identified.

Conclusion – The coupling HPLC-ESI-QTOF-MS led to the in-depth characterization of the olive leaf extracts on the basis of mass accuracy, true isotopic pattern and tandem mass spectrometry (MS/MS) spectra. We may conclude therefore that this analytical tool is very valuable in the study of phenolic compounds in plant matrices.

INTRODUCTION

The last decade has witnessed an ever increasing interest in phenolic compounds because of their health-giving properties with regard to the prevention of degenerative diseases. Phenolic compounds in general show a broad spectrum of bioactive properties, including antioxidant, anti-inflammatory, antimicrobial, antiproliferative, anti-arrhythmic, platelet anti-aggregant and vasodilatory effects (Scalbert et al., 2005). As a result there has been growing interest in the use of these phytochemicals as natural antioxidants in the food industry as a way of providing additional value to common foodstuffs.

Olea europaea L. leaves are a significant by-product in olive oil production, containing as they do high quantities of phenolic compounds, and may be considered a cheap, easily available natural source of these phytochemicals. As far as their antioxidant activity is concerned, several studies have described how exert hypoglycaemic, antihypertensive, antimicrobial, antiviral and anti-atherosclerotic effects (El et al., 2009).

In a previous study we evaluated the possibility of obtaining bioactive phenolic compounds from olive leaves by PLE for their use as natural antioxidants (Herrero et al., 2011). The alimentary use of these kinds of extract makes a comprehensive knowledge of their composition essential. Therefore, as a continuation of our previous study, we have undertaken a further, more detailed characterization of the most promising olive leaf extracts obtained.

The precise identification of phenolic compounds can be a complex task as they contain a wide variety of structures. Within this context, HPLC–MS has proved to be a very useful tool in the characterization of natural products (Careri et al., 1998; Xing et al.,

2007). ESI in particular, has been widely applied as it is a mild ionization technique resulting in both protonated and deprotonated molecules. Accurate mass measurement of small molecules is used to determine elemental formulas, thus enabling the identification of unknown substances. Sometimes, because compounds in real samples co-elute or MS is unable to distinguish between isobaric substances, structural information may be needed and this can be obtained via MS/MS by means of collision-induced dissociation (CID). QTOF-MS combines high sensitivity and mass accuracy for both precursor and product ions, providing the elemental composition of the parent and fragment ions. This feature helps to identify compounds thoroughly and to differentiate between isobaric compounds. The potential of HPLC-ESI-QTOF-MS for qualitative purposes has been highlighted in several studies (Rodríguez-Medina et al. 2009; Gómez-Romero et al., 2011).

The aim of our work here was to undertake a comprehensive characterization of two olive leaf extracts by HPLC-ESI-QTOF-MS and thus arrive at an in-depth knowledge of their composition.

EXPERIMENTAL

Chemicals

All chemicals were of analytical reagent grade and used as received. Acetic acid and acetonitrile for HPLC were from Fluka, Sigma-Aldrich (Steinheim, Germany) and Lab-Scan (Gliwice, Sowinskiego, Poland) respectively. Water was purified by a Milli-Q system from Millipore (Bedford, MA, USA). All the standard compounds were from Sigma-Aldrich (St. Louis, MO). The stock solutions containing these analytes were

prepared in methanol (Lab-Scan, Gliwice, Sowinskiego, Poland) and stored at -20°C until use.

Samples

Dried olive leaves (*Olea europaea* L., variety Hojiblanca), generated as by-products from the olive oil industry, were provided by “Cooperativa Sor Angela de la Cruz” (Sevilla, Spain). The leaves were dried following a traditional procedure: once the leaves were separated from the rest of plant materials they were covered to avoid direct light and left ventilated at ambient temperature to remove humidity for about 50 days, depending upon the relative humidity of the air. The dried leaves were then ground up under liquid nitrogen and stored in darkness at 4°C until use.

Pressurized Liquid Extraction (PLE)

The phenolic compounds were extracted from olive leaves as described by Herrero et al., 2011. In brief, an accelerated solvent extractor (ASE 200, Dionex, Sunnyvale, CA, USA) was used with ethanol or water as extracting solvents. The ethanol and water extracts were obtained at 150°C and 200°C respectively, with a static time equal to 20 min.

HPLC-ESI-QTOF-MS analyses

Analyses were made using an Agilent 1100 Liquid Chromatography system (Agilent Technologies, Palo Alto, CA, USA) equipped with a standard autosampler. The HPLC column was a Phenomenex Gemini C18 (3 µm, 2 x 150 mm). Separation was carried out at 25°C with a gradient elution program at a flow rate of 0.2 ml/min. The mobile phases consisted of water plus 0.5% acetic acid (A) and acetonitrile (B). The following multi-step linear gradient was applied: 0 min, 5% B; 5 min, 15% B; 25 min, 30% B; 35 min, 95% B; 40 min, 5% B. The initial conditions were maintained for 5 min. The injection volume in the HPLC system was 1 µl.

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3 130 The HPLC system was coupled to a micrOTOF-Q II mass spectrometer (Bruker
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5 131 Daltoniks, Bremen, Germany) equipped with an ESI interface (Bruker Daltoniks,
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7 132 Bremen, Germany) operating in negative ion mode using a capillary voltage of +4 kV.
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10 133 The other optimum values of the ESI-QTOF-MS parameters were drying gas
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12 134 temperature, 210°C; drying gas flow, 8 l/min; and nebulizing gas pressure, 2 bar.
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15 135 Detection was carried out within a mass range of 50-1100 m/z. Collision energy values
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17 136 for MS/MS experiments were adjusted as follows: m/z 100, 20 eV; m/z 500, 30 eV; m/z
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19 137 1000, 35 eV. Nitrogen was used as drying, nebulizing and collision gas.
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22 138 The accurate mass data of the molecular ions were processed using DataAnalysis 4.0
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24 139 software (Bruker Daltoniks), which provided a list of possible elemental formulas via
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26 140 the GenerateMolecularFormula Editor. The GenerateMolecularFormula Editor uses a
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28 141 CHNO algorithm, which provides standard functionalities such as minimum/maximum
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30 142 elemental range, electron configuration and ring-plus double-bond equivalents, as well
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32 143 as a sophisticated comparison of the theoretical with the measured isotope pattern
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34 144 (Sigma Value) for increased confidence in the suggested molecular formula. The widely
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36 145 accepted accuracy threshold for confirmation of elemental compositions was established
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38 146 at 5 ppm (Bringmann et al., 2005). It is important to point out that even with very high
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40 147 mass accuracy (<1ppm) many chemically possible formulas may be obtained,
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42 148 depending upon the mass regions considered and so high mass accuracy alone is not
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44 149 enough to exclude enough candidates with complex elemental compositions. The use of
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46 150 isotopic abundance patterns as a single further constraint, however, removes >95% of
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48 151 the false candidates. This orthogonal filter can reduce several thousand candidates down
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50 152 to only a small number of molecular formulas.
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53 153 During the development of the HPLC method, the instrument was calibrated externally
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55 154 with a 74900-00-05 Cole Palmer syringe pump (Vernon Hills, Illinois, USA) directly
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connected to the interface and injected with a sodium acetate cluster solution containing 5 mM sodium hydroxide and 0.2% acetic acid in water:isopropanol (1:1, v/v). The calibration solution was injected at the beginning of each run and all the spectra were calibrated prior to compound identification. By using this method, an exact calibration curve based on numerous cluster masses, each differing by 82 Da ($\text{NaC}_2\text{H}_3\text{O}_2$), was obtained. Due to the compensation of temperature drift in the micrOTOF-Q II, this external calibration provided accurate mass values of better than 5 ppm for a complete run without the need for a dual sprayer setup for internal mass calibration.

RESULTS AND DISCUSSION

Figure 1 shows the base peak chromatograms (BPC) of both olive leaf extracts obtained by PLE using ethanol at 150°C (OL150ET) and water at 200°C (OL200W) as extracting solvents.

The compounds were identified by comparing their retention times and MS/MS spectra provided by QTOF-MS with those of authentic standards whenever available. The remaining compounds were identified by interpretation of their MS and MS/MS spectra obtained by QTOF-MS combined with the data provided in the literature. Table 1 summarizes the MS data of the identified compounds, including experimental and calculated m/z for the molecular formulas provided, error, sigma value and the main fragments obtained by MS/MS, as well as the proposed compound for each peak. As can be seen from these data, the phenolic compounds identified belong to different classes such as secoiridoids, simple phenols, flavonoids, cinnamic acid derivatives and benzoic acids.

Secoiridoids

Olea europaea L. is rich in secoiridoids, especially in oleosides, which are oleaceae-specific secoiridoids commonly esterified to a phenolic moiety. In fact one of the major compounds found in both extracts was oleuropein (compound **38**), which was confirmed by comparison with the authentic standard. This compound has been described previously as being the main component of olive leaves (Benavente-García et al., 2000; Briante et al., 2002; Chiou et al., 2007; Pererira et al., 2007; Salta et al., 2007; Altioek et al., 2008; Mylonaki et al., 2008; Goulas et al., 2009, 2010; Laguerre et al., 2009; Fu et al., 2010). Two other oleuropein isomers with a similar fragmentation pattern (**39** and **41**) were present in the extracts. Oleuropein has traditionally been found in olive leaves together with its isomer oleuroside, which has been proposed as compound **41** on the basis of its elution order. Other oleuropein derivatives such as hydroxyoleuropein (**23**) and several oleuropein diglucoside isomers (**30**, **31** and **37**), were also found in both extracts.

Peak **6** has been identified as oleoside or secologanoside, both of which have been reported before in olive leaves (Di Donna et al., 2010; Kiritsakis et al., 2010; Poudyal et al., 2010). Because of the identical fragmentation pattern of these analytes (Fu et al., 2010), it was impossible to distinguish between them.

Oleoside methyl ester has been proposed as compound **14**. The presence of this oleoside derivative in the olive fruit has been reported before, together with its main fragments at m/z 119.0345 and 89.0231 (Bianco et al., 2003; Di Donna et al., 2007), and it has recently been identified in olive leaves by Di Donna et al., 2010. Peak **18** showed the same molecular formula as oleoside methyl ester but its MS/MS spectrum presented a fragment at m/z 223.0607, which corresponds to dehydrated elenolic acid and so this compound was proposed as being elenolic acid glucoside, which has been identified in

the same matrix by Fu et al., 2010. The diglucosidic form of elenolic acid has also been found in the ethanolic extract (**10**).

Compound **17** has been characterized as 7-epiloganin, an intermediate in the biosynthesis of oleoside-type secoiridoids (Jensen et al., 2002) that has been reported previously in olive leaves (Rovellini and Cortesi, 1998). This assignment was consistent with the presence in the MS/MS spectrum of a fragment at 169.0885, representing the loss of the glucose moiety and the methylester group, as shown in figure 2. Subsequent dehydration was responsible for producing the major product ion at 151.0760. Another fragment was detected at 357.1194, corresponding to the elimination of the methoxyl group from the precursor ion.

The software provided the same molecular formula for peaks **42**, **43** and **46** and they also showed a similar fragmentation pattern. They were tentatively identified as lucidumoside C and its isomers although it was impossible to assign any specific peak to lucidumoside C itself. The structure of this secoiridoid closely resembles that of oleuropein and it has been reported previously in other species belonging to the *Oleaceae* family, such as *Ligustrum lucidum* (He et al., 2001; Guo et al., 2011). The proposed fragmentation pathway is depicted in figure 3. The intense fragment at 403.1235 was due to cleavage of the phenolic moiety, after which it could undergo elimination of the methoxyl group (corresponding to fragment 371.0973) or glucose moiety (fragment 223.0601). The ion at 537.1603 could be attributed to the loss of the ethoxyl group from the precursor ion whilst the fragment at 351.1085 arose from the cleavage of the elenolic ring as described for oleuropein (Japón-Luján et al., 2008). The main fragment at 151.0389 matched with the elimination of the ethoxyl group from the phenolic moiety.

Compound **44** corresponded to 6'-O-[2,6-dimethyl-8-hydroxy-2-octenoyloxy]secologanoside, the structure and fragmentation pathway of which are shown in figure 4. Its assignment was consistent with the presence of fragments found at m/z 227.0550 and 183.0652, representing the loss of the glucose moiety and its subsequent decarboxylation. This compound has been described in the past in olive leaves with boron deficiency (Karioti et al., 2006).

Compound **45** was proposed as being ligstroside since its molecular formula and fragmentation pattern agreed with the data reported in the literature and it has also been widely described in olive leaves (Briante et al., 2002; Laguerre et al., 2009; Fu et al., 2010).

Simple phenols

Hydroxytyrosol has been widely described as one of the main components of olive leaves (Benavente-García et al. 2000; Briante et al., 2002; Chiou et al., 2007; Salta et al.; Altioek et al., 2008; Goulas et al., 2009, 2010; Fu et al., 2010) and it has been found in both extracts (**7**), identifying it by comparison with commercial standards. Its acetate derivative has also been identified (**28**).

Flavonoids

Flavonoids are another important group of phenolic compounds widely represented in olive leaves. Among these, luteolin-7-O-glucoside (**29**), rutin (**26**), apigenin-7-O-glucoside (**35**) and luteolin (**48**) are the most cited in the literature (Benavente-García et al. 2000; Meirinhos et al., 2005; Pereira et al., 2007; Altioek et al., 2008; Mylonaki et al., 2008; Goulas et al., 2009, 2010; Laguerre et al., 2009; Fu et al., 2010) and all of them were identified in the extracts by comparing their molecular formulas and fragmentation patterns with those reported in the literature and databases.

Other flavonoids found in the extracts were luteolin-7,4-O-diglucoside (**19**), luteolin-7-O-rutinoside (**25**), apigenin-7-O-rutinoside (**32**) and luteolin-4-O-glucoside or luteolin-3-O-glucoside (**36**). The latter compounds have also been reported in previous studies although with a narrower spread (Meirinhos et al., 2005; Pereira et al., 2007; Mylonaki et al., 2008; Goulas et al., 2009, 2010; Laguerre et al., 2009).

Cinnamic acids and derivatives

Compound **27** was identified as verbascoside according to the MS data and by comparison with the retention time and MS/MS spectrum of the standard. This cinnamic acid derivative is commonly present in all the derivatives of the olive tree (Benavente-García et al. 2000; Pereira et al., 2007; Altioek et al., 2008; Laguerre et al., 2009; Fu et al., 2010).

The aqueous extract also contained p-coumaric acid (**11**). The molecular ion of p-coumaric acid (m/z 163.0401) produced the major fragment ion at m/z 119.0497, corresponding to the loss of carbon dioxide from the precursor ion.

Benzoic acids

As far as benzoic acids are concerned, vanillin (**12**) and p-hydroxybenzoic acid (**9**) were identified in the extracts. These compounds have been described previously in the literature (Benavente-García et al., 2000; Chiou et al., 2007; Salta et al., 2007; Altioek et al., 2008). These assignments were supported by the fragment ions produced in MS/MS spectra. In the case of vanillin, just one ion, at m/z 123.0449, was yielded from the loss of the carbonyl group, whilst the p-hydroxybenzoic acid spectrum showed an ion at m/z 119.0318, resulting from dehydration of the parent ion.

Unknown compounds

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275 Table 2 shows a list of compounds for which it was impossible to elucidate a structure
276 due to a lack of sufficient evidence. The table includes retention times, experimental
277 m/z, molecular formulas, errors, sigma values and MS/MS fragments.
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279 In summary, a powerful analytical method has been used to characterize
280 comprehensively two olive leaf extracts obtained by PLE using ethanol and water as
281 solvents. The coupling HPLC-ESI-QTOF-MS enabled us to characterize tentatively
282 more than 30 different phenolic compounds, including secoiridoids, simple phenols,
283 flavonoids, cinnamic acid derivatives and benzoic acids. We may conclude therefore
284 that this analytical tool is very valuable in the study of phenolic compounds in plant
285 matrices. It is also important to highlight that, to the best of our knowledge, this is the
286 first time that lucidumoside C has been detected in olive leaves.
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FIGURE CAPTIONS

Figure 1. BPC (50–1100 *m/z*) of OL150ET extract (a) and OL200W extract (b), in which the peaks are identified with numbers 1-48 according to the order of elution.

Figure 2. Proposed fragmentation pathway for 7-epiloganin (compound 17).

Figure 3. Proposed fragmentation pathway for lucidumoside C (compound 42, 43 or 46).

Figure 4. Structure and fragmentation pathway of 6'-O-[2,6-Dimethyl-8-hydroxy-2-octenoyloxy]secologanoside (compound 44).

408 **Table 1.** Compounds identified in olive leaf extracts

Peak	RT (min)	Measured m/z	Formula	Theoretical m/z	Error (ppm)	mSigma	Fragments	Proposed compound	Extract
1	1.84	181.0717	C 6 H 13 O 6	181.0718	0.1	38.1	71.0105 (100), 89.0261 (50.2), 101.0239 (98.3), 113.0282 (54)	sugar	OL150ET OL200W
2	2.58	195.0516	C 6 H 11 O 7	195.0510	-2.8	1.3	75.0065 (100.0), 99.0075 (24.9), 129.0190 (79.5), 177.0378 (34.3), 192.0624 (45.6)	gluconic acid	OL200W
3	2.6	191.0559	C 7 H 11 O 6	191.0561	1.1	44.3	85.0274 (11.9), 93.0320 (5.6), 127.0387 (10.6)	quinic acid	OL150ET
6	6.61	389.1083	C 16 H 21 O 11	389.1089	1.6	18.9	89.0216 (20.8), 113.0211 (20.8), 119.0365 (25), 121.0645 (100), 165.0547 (32.4), 183.0641 (61.4)	oleoside/secologanoside	OL150ET OL200W
7	6.73	153.0553	C 8 H 9 O 3	153.0557	3	50.1	123.0446 (100)	hydroxytyrosol ^a	OL150ET OL200W
9	9.09	137.0276	C 7 H 5 O 3	137.0244	-23.6	11.9	108.0232 (100.0), 119.0318 (87.9)	p-hydroxybenzoic acid	OL200W
10	9.11	565.1753	C 23 H 33 O 16	565.1774	3.7	37.2		elenolic acid diglucoside	OL150ET
11	9.56	163.0401	C 9 H 7 O 3	163.0401	-0.1	41.6	107.0483 (55.7), 119.0497 (100.0), 121.0279 (65.2), 135.0381 (52.4)	p-coumaric acid	OL200W
12	9.58	151.0393	C 8 H 7 O 3	151.0401	4.8	779.3	123.04489 (100)	vanillin	OL150ET OL200W
14	10.79	403.1234	C 17 H 23 O 11	403.1246	3	20.9	89.0231 (86.6), 101.0222 (100), 113.0225 (59.9), 119.0345 (70.6)	oleoside methyl ester	OL150ET
17	11.74	389.1436	C 17 H 25 O 10	389.1453	4.3	23.5	101.0226 (21.4), 113.0239 (25.6), 151.0760 (100.0), 161.0479 (12.9), 169.0885 (16.7), 183.1028 (29.2), 313.1331 (14.8), 357.1194 (23.7)	7-epiloganin	OL150ET OL200W
18	12.56	403.1233	C 17 H 23 O 11	403.1246	3.3	20.4	89.0235 (13.6), 101.0229 (16.9), 113.0239 (22.9), 119.0347 (16.2), 121.0287 (100), 127.0373 (23.8), 139.0051 (24.7), 165.0542 (68.9), 181.0490 (19.1), 223.0607 (13.8), 371.0967 (13.7)	elenolic acid glucoside	OL150ET OL200W
19	12.62	609.1448	C 27 H 29 O 16	609.1461	2.1	38.5	285.0383 (10.3), 447.0920 (100)	luteolin-7,4-O-diglucoside	OL150ET
23	15.16	555.1718	C 25 H 31 O 14	555.1719	0.2	33.5	151.0392 (100), 223.0619 (12.6), 323.0792 (6.7)	hydroxyoleuropein	OL150ET OL200W
25	16.16	593.1514	C 27 H 29 O 15	593.1512	-0.3	37.7	285.0397 (60.8)	luteolin-7-O-rutinoside	OL150ET OL200W
26	16.33	609.1464	C 27 H 29 O 16	609.1461	-0.5	38.5	151.0030 (4.3), 178.9964 (4.5), 300.0264 (100.0)	rutin	OL150ET
27	16.62	623.1981	C 29 H 35 O 15	623.1981	0.1	42	161.0241 (100.0), 179.0341 (14.8), 461.1626 (14.4)	verbascoside ^a	OL150ET
28	16.85	195.0664	C 10 H 11 O 4	195.0663	-0.7	9.6	135.0527 (100)	hydroxytyrosol acetate	OL150ET OL200W
29	17.15	447.0931	C 21 H 19 O 11	447.0933	0.3	25	285.0392 (100)	luteolin-7-O-glucoside ^a	OL150ET OL200W
30	18.16	701.2294	C 31 H 41 O 18	701.2298	0.7	10.7	149.0245 (31.2), 179.0592 (28.7), 275.0907 (100.0), 307.0810 (75.4), 377.1216 (64.7), 539.1742 (69.1)	oleuropein diglucoside (isomer 1)	OL150ET
31	18.63	701.2294	C 31 H 41 O 18	701.2298	0.7	48.9	179.0547 (11.7), 275.0870 (15.3), 307.0841 (15.2), 377.1175 (18.1), 539.1782 (100)	oleuropein diglucoside (isomer 2)	OL150ET OL200W
32	18.86	577.1561	C 27 H 29 O 14	577.1563	0.2	37.2	269.044 (100)	apigenin-7-O-rutinoside	OL150ET OL200W

35	20.25	431.0984	C 21 H 19 O 10	431.0984	-0.2	23.1	268.0371 (41.3)	apigenin 7-O-glucoside	OL150ET OL200W
36	20.67	447.0936	C 21 H 19 O 11	447.0933	-0.7	25.1	285.0396 (100)	luteolin 4-O-glucoside/ luteolin 3-O-glucoside	OL150ET OL200W
37	21.37	701.2297	C 31 H 41 O 18	701.2298	0.1	49.3	113.0221 (25.8), 149.0232 (25.1), 161.0451 (31.3), 179.0561 (16.8), 275.0931 (100), 307.0829 (66.4), 345.0988 (23.1), 377.1246 (96.6)	oleuropein diglucoside (isomer 3)	OL150ET OL200W
38	21.92	539.1768	C 25 H 31 O 13	539.1770	0.3	32.5	139.0369 (25.3), 149.0242 (44.5), 179.0562 (19.7), 223.0611 (45.7), 275.0878 (100.0), 307.0814 (89.0), 377.1238 (19.8)	oleuropein ^a	OL150ET OL200W
39	22.84	539.1776	C 25 H 31 O 13	539.1770	-1.1	32.4	139.0382 (45.7), 149.0243 (78.3), 223.0617 (45.6), 275.0871 (100.0), 307.0833 (95.3), 377.1282 (30.9)	oleuropein isomer	OL150ET
41	23.63	539.1776	C 25 H 31 O 13	539.1770	-1.1	32.5	149.0247 (26.9), 165.0545 (33.3), 179.0577 (42.3), 223.0602 (68.1), 275.0830 (61.2), 307.0816 (100.0), 371.0953 (62.2), 403.1236 (37.2)	oleuroside	OL150ET OL200W
42	24.33	583.2036	C 27 H 35 O 14	583.2032	-0.6	36.8	151.0396 (100), 179.0555 (19.9), 223.0603 (43.4), 403.1210 (13.4)	lucidumoside C (isomer 1)	OL150ET
43	24.65	583.2038	C 27 H 35 O 14	583.2032	-1	36.8	151.0396 (100), 179.0561 (19.5), 223.0596 (40), 403.1240 (11.7), 537.1585 (8.2)	lucidumoside C (isomer 2)	OL150ET
44	24.79	557.2218	C 26 H 37 O 13	557.2240	3.8	17.5	121.0638 (34.6), 183.0664 (85.3), 185.1184 (40.0), 227.0573 (44.0), 227.1279 (52.6)	6'-O-[2,6-dimethyl-8-hydroxy-2-octenoyloxy]secologanoside	OL200W
45	25.82	523.1832	C 25 H 31 O 12	523.1821	-2	31.5	101.0234 (11.5), 127.0392 (14), 259.0953 (20.4), 291.0862 (100), 361.1282 (8.1)	ligstroside	OL150ET OL200W
46	26.21	583.2040	C 27 H 35 O 14	583.2032	-1.3	36.9	151.0393 (100), 179.0564 (68.1), 223.0591 (79), 351.1042 (18.9), 371.0943 (71.2), 403.1223 (56.9), 537.1616 (22.7)	lucidumoside C (isomer 3)	OL150ET
48	28.32	285.0411	C 15 H 9 O 6	285.0405	-2.4	14.4	133.0295 (6.1), 151.0013 (7.5), 175.0387 (4.5), 255.0299 (6.9)	luteolin ^a	OL150ET OL200W

^a Identification confirmed using commercial standards.

411 **Table 2.** Unknown compounds from olive leave extracts

Peak	RT (min)	Measured m/z	Formula	Theoretical m/z	Error (ppm)	mSigma	Fragments	Extract
4	2.87	128.0354	C 5 H 6 N O 3	128.0353	-0.3	2.8		OL200W
5	3.48	217.0712	C 9 H 13 O 6	217.0718	2.7	4.3	111.0063 (26.2), 129.0556 (100.0), 155.0634 (20.1), 173.0436 (33.8)	OL200W
8	8.32	199.0614	C 9 H 11 O 5	199.0612	-0.8	16.8	69.0327 (49.3), 85.0310 (57.3), 95.0472 (68.3), 111.0822 (100.0)	OL150ET OL200W
13	10.22	489.1602	C 21 H 29 O 13	489.1614	2.4	27	145.0293 (100), 163.0404 (92.3), 205.0504 (22.6), 235.0621 (15.2), 265.0695 (20.9)	OL150ET OL200W
15	10.82	445.2060	C 21 H 33 O 10	445.2080	4.3	21.0		OL200W
16	11.35	377.1444	C 16 H 25 O 10	377.1453	2.5	17.7	153.0925 (65.9), 197.0810 (100)	OL150ET OL200W
20	13.07	245.1028	C 11 H 17 O 6	245.1031	1.2	11.8	111.0811 (100), 155.0709 (51.7), 201.1113 (68.7)	OL150ET
21	13.61	191.0347	C 10 H 7 O 4	191.0350	1.3	10.5	108.0203 (77.8), 119.0533 (29.5), 121.0298 (35.9), 135.0492 (45.9), 147.0426 (18.6), 163.0410 (100.0)	OL200W
22	14.44	511.2376	C 22 H 39 O 13	511.2396	4	29.2	185.1158 (100), 199.1341 (60.2), 227.1284 (47), 343.1228 (38.1)	OL150ET OL200W
24	15.33	245.1032	C 11 H 17 O 6	245.1031	-0.5	10.9	111.0818 (100), 137.0606 (7), 155.0720 (48.5), 201.1127 (53.4), 227.0884 (6.6)	OL150ET
33	19.49	491.1766	C 21 H 31 O 13	491.1770	0.7	26.9	101.0232 (10.9), 167.0703 (14.5), 199.0603 (100), 269.1010 (13.2)	OL150ET
34	19.85	187.0972	C 9 H 15 O 4	187.0976	2.1	12.2	97.0658 (6.8), 123.0788 (8.8), 125.0964 (100.0), 169.0898 (5.0)	OL150ET OL200W
40	23.27	537.1614	C 25 H 29 O 13	537.1614	-0.1	31.9	151.0399 (100), 223.0615 (17.4), 275.0900 (15.6)	OL150ET
47	27.07	623.1416	C 31 H 27 O 14	623.1406	-1.5	43.6	161.0225 (6.2), 285.0383 (81.1), 299.0558 (16), 323.0772 (22.8)	OL150ET

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Figure 1. BPC (50–1100 m/z) of OL150ET extract (a) and OL200W extract (b), in which the peaks are identified with numbers 1-48 according to the order of elution.
166x202mm (150 x 150 DPI)

Figure 2. Proposed fragmentation pathway for 7-epiloganin (compound 17).
242x123mm (300 x 300 DPI)

Figure 3. Proposed fragmentation pathway for lucidumoside C (compound 42, 43 or 46).
249x158mm (300 x 300 DPI)

Figure 4. Structure and fragmentation pathway of 6'-O-[2,6-Dimethyl-8-hydroxy-2-octenoyloxy]secologanoside (compound 44).
190x74mm (300 x 300 DPI)