

## Supporting Information

### Materials and Methods

#### S1. NMR Experiments

NMR experiments were performed on a Bruker AV-500 spectrometer. Spectra were obtained from hexane extract dissolved in pyridine-*d*<sub>5</sub> (20 mg of material in 0.5 mL of solution in pyridine-*d*<sub>5</sub>). For the preliminary experiments, of 1D and 2D NMR spectra a 1:1:1 mixture of standard oleanolic acid, erythrodiol and uvaol in pyridine-*d*<sub>5</sub> was used ( 4.4 mM for oleanolic acid, 4.5 mM for erythrodiol and for uvaol) in order to reveal the differences between the connectivities of these triterpenoids. Chemical shifts were measured with reference to the internal standard *d*<sub>4</sub>-TMSP ( $\delta$ =0,000), of known concentration (0.012M). Data acquisition parameters for <sup>1</sup>H-NMR spectra were:  $\pi/2$  excitation pulse, spectral width 6684 Hz, 32 K real data points, acquisition time 3.27 s, relaxation delay 5 s (as estimated for adequate relaxation, see below). In order to plot the calibration curves of oleanolic acid, erythrodiol and uvaol, a serial dilution was made on each stock solution with pyridine-*d*<sub>5</sub> and standard solutions at concentrations 4.4 – 30.6 mM were prepared, in which 50  $\mu$ L of *d*<sub>4</sub> -TMSP solution (0.012M) was added. For the analysis of triterpenoids, 20 mg of olive leaf extract were dissolved in 0.5 mL of pyridine-*d*<sub>5</sub> and 50  $\mu$ L of the *d*<sub>4</sub> -TMSP solution was added. All measurements were performed in triplicates (on three different extracts of the same sample). For measuring limits of detection and quantification, standards were sequentially diluted and their spectra were acquired.

A quantitative 2D <sup>1</sup>H-<sup>13</sup>C NMR strategy previously published by our research group for the quantitation of oleanolic acid and ursolic acid in plant extracts of the *Lamiaceae* and *Oleaceae* family (Kontogianni *et al.*, 2009) was applied. <sup>1</sup>H-<sup>13</sup>C

HSQC spectra were collected with 2 and 4 scans. A six point calibration curve was constructed for oleanolic acid, erythrodiol and uvaol by regressing absolute peak intensities from the reference samples of known concentrations. The method, therefore, is independent of the relaxation properties of the spin system. The mean value of the absolute peak intensities of two well-dispersed cross peaks of H18-C18 and H12-C12 in the  $^1\text{H}$ - $^{13}\text{C}$  HSQC spectra for oleanolic acid, were used. For erythrodiol and uvaol the mean value of the absolute intensities of the well-dispersed cross peaks of H12-C12 and H28a,b-C28 were used.

In order to plot the calibration curves of the triterpenoids, a serial dilution was made on each stock solution with pyridine- $d_5$  and standard solutions with concentrations 4.4 – 30.6 mM were prepared, in which 50  $\mu\text{L}$  of  $d_4$ -TMSP solution (0.012 M) was added. Each quantitative  $^1\text{H}$ - $^{13}\text{C}$  HSQC spectrum required 14 min to collect. Similar results were obtained with double experimental time (28 min, 4 scans). The amount of starting material used was again 20 mg of extract dissolved in 0.5 mL of pyridine- $d_5$  with 50  $\mu\text{L}$  of the  $d_4$ -TMSP solution. All measurements were performed in triplicates (on three different extracts of the same sample).

2D acquisition parameters were as follows:  $^1\text{H}$ - $^{13}\text{C}$  HSQC experiments were acquired with a spectral width of 6684 Hz in the  $F_2$  dimension and 20833 Hz in the  $F_1$  dimension, acquisition time was 0.077 s, relaxation delay was 1.5 s, the  $t_1$  dimension was zero-filled to 1 K real data points and 8 scans were acquired per increment; the  $^1\text{H}$ - $^{13}\text{C}$  HMBC spectra were acquired with spectral widths of 6510 Hz in the  $F_2$  dimension and 27933 Hz in the  $F_1$  dimension, acquisition time was 0.31 s, relaxation delay was 1.5 s, the  $t_1$  dimension was zero-filled to 4 K real data points and 16 scans per increment were acquired. NMR data were processed using Topspin 1.3 NMR (Bruker) software.

## S2. HPLC experiments

A Shimadzu liquid chromatography apparatus consisting of a LC-10AD VP quaternary pump, a SCL-10A VP control system coupled with a SPD-M10A VP photo-diode array detector was used for the HPLC analysis. Injection was through a Rheodyne 7125 injector equipped with a 20  $\mu$ L loop. The temperature control of the column was made with a CT0-10AS oven (Shimadzu, Tokyo, Japan). Separation was achieved on a 25 cm x 4.6 mm i.d., 5  $\mu$ m ODS HYPERSIL C18 analytical column provided by Supelco (Bellefonte, USA). Data acquisition and integration was performed with CLASS-VP software. Solvent A was acetonitrile and Solvent B 0.02M phosphate buffer pH 3.5 (60/40 %, v/v) with 7.5 mM HP- $\gamma$ -CD, according to *Claude et al. 2004*. An isocratic mobile phase elution was performed at 1 mL min<sup>-1</sup> flow-rate, the absorbance changes were monitored at 210 nm and the injection volume was 20  $\mu$ L. The column was thermostated at 25°C. The stock solution was prepared by dissolving 1 mg of triterpenoid into 1 mL of methanol (2.2 mM). For the analysis of triterpenoids the olive leaf extract was dissolved in methanol in the ratio of 1 mg mL<sup>-1</sup>. Identification of the triterpenoids was based on their retention time and UV spectra and spiking. Quantitative analysis was achieved by applying seven point calibration curves for oleanolic acid, erythrodiol and uvaol at concentrations of 25-300  $\mu$ g mL<sup>-1</sup> (0.06 – 0.66 mM). Real samples were diluted accordingly to fit the dynamic linear range of the regression line, when necessary. All measurements were performed in triplicates (on three different extracts of the same sample).

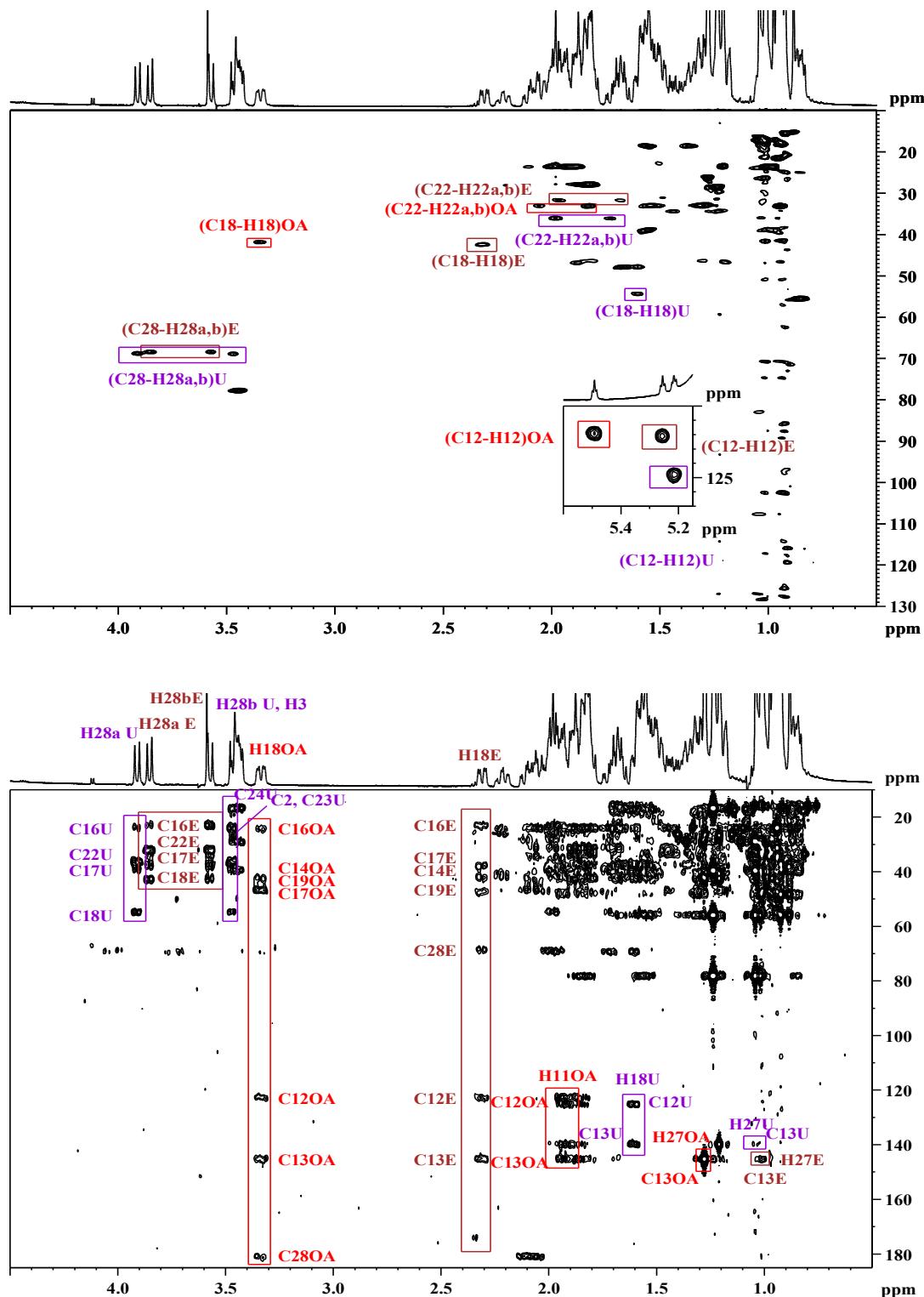


Figure S1. (A) 500 MHz  $^1\text{H}$ - $^{13}\text{C}$  HSQC spectrum of the mixture of oleanolic acid, erythrodiol and uvaol 1:1:1 (4.5 mM) (ns = 2, experimental time: 14 min), (B) 500 MHz  $^1\text{H}$ - $^{13}\text{C}$  HMBC spectrum of the mixture of oleanolic acid, erythrodiol and uvaol 1:1:1 (4.5 mM) (ns = 16, mixing time 50 ms, experimental time: 1 h).

Table S1:  $^1\text{H}$  and  $^{13}\text{C}$  NMR chemical shifts ( $\delta$ , ppm) of erythrodiol and uvaol NMR signals and their long range connectivities that were utilized for their identification.

Erythrodiol				Uvaol				
C	$\delta$ C	$\delta$ H	Long connectivities	range	$\delta$ C	$\delta$ H	Long connectivities	range
12	122.1	5.24			125.8	5.22		
18	42.4	2.32	C16 (22.6), C17 (36.6), C14 (41.7), C19 (46.8), C28 (68.2) C12 (122.1), C13 (144.0)		55.5	1.62	C12 (125.8), C13 (139.7)	
22	31.5	1.69 $\alpha$ 1.98 $\beta$			37.0	1.73 $\alpha$ 2.00 $\beta$		
27		1.01	C13 (144.0)			1.04	C13 (139.7)	
28	68.2	3.84 $\alpha$ 3.57 $\beta$	C16 (22.6), C22 (31.5), C17 (36.6), C18 (42.4)	70.0	3.91 $\alpha$ 3.46 $\beta$		C16 (24.5), C22 (37.0), C17 (36.7), C18 (55.5)	

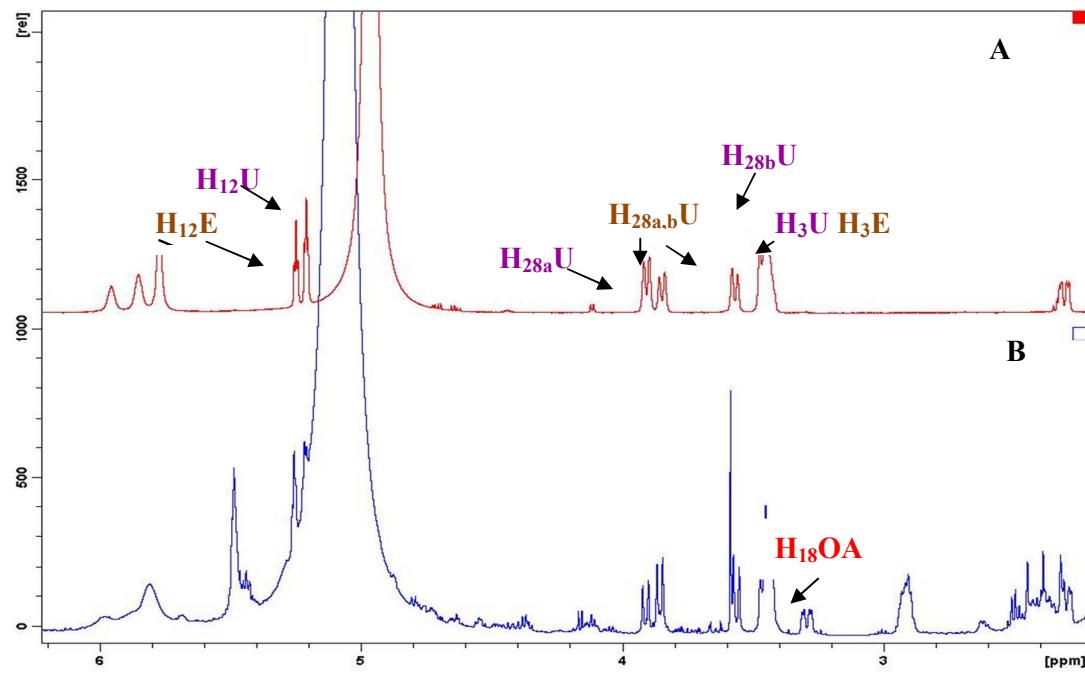


Figure S2. (A) 500 MHz <sup>1</sup>H- NMR spectrum of the mixture of oleanolic acid, erythrodiol and uvaol 1:1:1 (4.5 mM) (ns =64, experimental time: 7 min). (B) <sup>1</sup>H- NMR spectrum of the hexane olive leaf extract (40 mg/ ml) (ns =64, experimental time: 7 min).