

## **File S1: Chromatography and *in vitro* cells culture methods**

### **1. Fatty acid analysis**

The FA profiles were obtained after direct derivatization of the oils contained in seeds to FA methyl esters (FAMES). Seeds (200 mg) were accurately weighed and placed in 10-mL glass test tubes. Then, 1 mL of *n*-hexane (chromatographic grade) was added, the tubes were capped, and seeds were allowed to macerate for 24 h at room temperature, darkness and inert atmosphere of nitrogen. After that, 2 mL of a methylating mixture (methanol:acetyl chloride, 20:1, v/v) were carefully poured over the described material. Tubes were then capped and heated at 100 °C for 30 min. Then, tubes were cooled to room temperature and 1 mL of distilled water was added to each tube which were then centrifuged for 5 min at 2,000 g on a Heraeus Labofuge 200 centrifuge (Thermo Scientific, USA). Subsequently, the upper hexane layer was collected for GC-FID analysis. FAMES were analyzed in a Focus GC (Thermo Electron, Cambridge, UK), equipped with a flame ionization detector (FID) and an Omegawax™ 250 Fused Silica capillary column (30 m × 0.25 mm × 0.25 µm film thickness; Supelco, Bellefonte, USA), as previously described (Lyashenko et al., 2019). The peak area of each FAME was used to calculate their proportion on total FAME in the samples.

The quality control for FA analyses was carried out as previously described (Barroso et al., 2019). The repeatability of the direct methylation was checked by analyzing replicates of the same sample daily. The intermediate precision was evaluated by measuring samples on different days throughout the study. Also, blank samples were analyzed every time when the methylations were performed. Control oil samples were analyzed before and after running samples. As quality control of GC, a blank sample (hexane) was run together with the samples in every batch. The limits of detection (LOD) and quantification (LOQ) were determined with pure oleic acid (OA, 18:1*n*-9; purity, 98%) and linoleic acid (LA, 18:2*n*-6; purity 97.5%), which were diluted in toluene in the 0.001-20 mg/mL range, methylated and quantified in triplicate by GC-FID. Negative controls were also analyzed. The LOD was defined as the minimum concentration at which distinct peaks could be detected above the baseline noise. The estimated LOD for 18:2*n*-6 and  $\alpha$ -linolenic acid (ALA, 18:3*n*-3) were in the range of 0.8-0.9 mg/mL, while LOQ were in the range of 2.4-3.8 mg/mL.

### **2. Extraction of phenolic compounds from Areaceae seeds**

Extraction and analysis of phenolic compounds were carried out according to Lyashenko et al. (2021) with some modifications. Grounded seeds (~0.8 g) were extracted three times with 3 mL of methanol:water, (60:40, v/v) as solvent and adjusted to pH 3.2 with formic acid, under stirring for 4 h. After centrifuging at 1,000×g for 10 min, the supernatants were collected, combined and the solvent was evaporated under vacuum at 60 °C to dryness. After weighing the resulting residue, it was dissolved in 1 mL of methanol:water (60:40, v/v), and passed through a 0.22 µm membrane filter prior to the chromatographic analysis.

### **3. Characterization of phenolic compounds by HPLC-DAD**

Identification of phenolic compounds by HPLC-DAD was carried out using a Finnigan Surveyor chromatograph and a reverse-phase C18 column (Hypersil Gold, 250 mm × 4.6 mm i.d., 5 µm particle size) (Thermo Electron, Cambridge, UK). Compounds were

separated with a gradient elution with acidified water (4% acetic acid) (A) and methanol (B) as mobile phase at 30 °C. Ultrapure water Milli-Q® and chromatographic-grade methanol were filtered through a 0.45 µm membrane filter (Millipore, Durapore®, Ireland) prior to their use as mobile phase. The gradient started at 100% solvent A (10 min) which was reduced to 80% (15 min), 70% (15 min), 60% (15 min), 50% (10 min), 20% (10 min) and 0% (15 min). The total running time was 90 min. The flow rate was kept at 0.4 mL/min and the injection volume was 10 µL. Peaks were monitored at 240, 280 and 300 nm and identified by their retention times compared with analytical standards. Quantification was performed using external calibration curves from pure standards (Sigma-Aldrich, St. Louis, MO, USA). Standards for gallic acid (97.5-102.5%, 91215), vanillic acid (97%, H36001), protocatechuic acid (≥97%, 37580), salicylic acid (≥99%, 247588), 4-hydroxybenzoic acid (≥99%, 92596), DL-*p*-hydroxyphenyl lactic acid (≥97%, H3253), 2,4-dihydroxyhydrocinnamic acid (97%, 663158), 3,4-dihydroxyhydrocinnamic acid (98%, 102601), chlorogenic acid (≥95%, C3878), caffeic acid (≥98%, C0625), (-)-catechin (≥97% C0567, syringic acid (≥95%, S6881), *trans-p*-coumaric acid (≥98%, 55823), ferulic acid (99%, 128708), sinapic acid (≥98%, D7927), rutin (≥94%, R5143), rosmarinic acid (≥98%, R4033), 2-Hydroxy-4-methoxybenzoic acid (99%, 173479), naringenin (≥95%, N5893), quercetin (≥95%, Q4951), luteolin (≥97%, 72511), and kaempferol (≥90%, K0133) were purchased from Merck (Darmstadt, Germany) (Lahlou et al., 2022).

#### 4. Characterization of phenolic compounds by LC-MS

The chromatographic separation was performed on a Vanquish Flex Quaternary LC (Thermo Fisher Scientific, San Jose, CA, USA) equipped with a reverse-phase C18 column (Hypersil Gold, 100 mm × 2.1 mm, 1.9 µm, Thermo Fisher Scientific) at a flow rate of 0.2 mL/min. The compounds were separated under a gradient elution using acidified water (0.1% formic acid) (A) and methanol (MeOH) (B) as eluents at room temperature (25 °C). The step gradient was as follows: 0-1 min 95% A; then, the proportion of solvent A was linearly decreased to 70% in 2.5 min, and to 0% in 2.5 min, remaining constant for 8 min. Finally, the proportion of solvent A was increased to 95% in 2 min and remained constant for 5 min. Total running time was 20 min. The injection volume was 10 µL.

The LC system was coupled to a hybrid mass spectrometer Q-Orbitrap Q-Exactive™ (Thermo Fisher Scientific, Bremen, Germany) with electrospray ionization (ESI) (HESI-II, Thermo Fisher Scientific, San Jose, CA, USA) in positive and negative ion mode. ESI parameters were as follows: spray voltage, 4 kV; sheath gas (N<sub>2</sub>, 95%), 35 (arbitrary units); auxiliary gas (N<sub>2</sub>, 95%), 10 (arbitrary units); S-lens RF level, 50 (arbitrary units); heater temperature, 305 °C, and capillary temperature, 300 °C. The mass spectra were acquired employing four alternating acquisition functions: (1) full MS, ESI + , without fragmentation (the higher collisional dissociation (HCD) collision cell was switched off), mass resolving power = 70,000 Full Width at Half Maximum (FWHM); AGC target = 1e<sup>6</sup>, scan time = 250 ms; (2) full MS, ESI - , without fragmentation (the higher collisional dissociation (HCD) collision cell was switched off), mass resolving power = 70,000 Full Width at Half Maximum (FWHM); AGC target = 1e<sup>6</sup>, scan time = 250 ms; (3) data independent analysis (DIA), ESI + , setting higher energy collisional dissociation (HCD) on, and collision energy = 30 eV, mass resolving power = 35,000 FWHM, scan time = 125 ms; (4) DIA, ESI - (setting HCD on, and collision energy = 30 eV), mass resolving power = 35,000

FWHM, scan time = 125 ms. The mass range in the full scan MS experiments was set to  $m/z$  50–750. Chromatograms were acquired using the external calibration mode and processed using Xcalibur™ version 3.0, with Qual browser and Trace Finder 4.0 (Thermo Fisher Scientific, Les Ulis, France). Unknown analysis was carried out with Compound Discoverer™ version 2.1 (Thermo Scientific, Les Ulis, France).

### **5. Accuracy and validity of the HPLC methodologies**

HPLC-DAD and LC-MS analyses were performed using the experimental set-up described in Supplemental Table 3. Precision/injection repeatability test (expressed as relative standard deviation, %) was performed by five replicated injections of the standard mixture of phenolic acids (PAs) at a concentration of 1.25, 2.5, 5.0, 10.0 and 50.0 ppm. The accuracy was also evaluated by the standard addition procedure (% of recovery) with three addition levels (30% of the expected values, in duplicate). Standard mixture was added to the samples, and all the extraction procedures were carried out. Recovery rates were calculated by the following equation:

$R\% = [(C_s - C_p)/C_a]$ , where R (%) is the percent recovery of added standard;  $C_s$  the compound content in spiked sample;  $C_p$  the compound content in sample; and  $C_a$  the compound standard added. To determine the limits of detection (LOD) and quantification (LOQ) of the assays, pure phenolic compounds were diluted in the 0.001–20 mg/mL range in methanol in triplicate and quantified by HPLC-DAD. Negative controls made without phenolics addition were also analyzed. The LOD was defined as the minimum concentration at which distinct peaks could be detected above the baseline noise. The LOQ was defined as the lowest concentration of compounds that could be quantified with an accuracy and precision within 15%.

### **6. Antiproliferative assays of phenolic extracts on the HT-29 cell line**

The antiproliferative activity of hydroalcoholic (methanol:water, 60:40, v/v) extracts from Areaceae fruits was assayed on the HT-29 human colon cancer cell line as described by Lyashenko et al. (2021). Cultures were supplied by the Technical Instrumentation Service of University of Granada (Granada, Spain). After being checked for the absence of *Mycoplasma* and bacteria, cells were grown at 37 °C and 5% CO<sub>2</sub> humidified atmosphere in RPMI-1640 medium supplemented with 5% fetal bovine serum, 2 mM L-Glutamine, 1 mM Sodium pyruvate, 0.125 mg/mL Amphotericin B and 100 mg/mL Penicillin-Streptomycin. Cultures were plated in 25 cm<sup>2</sup> plastic tissue culture flasks (Sarstedt, USA). All culture media and reagents were purchased from Sigma-Aldrich (St. Louis, MO, USA). Cell culture and cell assay, that is, the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) test were accomplished as previously described (Ramos-Bueno et al., 2016). Briefly, cells were divided into 96-well microtiter plates, adjusted at  $1 \times 10^4$  cell/well and cultivated in medium at 37 °C, 5% CO<sub>2</sub>. The extracts of Areaceae seeds were supplied to cells dissolved in a mixture of distilled water-methanol (40:60, v/v) and then the culture medium was used to obtain dilutions at designed concentrations (1,200–2,000 µg/mL). Pure phenolics (3,4-dihydroxyhydrocinnamic, gallic, rosmarinic and ferulic acids) were supplied to cells dissolved in dimethylsulfoxide (DMSO) at the same concentrations. After 72 h of the extract addition to the cells, 5 mg/mL of an MTT solution was added to the culture medium to determine the cell viability. The absorbance was recorded at 570 nm on an enzyme-linked immunosorbent assay (ELISA) plate reader (Thermo Electron

Corporation, Sant Cugat del Valles, Barcelona, Spain) (Ramos-Bueno et al., 2016). The formazan crystals produced were solubilized using 100  $\mu$ L of DMSO. Cells with no added phenolic extracts were considered as negative controls. Cell survival in exposed cultures relative to unexposed cultures (negative controls) was considered, and the number of viable cells was calculated using the following equation:

Percentage of viable cell (%) = (Absorbance of treated cells/Absorbance of untreated cells)  $\times$  100.

The concentrations causing 50% cell growth inhibition (GI<sub>50</sub>) were calculated from the growth curves. Phenolic extracts, pure phenolics and controls were evaluated in three independent assays, and values are shown as mean  $\pm$  standard error of the mean.

## References

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