## Supporting information

**Action of polyphenols in bone formation**

**The impact of polyphenols in chondrocyte growth and survival: a preliminary report**

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**Chromatographic methods to assess the composition of plant extracts.** Polyphenols and other polar compounds (e.g., organic acids or simple phenols) were characterised using high-performance liquid chromatography (HPLC) in a suitable apparatus equipped with a C18 reverse phase column coupled to mass spectrometry (MS) utilising an electrospray ionisation source (ESI). In particular, *Aspalathus linearis* (Family *Fabaceae*; rooibos leaves) was characterised utilising a time-of-flight (TOF) and an ion trap (IT) as mass spectrometers (1). To analyse *Lippia citriodora* (Family *Verbenaceae*; lemon verbena leaves), we employed capillary electrophoresis coupled to an ESI-TOF/IT-MS platform (2). *Olea europaea* (Family *Oleaceae*; olive) leaves were characterised utilising a quadrupole-TOF as mass spectrometer (HPLC-ESI-QTOF-MS) (3). *Vitis vinifera* (Family *Vitaceae*; grape) seeds were analysed using an HPLC system coupled to a photodiode-array detector (4). For *Citrus aurantium* (Family *Rutaceae*; bitter orange), the characterisation was conducted using a diode-array detector (DAD) (5). Finally, the phenolic and other polar compoundsfrom *Hibiscus sabdariffa* (Family *Malvaceae*; karkade) were characterised utilising an HPLC-ESI-DAD-IT-MS (6) and quantitated with an HPLC-ESI-DAD-TOF-MS (7); the concentrated polyphenolic extract of *Hibiscus sabdariffa* was prepared as described (8).

**Table S1.** Qualitative composition of phenolic compounds in the assayed plant extracts

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| ***Aspalathus*** ***linearis*** | ***Citrus*** ***aurantium*** | ***Lippia*** ***citriodora*** | ***Olea europaea*** **leaves** | ***Vitis vinifera*** **seeds** |
| AspalanininAspalathinCarlinoside (and derivatives)EsculinIso-orientinIsovitexinLuteolin (and glycoside derivatives)NothofaginOrientinPatuletin-7-glucosideQuercetin (and glycoside derivatives)SecoisolariciresinolVicenin-2 | CatechinChlorogenic acidCinnamic acid (and derivatives)EpicatechinFerulic acidGallic acidHydroxybenzoic acidNaringin*p*-Coumaric acidRosmarinic acidRutinSyringic acidTyrosolVanillic acid | Acacetin-7-diglucuronideApigenin-7-diglucuronideCampneoside IChrysoeriol-7-diglucuronideCistanoside FEukovosideForsythoside AGardosideLuteolin-7-diglucuronideMartinosideThevesideVerbascoside (and derivatives) | 7-epiloganinApigenin (and glycoside derivatives)Elenolic acid derivativesHydroxybenzoic acidHydroxytyrosolLigstrosideLucidumoside CLuteolin (and glycoside derivatives)Oleoside (and derivatives)Oleuropein (and derivatives)RutinVanillinVerbascoside | Caffeic acidCatechinChlorogenic acidEpicatechinEpicatechin-gallateGallic acid*p*-Coumaric acidProcyanidin B1Procyanidin B2Procyanidin B3Procyanidin B4Protocatechuic acidQuercetinRutinSyringic acid*t*-piceatannol*t*-resveratrolVanillic acid |

|  |  |  |
| --- | --- | --- |
| Compound | *Hibiscus sabdariffa* | Polyphenolic extract of *Hibiscus sabdariffa* |
| Hydroxycitric acid | 0.8288 | - |
| Hibiscicus acid | 3.1122 | 1.2813 |
| Delphinidin-3-*O*-sambubioside | 0.2701 | 2.0732 |
| Cyanidin-3-*O*-sambubioside | 0.1939 | 0.8714 |
| Chlorogenic acid | 0.572 | 1.0647 |
| Hibiscus acid dimethyl ester | - | 0.0530 |
| Methyl digallate | - | 0.0280 |
| Myricetin-3-*O*-arabinogalactose | 0.0057 | 0.0476 |
| Coumaroylquinic acid | - | 0.0077 |
| Quercetin-3-*O*-sambubioside | 0.0304 | 0.0767 |
| Quercetin-3-*O*-rutinoside | 0.0495 | 0.0495 |
| 5-*O*-Caffeoylshikimic acid | 0.0172 | 0.0353 |
| Kaempferol-3-*O*-sambubioside | - | 0.0112 |
| Quercetin-3-*O*-glucoside | 0.0144 | 0.0307 |
| Kaempferol-3-*O*-rutinoside | 0.0092 | 0.0219 |
| Methyl epigallocatechin | - | 0.0031 |
| Myricetin | - | 0.0477 |
| *N*-feruloyltyramine | 0.0099 | 0.0087 |
| Prodelphinidin B3 | 0.1839 | 0.0033 |
| Quercetin | 0.0121 | 0.0580 |

**Table S2**. Phenolic compounds characterised in *Hibiscus sabdariffa* and its polyphenolic extract. The values indicate the concentration (in g/mL) of each compound in the culture medium. To provide the same total amount of compounds, we added 100 g/mL of the *Hibiscus sabdariffa* extract or 10 g/mL of the polyphenolic extract of *Hibiscus sabdariffa*.

**Additional references**

1. Iswaldi I, Arraez-Roman D, Rodriguez-Medina I, Beltran-Debon R, Joven J, Segura-Carretero A, et al. Identification of phenolic compounds in aqueous and ethanolic rooibos extracts (*Aspalathus linearis*) by HPLC-ESI-MS (TOF/IT). Anal Bioanal Chem. 2011;400:3643-54.

2. Quirantes-Pine R, Arraez-Roman D, Segura-Carretero A, Fernandez-Gutierrez A. Characterization of phenolic and other polar compounds in a lemon verbena extract by capillary electrophoresis-electrospray ionization-mass spectrometry. J Sep Sci. 2010;33: 2818-27.

3. Quirantes-Pine R, Lozano-Sanchez J, Herrero M, Ibañez E, Segura-Carretero A, Fernandez-Gutierrez A. HPLC-ESI-QTOF-MS as a powerful analytical tool for characterising phenolic compounds in olive-leaf extracts. Phytochem Anal. 2013;24:213-23.

4. Doshi P, Adsule P, Banerjee K, Oulkar D. Phenolic compounds, antioxidant activity and insulinotropic effect of extracts prepared from grape (Vitis vinifera L) byproducts. J. Food Sci Technol. 2015;52:181-90.

5. Karoui IJ, Marzouk B. Characterization of bioactive compounds in tunisian bitter orange (*Citrus aurantium* L.) peel and juice and determination of their antioxidant activities. Biomed Res Int. 2013;Article ID 345415.

6. Rodriguez-Medina IC, Beltran-Debon R, Micol V, Alonso-Villaverde C, Joven J, Menendez JA, et al. Direct characterization of aqueous extract of *Hibiscus sabdariffa* using HPLC with diode array detection coupled to ESI and ion trap MS. J Sep Sci. 2009;32:3441-8.

7. Fernández-Arroyo S, Rodríguez-Medina IC, Beltrán-Debón R, Pasini F, Joven J, Micol V, et al. Quantification of the polyphenolic fraction and in vitro antioxidant and in vivo anti-hyperlipemic activities of *Hibiscus sabdariffa* aqueous extract. Food Res Int. 2011;44:1490-5.

8. Herranz-Lopez M, Fernandez-Arroyo S, Perez-Sanchez A, Barrajon-Catalan E, Beltran-Debon R, Menendez JA, et al. Synergism of plant-derived polyphenols in adipogenesis: Perspectives and implications. Phytomedicine 2012;19:253-61.