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Electronic Supplementary Information

Sugars and minerals enriched fraction from olive mill wastewater for promising cosmeceutical application: characterization, i*n vitro* and *in vivo* studies.

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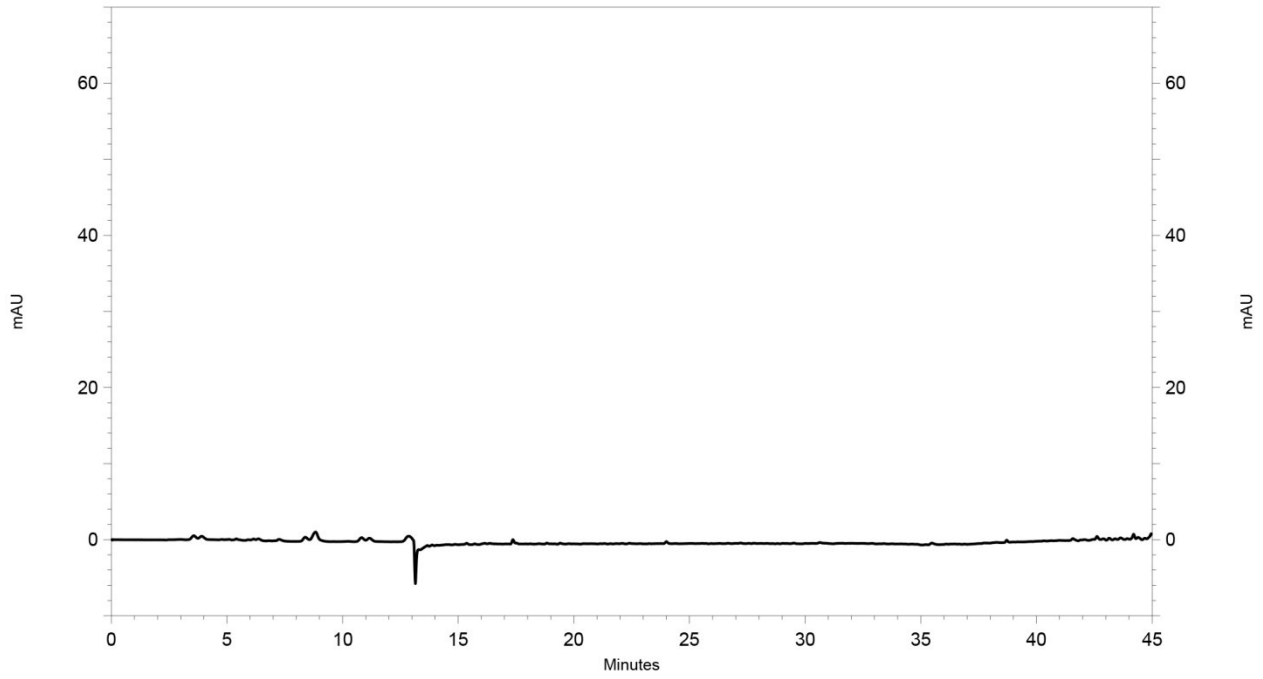

Fig. S1. Chromatogram of lyophilized sugars and minerals enriched fraction at 280 nm. HPLC-DAD analysis was performed using a Kinetex C-18 (4.6x250mm, 5µm) column (Phenomenex) with a security guard cartridge (Phenomenex) maintained at 30±1°C. The sample (about 0.1g), previously dissolved in water (10 ml), filtered through 0.45 µm regenerated cellulose syringe filter and diluted both 1:10 and 1:20, was eluted with acetonitrile (A) and water (B) both added with 0.1% trifluoroacetic acid, with the following gradient: 100% B as initial condition, maintained for 5min; 58% B in 25min; 100% A in 15min, maintained for 5min. Flow rate was 0.8mL/min. Injection volume was 10 µl.

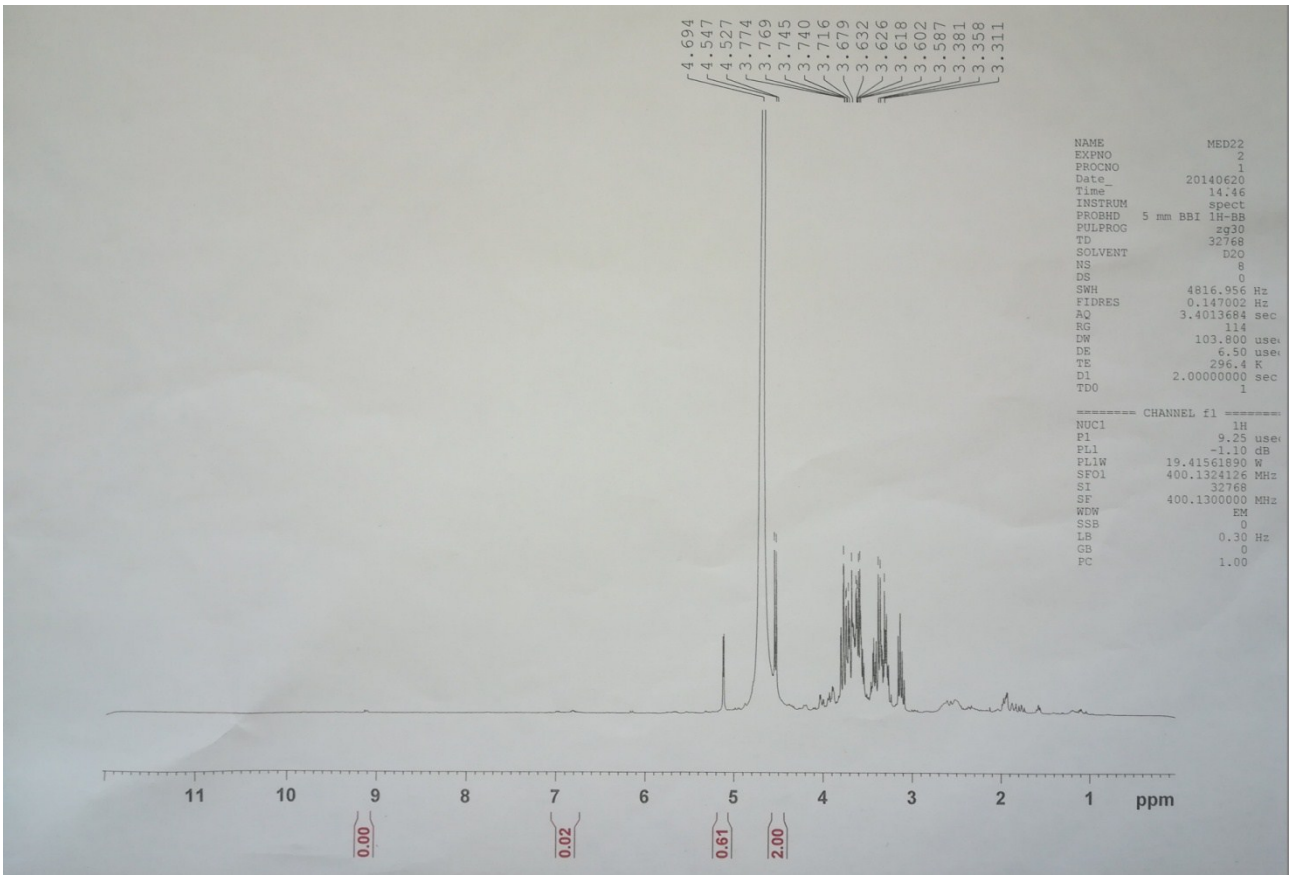

Fig. S2. ^{1}H-NMR spectrum of lyophilized sugars and minerals fraction. The spectrum was recorded on a Bruker AvanceTM 400 spectrometer at 400.13 MHz. All samples, previously dried, were dissolved in deuterium oxide. Chemical shifts (δ) are given as parts per million relative to the residual solvent peak.

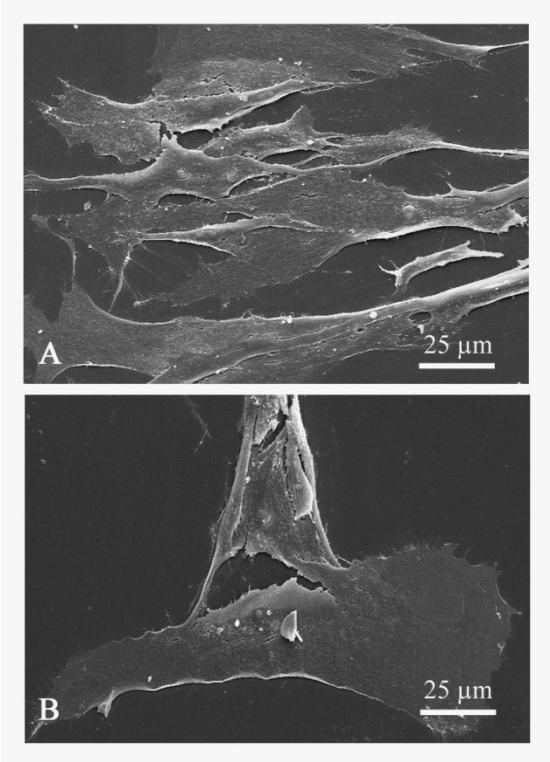

Fig. S3. SEM micrographs of MRC-5 cells. A) treated with 0.5 % w/w of lyophilized SMEF; B) treated with 1% w/w of lyophilized SMEF.

Fig. S4. Picture of emulsion containing lyophilized sugars and minerals enriched fraction (1% w/w) stored at 25°C±2°C with 60±5% relative humidity (R.H.) for 12 months (left) and at 40°C±2°C with 60±5% R.H. for 6 months (right) at light.

Table S1 Metals content of emulsion containing lyophilized SMEF (1% w/w) determined by ICP-MS in our experimental model, represented in comparison with limit content provided by some countries (Italy, Germany, Canada).

		Limits as impurities in cosmetics (mg/kg)		
Metal	**mg/kg**	**Italy[32]**	**Germany[31]**	**Canada[33]**
As	< 0.05	1	5	3
Cd	< 0.05	5	5	3
Hg	< 0.05	1	1	3
Pb	0.72 ± 0.01	20	20	10
Sb	< 0.05	10	10	5
Cr	0.51 ± 0.02	1	-	-
Co	< 0.05	5	-	-
Ni	0.50 ± 0.01	10	-	-
Al	6.11 ± 0.11	-	-	-
Cu	0.21 ± 0.01	-	-	-
Zn	5.95 ± 0.21	-	-	-
Fe	2.62 ± 0.05	-	-	-
Mn	0.52 ± 0.01	-	-	-
Sn	0.14 ± 0.01	-	-	-
Mo, Tl, Be,Ag, Bi, V	< 0.05	-	-	-

Values are the mean ± SD of three experiments in triplicate.

[31] BfR, Bundesinstitut für Risikobewertung. Kosmetische Mittel: BfR empfiehlt Schwermetallgehalte über Reinheitsanforderungen der Ausgangsstoffe zu regeln, Stellungnahme Nr. 025/2006 des BfR vom 05. April 2006. Available at: <http://www.bfr.bund.de/cm/343/kosmetische_mittel_bfr_empfiehlt_schwermetallgehalte_ueber.pdf>.

[32] B. Bocca, A. Pino, A. Alimonti and G. Forte, Toxic metals contained in cosmetics: A status report, *Regul. Toxicol. Pharmacol.,* 2014, **68**, 447-467.

[33] HC-SC, Health Canada-Santé Canada, 2012. Guidance on Heavy Metal Impurities in Cosmetics. Available at: <http://www.hc-sc.gc.ca/cps-spc/pubs/indust/heavy_metals-metaux_lourds/index-eng.php>.