High-performance liquid chromatography (HPLC) analysis

Phenolic profile was performed using an Agilent 1100 model HPLC with automatic injection and equipped with an 1100 quaternary gradient pump, in-line degasser, auto sampler, dual wavelength UV/VIS detector, and acquisition system (Agilent Software 1100, Santa Clara, Calif., U.S.A.). Chromatographic separations were performed on a C_{18} reverse phase column LiChroCART (25 x 0.4 cm, particle size 5 mm), using water/formic acid (99:1) (A) and acetonitrile (B) as mobile phase at 1 mL min^{-1} and samples of 10 microliters. Gradient was applied, starting with 2 % B in A, uploading to 32 % B at 30 min, isocratic for 18 min, up to 55 % B at 48 min, 95 % B in A for 53 min, isocratic for 4 min and returning to initial conditions (2 % B) to 65 min. The resolved compounds were detected at 280 nm, identified, and quantified on the basis of chromatographic retention times of coeluted pure standards. Eight pure commercial phenolic acids (gallic, vanillic, chlorogenic, caffeic, ellagic, rosmarinic, coumaric, and ferulic acids), catechin, epicatechin, epigallocatechin gallate, rutin, quercetin, kaempferol, naringin, hesperidin, and umbelliferone were used for calibration and quantification (Sigma, St Louis, MO, USA).

Table 1. Phenolic profile and composition of lyophilized infusions. Values are expressed as mean ± standard error (n = 3).

Compound (μg g^{-1})	HS	OS	CP
Caffeic acid	1.01 ± 0.02	1.87 ± 0.07	1.35 ± 0.03
Chlorogenic acid	ND	3.33 ± 0.12	ND
Naringin	ND	ND	9.35 ± 0.04
Rutin	2.30 ± 0.05	ND	15.68 ± 0.14
Vanillic acid	9.56 ± 0.30	ND	0.22 ± 0.01
Coumaric acid	22.13 ± 1.63	7.62 ± 0.51	ND
Quercetin	0.42 ± 0.01	ND	1.82 ± 0.01
Ellagic acid	ND	1.35 ± 0.36	ND
Rosmarinic acid	ND	37.52 ± 1.96	ND
Hesperidin	17.02 ± 0.35	13.38 ± 1.34	2.94 ± 0.04
Epigallocatechin gallate	ND	5.45 ± 0.22	ND

Values in a row followed by different letters are significantly different ($p < 0.05$).
ND = not detected. *Hibiscus sabdariffa* (HS), *Citrus paradisi* (CP), *Ocimum sanctum* L. (OS).

The three infusions presented different profiles, as expected. For the OS, we identify rosmarinic acid, hesperidin, and coumaric acid, and rosmarinic acid, being the compound with the highest concentration quantified in this work (Table 1). For the HS infusion, coumaric and vanillic acids and the flavonoid hesperidin were the major compounds identified. Finally, rutin and naringin were the main compounds identify for CP.