Supplementary information

Quantification of caffeine, catechin and their metabolites by HPLC-ECD

Analyses were performed in a CoulArray multichannel ECD array system (ESA-Dionex) comprised of: 2 pumps (Model 584), autosampler (Model 542), pulse damper and high-pressure mixer, 8 channel Coularray 5600A detector and UV-vis detector (Model 528). Samples were kept at 4 °C, and the injection volume was 25 µL. Separations were achieved using an Atlantis T3 column (4.6×150 mm, 5 µm, Waters) fitted with a pre-column (Atlantis T3 4.6×20 mm, Waters), kept at 30 °C, with mobile phases A (water:acetonitrile, 98:2, v:v, 5 mM ammonium acetate) and B (water-acetonitrile, 30:70, v:v, 5 mM ammonium acetate) flowing at 0.8 mL/min with the following gradient: 0 min, 5% B; 40 min, 10% B, 100 min, 30% B, 110 min, 100% B; 120 min 100% B; 122 min, 5% B, 135 min, 5% B. Channels 1 to 7 were set to 100-700 mV with 100 mV increments. The channel 8 monitored the UV-vis detector, set to 272 nm for detection of caffeine. LOD and LOQ values were 5.71 and 19.0 pmol/mL for catechin, 4.01 and 13.4 pmol/mL for epicatechin and 0.214 and 0.715 nmol/ml for caffeine, as concentration in plasma for 25 µL injections.

Compounds were identified by comparison of retention times and voltamograms with authentic standards. (+)-Catechin, (-)-epicatechin, procyanidin B1 and B2 were purchased from Sigma-Aldrich, and *O*-methylated catechins, namely 3`- and 4`-*O*-methyl-(+)-catechin, 3`- and 4`-*O*-methyl-(-)-epicatechin, were prepared in house by chemical methylation with methyl iodide [1] and purified by preparative HPLC. Confirmation of methylation was done by HPLC-MS and the amount was estimated by UV spectrophotometry using the same extinction coefficients of the respective unmethylated precursor. Quantification of catechins and their metabolites in plasma and guaraná extracts was done by peak height in the dominant channel with an external standard curve of the respective compounds.

[1] Donovan, J. L.; Luthria, D. L.; Stremple, P.; Waterhouse, A. L., Analysis of (+)-catechin, (-)-epicatechin and their 3'- and 4'-O-methylated analogs: A comparison of sensitive methods. *Journal of Chromatography B: Biomedical Sciences and Applications* **1999,** *726* (1-2), 277-283.

Supplementary Table 1. LOD and LOQ of catechins and methylxanthines in human plasma based on 25 µL injections. Values are means ± SD, n=3.

	Coularray dominant channel or wavelength	LOD	LOQ
		*pmol/mL**	*pmol/mL**
C	2	5.71 ± 0.81	19.03 ± 2.69
EC	2	4.01 ± 0.57	13.36 ± 1.89
ECG	2	2.34 ± 0.33	7.80 ± 1.10
EGCG	2	1.67 ± 0.24	5.57 ± 0.79
EGC	1	6.59 ± 32	21.31 ± 1.05
PCB1	3	8.21 ± 1.32	27.36 ± 4.41
PCB2	3	2.93 ± 0.47	9.75 ± 1.57
Caffeine	272 nm	214 ± 70	715 ± 232
Theobromine	273 nm	208 ± 68	693 ± 225
Theophylline	274 nm	245 ± 80	816 ± 265

* for a 25 µL injection

Supplementary table 2. Recovery percentages of catechin and methylxanthine standards added at four levels to blank plasma. Values are means ± SD, n=3.

	level 1	level 2	level 3	level 4	
	0.27 nmol/mL	*0.54 nmol/mL*	*1.08 nmol/mL*	*5.04 nmol/mL*	
Theobromine	140.4 ± 8.2	118.6 ± 2.4	103.1 ± 1.5	95.7 ± 0.8	small coeluting peak in blank plasma
Theophylline	56.5 ± 10.5	78.3 ± 9.3	139.3 ± 9.2	96.6 ± 2.3	elutes on the tail of a big peak
Caffeine	103.4 ± 4.2	92.2 ± 8.4	105.5 ± 1.6	99.5 ± 1.4	
	0.027 nmol/L	*0.054 nmol/L*	*0.108 nmol/L*	*0.504 nmol/L*	
C	107.1 ± 4.9	106.3 ± 4.8	95.8 ± 0.8	85.3 ± 2.0	
PCB1	57.5 ± 16.4	66.2 ± 6.3	70.5 ± 1.6	74.1 ± 2.8	low recovery, probably due
PCB2	54.7 ± 8.2	78.1 ± 6.7	80.8 ± 1.2	97.4 ± 12.7	to adsorption to protein
EC	79.5 ± 2.7	83.9 ± 2.1	92.6 ± 0.1	85.0 ± 1.5	
EGCG	74.0 ± 3.7	84.2 ± 3.2	91.0 ± 0.4	82.6 ± 1.5	
ECG	73.1 ± 4.9	77.1 ± 3.4	87.6 ± 0.9	80.2 ± 1.7	