SUPPLEMENTARY MATERIAL

Quality assessment of Coffea arabica commercial samples

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Abstract

A simple and reliable HPLC method was developed and validated for the quality consistency evaluation of *Coffea arabica* commercial samples through establishing chromatographic fingerprint and simultaneous determination of bioactive constituents. In the HPLC fingerprint, thirteen common peaks were selected to assess the similarities of coffee samples of different geographical origin. A similarity analysis showed values from 0.434 to 0.950 for the analyzed samples, while quantitation of selected bioactive compounds revealed the highest content of caffeine and the lowest of *p*-coumaric acid and theobromine in coffee samples. Since phenolic compounds and alkaloids are commonly recognized as natural antioxidants, the antioxidant activity of coffee extracts was also evaluated. The correlation analysis and principal component analysis indicated that the combination of HPLC fingerprint and quantitative analysis can be readily utilized as a quality assessment tool for coffee and other plant products.

Key words: *Coffea Arabica;* chromatographic fingerprint; secondary metabolites; antioxidant activity; chemometric analysis

Experimental

Coffee samples

Twenty-four commercially available samples of roasted, ground *C. arabica* compiled in Table S2 were obtained from two distributing markets in Poland – Progressive, Czas na Herbate (Wroclaw, Poland) and Herbata i Kawa Swiata (Koscierzyna, Poland). The samples were of different geographical origin (geographical specifications were acquired from coffee distributing markets): Central America (CA1-CA6), South America (SA1-SA8), Africa (AF1-AF8), Asia (AZ1) and Australia (AU1). The identification of the coffee arabica constituents was based on the declaration of coffee quality and origin in line with UE standards. The homogenized samples were stored in a desiccator which was protected from light.

Chemicals and instruments

Standards of phenolic acids - caffeic, chlorogenic, gallic, vanillic, *p*-coumaric, ferulic, flavonoid - rutin, and alkaloids - caffeine, theobromine, theophylline were purchased from ChromaDex (California, USA). Acetonitrile and HPLC grade methanol were purchased from J.T. Baker (Phillipsbusg, USA) and POCh (Gliwice, Poland), respectively. Analytical grade methanol, ethanol, acetic acid were purchased from POCh (Gliwice, Poland) and trifluoroacetic acid (TFA) was obtained from Sigma Aldrich (St. Louis, MO, USA). Redistillated water was prepared by triple distillation of water in a Destamat bi-18 system (Heraeus Quarzglas, Hanau, Germany).

Separation and quantitation of phenolic compounds and alkaloids were performed using a HPLC Merck-Hitachi LaChrome device (Darmstadt, Germany) equipped with a L-7420 UV–vis detector, a L-7200 autosampler and a L-7360 thermostat. Chromatographic data were collected using a D-7000 HPLC System Manager, ver. 3.1 (Merck-Hitachi, Darmstadt, Germany). The method developed for quantitation of seven phenolic compounds and three alkaloids was validated by linear range, limit of detection (LOD), limit of quantitation (LOQ), precision and accuracy according to the procedure described previously (Viapiana et al., 2016).

Sample preparation

In this study hydromethanolic extracts were prepared. Sample of coffee (0.5 g) was sonicated with 4 mL of methanol-water mixture (80:20, v/v) for 15 min at 25 °C using an ultrasonic bath (Emag, Salach, Germany). The suspension was centrifuged in an EBA-20S centrifuge (Hettich, Tuttlingen, Germany) for 10 min at 8,000 rpm and the supernatant transferred into a 25 mL volumetric flask. This procedure was repeated twice, after which the extracts obtained were combined and diluted up to 25 mL with a mixture of methanol-water (80:20, v/v). Before HPLC analysis, hydromethanolic extracts were filtered through a 0.25 μm nylon filter film (Mecherey, Nagel, Germany) and 20 μL of the filtrate was injected into the HPLC system.

HPLC analysis

The chromatographic separation and quantitation of phenolic compounds: gallic acid, caffeic acid, chlorogenic acid, vanillic acid, *p*-coumaric acid, ferulic acid, rutin and alkaloids: caffeine, theobromine, theophylline were performed on a Hypersil Gold C18 column (250 × 4.6 mm, 5 μm particles) (Thermo Scientific, Runcorn, UK), maintained at 30 °C, using methanol-0.1% TFA solution (solvent A) and water-0.1% TFA solution (solvent B) as mobile phase. The HPLC mobile phase was freshly prepared on a daily basis, filtered through a 0.25 μm membrane filter. The separation was performed at a constant flow rate (1 mL/min) with the following condition: linear gradient from 5% to 25% of A in 30 min, from 25 to 55% in 15 min, from 55 to 83% in 5 min, then isocratic elution for 5 min and a linear gradient from 83 to 5% in 5 min. The absorbance was monitored at 280 nm, and the volume of injected sample and standard solutions was 20 µL. The identification of the analytes compounds was based on comparison of retention time of their standard compounds. Additionally, selected coffee sample (CA3) was spiked with the standard compounds and analyzed again.

A mixed stock solutions containing 1.04 mg/mL gallic acid, 0.84 mg/mL caffeic acid, 0.85 mg/mL chlorogenic acid, 1.33 mg/mL ferulic acid, 1.36 mg/mL rutin, 0.71 mg/mL vanillic acid, 0.88 mg/mL *p*-coumaric acid, 1.27 mg/mL theophylline, 0.93 mg/mL theobromine, 1.03 mg/mL caffeine were prepared by adding accurately weighed standard substances in the mixture of methanol-water (80:20, v/v), which were then diluted to six different working standard solutions.

DPPH radical scavenging activity

The radical scavenging activity of *C. arabica* extracts using DPPH assay was determined with the method developed by Tuberoso et al. (2010). About 400 µL of the sample extract solution were mixed with 1.6 mL of a 0.076 mM methanolic solution of DPPH. The mixture was incubated for 10 min. Absorbance was measured at 517 nm by the use of Metertech UV/Vis spectrophotometer (Nankang, Taiwan), and compared with Trolox calibration curve. The results were expressed as mg of Trolox per g of dry weight (mg TE/g DW).

Data analysis

All analyses were carried out in triplicate. Data were analyzed using both one-way analysis of variance (ANOVA) test, followed by Duncan test. A reference chromatographic fingerprint was calculated by Matlab 9.1 software as a result of analyzing all *C. arabica* samples. Next, similarity analysis was performed using a simulated mean reference chromatogram, which resulted for each coffee samples. The relationship among different coffee extracts based on the chemical composition and antioxidant activity was analyzed by a Pearson correlation analysis, and principal component analysis (PCA) was conducted for evaluation the variation of characteristic parameters among the coffee samples. Chemometric data analysis was performed using Statistica 10 (StatSoft Inc., Tulsa, OK, USA) software on the basis of parametric tests with the level of significance of $p < 0.05$.

References

Tuberoso CIG, Rosa A, Bifulco E, Melis MP, Atzeri A, Pirisi FM, Dessi MA. 2010. Chemical composition and antioxidant activities of *Myrtus communis* L., berries extracts. Food Chem. 123:1242-1251.

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Table Captions

Table S1. The retention time (T_R), relative retention time (RRT), peak area (PA) and relative peak area (RPA) of 13 common peaks in *C. arabica* extracts (n=6)

Table S2. List of *Coffea arabica* code names and place of origin

Table S3. Validation report of the methods for quantitation of phenolic compounds in *C. arabica* samples (n = 3)

Table S4. Results of the quantification of phenolic acids in the *C. arabica* samples (arithmetic mean ± standard deviation)

Table S5. Results of the quantification of alkaloids and rutin in the *C. arabica* samples (arithmetic mean ± standard deviation)

Figure Captions

Figure S1. Chromatographic fingerprint for all the *C. arabica* samples

Figure S2. (A) Reference chromatographic fingerprint for the extracts of *C. arabica* samples. The retention times [min] for quantified compounds were as follow: 5.29 (GA, peak 1), 11.34 (THB, peak 4), 19.81 (CA, peak 5), 20.81 (CGA, peak 6), 22.61 (CAF, peak 7), 25.11 (*p*CA, peak 8), 30.23 (FA, peak 10), 40.35 (RUT, peak 12); (B) The HPLC chromatographic profile of ten standards

Figure S3. PCA loading plot describing the contribution of the target metabolites to the variation of data matrix

Table S1. The retention time (T_R), relative retention time (RRT), peak area (PA) and relative peak area (RPA) of 13 common peaks in *C. arabica* extracts (n=6).

Components	T_R (min)	RRT		PA	RPA	
		Average	CV (%)		Average	CV(%)
1	5.21	0.251	0.89	2802621	0.168	44.43
2	7.63	0.367	1.05	959403	0.048	63.95
3	8.72	0.420	1.04	729129	0.039	47.06
4	11.19	0.539	1.45	4498195	0.224	16.55
5	18.92	0.912	1.50	5788880	0.334	36.98
6	19.95	0.961	1.00	8256846	0.471	27.58
7 (S)	20.75	1.000	0.00	22915933	1.000	0.00
8	24.33	1.172	0.39	1911683	0.241	42.95
9	26.55	1.279	0.35	2049180	0.121	36.55
10	29.43	1.418	0.48	1126256	0.066	31.59
11	39.04	1.881	0.89	741061	0.041	16.57
12	39.88	1.922	0.94	461989	0.027	28.86
13	43.59	2.101	1.08	1290458	0.082	69.69

S- reference peak

Table S2. List of *Coffea arabica* code names and place of origin.

Sample code	Place of origin	Continent of origin	Similarity
CA1	Panama, Boguete	Central America	0.846
CA2	Jamaica, Blue Montain		0.535
CA3	Nicaragua		0.891
CA4	Cuba, Lavado		0.868
CA5	Mexic		0.769
CA6	Honduras		0.914
SA1	Ecuador, organic	South America	0.842
SA2	Guatemala, Marogogype		0.434
SA3	Guatemala		0.908
SA4	Columbia, Supremo		0.865
SA5	Columbia, Excelso		0.909
SA6	Costa Rica		0.940
SA7	Peru		0.906
SA8	Brasile, Santos		0.524
AF1	Kenya	Africa	0.574
AF2	Rwanda		0.748
AF3	Burundi		0.950
AF4	Ethiopia, Sidamo		0.945
AF5	Ethiopia, Djimmah		0.938
AF6	Tanzania		0.878
AF7	Kenya		0.913
AF8	Tanzania North		0.653
AZ1	Indie, Malabar	Asia	0.946
AU1	Papua	Australia	0.879

Table S3 Validation report of the methods for quantitation of phenolic compounds in *C. arabica* samples (n = 3).

Phenolic compounds	Gallic acid	Theobromine	Theophylline	*p*-Coumaric acid	Ferulic acid	Rutin	Caffeic acid	Vanillic acid	Chlorogenic acid	Caffeine
Range (µg/mL)	10.6-95.4	9.3-83.7	12.7-114.3	8.3-92.7	13.3-119.7	14.5-130.5	18.3-150.2	12.5-163.3	13.5-122.1	10.3-92.7
r	0.9990	0.9868	0.9993	0.9944	0.9895	0.9994	0.9856	0.9976	0.9861	0.9951
LOD (µg/mL)	3.03	1.8	3.93	1.7	3.36	2.5	5.32	1.8	2.93	2.61
LOQ (µg/mL)	10.0	5.4	11.89	5.3	10.8	7.64	16.47	5.4	8.98	8.84
Intra-day precision										
Nominal conc. (µg/mL)	53.0	46.5	63.5	62.5	66.5	72.5	75.4	44.0	71.6	51.5
Assayed conc. (µg/mL)	48.4	43.8	58.8	59.7	64.7	70.0	71.3	40.9	66.2	49.6
Recovery (%)	91.4	93.5	92.7	95.6	97.3	96.6	94.8	93.1	92.5	96.4
CV (%)	0.5	1.2	0.9	1.4	1.0	0.9	0.8	1.1	1.5	1.4
Inter-day precision										
Nominal conc. (µg/mL)	53.0	46.5	63.5	62.5	66.5	72.5	75.4	44.0	71.6	51.5
Assayed conc. (µg/mL)	46.2	41.1	54.9	57.3	60.8	65.2	67.2	39.4	62.9	44.4
Recovery (%)	87.3	88.4	86.6	91.7	91.5	90.0	89.2	89.6	87.9	86.3
CV (%)	0.8	2.1	1.9	2.6	1.7	2.7	3.5	2.9	4.0	2.7
Recovery										
Mean	93.8	101.5	96.5	94.8	103.2	98.6	96.1	97.4	95.8	98.7
RSD (%)	2.8	3.4	3.6	2.5	1.4	3.1	4.2	1.5	1.2	3.8

Table S4. Results of the quantification of phenolic acids in the *C. arabica* samples (arithmetic mean ± standard deviation).

Sample code	Gallic acid mg/g DW	Caffeic acid mg/g DW	Vanillic acid µg/g DW	*p*-Coumaric acid µg/g DW	Chlorogenic acid mg/g DW	Ferulic acid µg/g DW
CA1	1.14^{abc} ± 0.44	5.99^{ab} ± 2.85	257.23^{a} ± 2.91	58.6^{ab} ± 4.10	4.95^{ab} ± 1.81	148.29^{ab} ± 1.59
CA2	1.02^{abc} ± 0.27	10.22^{cde} ± 1.85	382.75^{abcd} ± 5.38	177.5^{ab} ± 3.02	4.67^{ab} ± 0.41	185.94^{def} ± 2.36
CA3	1.29^{bc} ± 0.03	8.78^{de} ± 0.51	718.20^{e} ± 6.06	70.5^{ab} ± 5.60	7.52^{ab} ± 0.13	212.67^{abcde} ± 8.51
CA4	1.08^{abc} ± 0.25	6.22^{e} ± 3.03	491.52^{abcde} ± 5.65	119.7^{ab} ± 2.00	5.40^{ab} ± 0.28	196.91^{abcde} ± 2.24
CA5	0.84^{abc} ± 0.27	6.13^{abc} ± 2.37	237.72^{a} ± 1.72	102.6^{ab} ± 1.69	4.22^{a} ± 1.88	122.14^{a} ± 5.01
CA6	1.01^{abc} ± 0.25	7.50^{ab} ± 2.68	303.20^{ab} ± 8.79	92.2^{ab} ± 7.06	5.37^{ab} ± 1.95	138.11^{abcde} ± 6.56
SA1	1.05^{abc} ± 0.11	7.56^{abc} ± 1.41	394.43^{abcd} ± 1.01	112.2^{ab} ± 5.92	5.37^{ab} ± 0.78	177.53^{abcde} ± 3.38
SA2	0.77^{ab} ± 0.18	7.16^{abc} ± 1.87	215.13^{a} ± 2.53	44.3^{ab} ± 2.42	4.73^{ab} ± 0.31	194.71^{abc} ± 4.50
SA3	1.03^{abc} ± 0.38	9.84^{bcd} ± 1.11	390.05^{abcd} ± 9.56	141.5^{ab} ± 2.65	6.41^{ab} ± 1.06	183.30^{cdef} ± 6.18
SA4	1.34^{bc} ± 0.03	9.27^{abcd} ± 1.07	644.12^{de} ± 1.89	236.6^{ab} ± 1.04	6.94^{ab} ± 0.35	244.13^{ef} ± 5.70
SA5	0.52^{a} ± 0.68	7.57^{abcd} ± 2.41	561.99^{bcde} ± 3.69	166.8^{ab} ± 8.30	6.06^{ab} ± 0.39	234.20^{bcdef} ± 1.64
SA6	1.40^{bc} ± 0.34	11.71^{abc} ± 1.71	501.52^{abcde} ± 3.59	126.9^{ab} ± 2.41	7.82^{ab} ± 0.14	217.20^{cdef} ± 2.51
SA7	1.45^{c} ± 0.41	11.44^{ab} ± 1.31	483.93^{abcde} ± 5.29	195.8^{ab} ± 3.42	7.42^{ab} ± 0.50	234.68^{f} ± 2.40
SA8	1.12^{abc} ± 0.16	6.68^{abcd} ± 1.60	247.18^{a} ± 1.32	62.0^{ab} ± 2.22	4.86^{ab} ± 0.30	179.99^{abc} ± 1.72
AF1	1.07^{abc} ± 0.48	5.90^{a} ± 1.57	376.52^{abcd} ± 12.12	78.8^{ab} ± 4.28	4.44^{a} ± 1.15	157.73^{abc} ± 3.16
AF2	1.29^{bc} ± 0.43	7.31^{abc} ± 1.39	462.25^{abcde} ± 1.71	114.0^{ab} ± 8.12	5.46^{ab} ± 1.31	161.62^{abcde} ± 6.7
AF3	1.02^{abc} ± 0.32	8.57^{abcd} ± 2.05	649.05^{de} ± 2.45	25.3^{a} ± 2.04	6.57^{ab} ± 1.14	178.48^{abcde} ± 3.07
AF4	1.34^{bc} ± 0.55	8.07^{abcd} ± 3.78	343.05^{abc} ± 2.21	134.4^{ab} ± 1.16	5.48^{ab} ± 2.27	169.91^{abcd} ± 8.45
AF5	1.31^{bc} ± 0.37	13.75^{abcd} ± 4.38	604.32^{cde} ± 7.84	134.2^{ab} ± 1.97	7.16^{ab} ± 0.48	191.75^{cdef} ± 1.74
AF6	1.33^{bc} ± 0.14	9.69^{de} ± 1.94	478.77^{abcde} ± 6.81	138.7^{ab} ± 1.87	6.45^{ab} ± 0.90	261.82^{cdef} ± 1.85
AF7	0.99^{abc} ± 0.23	9.23^{abc} ± 2.72	334.71^{abc} ± 8.78	113.8^{ab} ± 1.15	6.00^{ab} ± 2.11	179.38^{abcde} ± 9.01
AF8	0.90^{abc} ± 0.08	5.95^{abcd} ± 0.80	369.08^{abcd} ± 12.61	173.8^{ab} ± 5.28	4.53^{a} ± 0.57	170.53^{abc} ± 4.43
AZ1	1.28^{bc} ± 0.04	7.98^{abcd} ± 0.96	431.19^{abcde} ± 9.18	97.8^{ab} ± 2.60	6.22^{ab} ± 0.01	189.26^{abcde} ± 1.97
AU1	1.16^{abc} ± 0.35	7.30^{abc} ± 1.83	407.62^{abcd} ± 1.02	95.3^{ab} ± 4.27	5.35^{ab} ± 1.06	180.90^{abcde} ± 3.63

Means followed by the same letter within a column indicate no significant difference ($p < 0.05$) in Duncan test.

Table S5. Results of the quantification of alkaloids and rutin in the *C. arabica* samples (arithmetic mean ± standard deviation).

Sample Code	Rutin mg/g DW	Theobromine mg/g DW	Theophylline µg/g DW	Caffeine mg/g DW
CA1	$0.59^{a} \pm 0.21$	$1.14^{abc} \pm 0.01$	$93.29^{abcd} \pm 2.84$	$5.27^{abcd} \pm 1.40$
CA2	$0.76^{a} \pm 0.51$	$2.30^{bcdefg} \pm 0.48$	$97.87^{abcd} \pm 1.87$	$3.42^{a} \pm 1.60$
CA3	$8.90^{b} \pm 1.20$	$3.05^{efg} \pm 0.19$	$140.47^{bcde} \pm 2.01$	$5.86^{bcd} \pm 0.03$
CA4	$0.64^{a} \pm 0.19$	$2.63^{defg} \pm 0.45$	$97.23^{abcd} \pm 1.66$	$6.27^{d} \pm 0.23$
CA5	$0.51^{a} \pm 0.10$	$0.93^{ab} \pm 0.01$	$54.33^{a} \pm 1.77$	$3.87^{abc} \pm 1.82$
CA6	$0.85^{a} \pm 0.30$	$2.52^{cdefg} \pm 1.30$	$96.66^{abcd} \pm 4.99$	$4.58^{abcd} \pm 1.54$
SA1	$0.94^{a} \pm 0.15$	$1.69^{bcde} \pm 0.33$	$112.40^{abcde} \pm 4.95$	$4.57^{abcd} \pm 0.69$
SA2	$5.76^{b} \pm 1.57$	$1.50^{abcd} \pm 0.04$	$77.43^{ab} \pm 7.26$	$3.73^{ab} \pm 0.28$
SA3	$1.00^{a} \pm 0.34$	$2.41^{cdefg} \pm 1.08$	$88.42^{abc} \pm 6.35$	$4.76^{abcd} \pm 0.65$
SA4	$0.69^{a} \pm 0.07$	$2.26^{bcdef} \pm 0.12$	$118.50^{abcde} \pm 5.32$	$5.99^{bcd} \pm 0.12$
SA5	$0.53^{a} \pm 0.20$	$0.25^{a} \pm 0.03$	$154.74^{cde} \pm 6.98$	$5.25^{abcd} \pm 0.42$
SA6	$7.39^{b} \pm 0.22$	$2.54^{cdefg} \pm 0.47$	$136.96^{bcde} \pm 7.65$	$6.16^{cd} \pm 0.01$
SA7	$1.38^{a} \pm 0.64$	$3.21^{fg} \pm 1.46$	$137.87^{bcde} \pm 1.47$	$5.82^{bcd} \pm 0.11$
SA8	$0.29^{a} \pm 0.02$	$1.52^{abcd} \pm 0.10$	$110.72^{abcde} \pm 3.26$	$3.45^{ab} \pm 0.31$
AF1	$0.71^{a} \pm 0.29$	$0.90^{ab} \pm 0.05$	$93.52^{abcd} \pm 2.70$	$3.06^{abc} \pm 1.07$
AF2	$1.03^{a} \pm 0.31$	$1.63^{bcd} \pm 0.58$	$150.73^{cde} \pm 1.62$	$4.49^{abcd} \pm 1.21$
AF3	$1.13^{a} \pm 0.22$	$1.59^{abcd} \pm 0.67$	$120.07^{abcde} \pm 2.96$	$5.07^{abcd} \pm 0.67$
AF4	$0.86^{a} \pm 0.01$	$2.30^{bcdefg} \pm 0.08$	$159.98^{de} \pm 2.30$	$4.26^{abcd} \pm 2.17$
AF5	$6.47^{b} \pm 1.14$	$3.66^{g} \pm 0.88$	$127.14^{bcde} \pm 1.67$	$5.95^{bcd} \pm 0.01$
AF6	$0.85^{a} \pm 0.04$	$3.24^{fg} \pm 0.79$	$136.73^{bcde} \pm 1.03$	$4.75^{abcd} \pm 0.54$
AF7	$0.81^{a} \pm 0.18$	$1.87^{bcdef} \pm 0.69$	$135.04^{bcde} \pm 3.76$	$4.43^{abcd} \pm 1.51$
AF8	$0.38^{a} \pm 0.13$	$2.09^{bcdef} \pm 0.67$	$101.42^{abcd} \pm 8.10$	$3.73^{ab} \pm 0.42$
AZ1	$1.35^{a} \pm 0.84$	$2.12^{bcdef} \pm 0.19$	$139.06^{bcde} \pm 3.10$	$5.59^{abcd} \pm 0.09$
AU1	$0.75^{a} \pm 0.39$	$1.28^{abcd} \pm 0.24$	$172.86^{e} \pm 8.26$	$4.84^{abcd} \pm 0.91$

Means followed by the same letter within a column indicate no significant difference ($p < 0.05$) in Duncan test.

Figure S1. Chromatographic fingerprint for all the *C. arabica* samples.

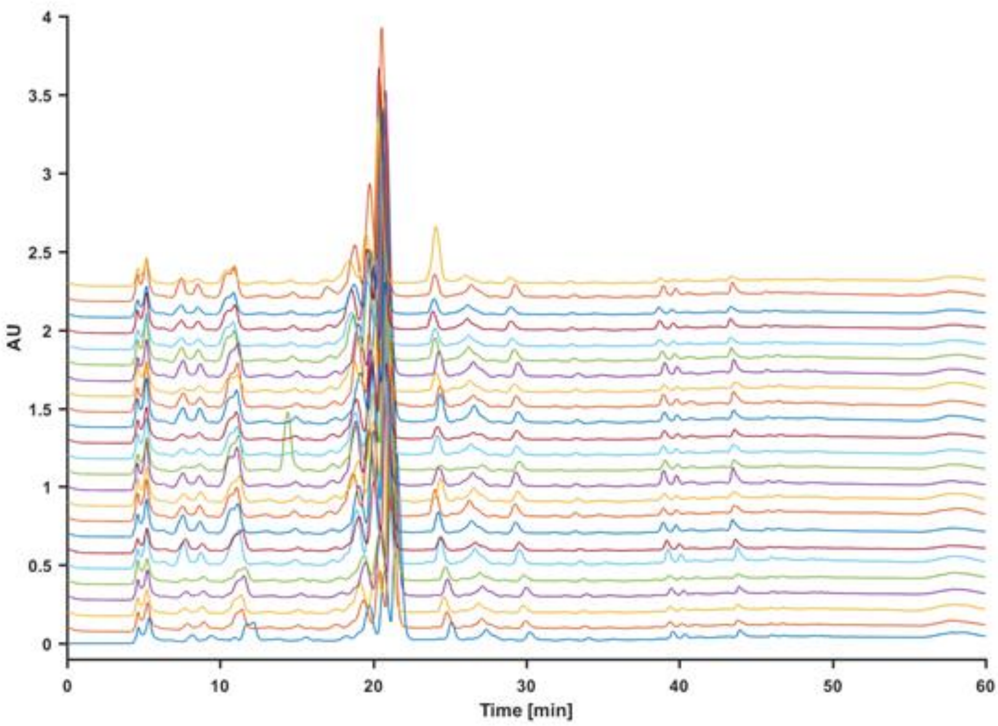

Figure S2. (A) Reference chromatographic fingerprint for the extracts of *C. arabica* samples. The retention times [min] for quantified compounds were as follow: 5.29 (GA, peak 1), 11.34 (THB, peak 4), 19.81 (CA, peak 5), 20.81 (CGA, peak 6), 22.61 (CAF, peak 7), 25.11 (*p*CA, peak 8), 30.23 (FA, peak 10), 40.35 (RUT, peak 12); (B) The HPLC chromatographic profile of ten standards.

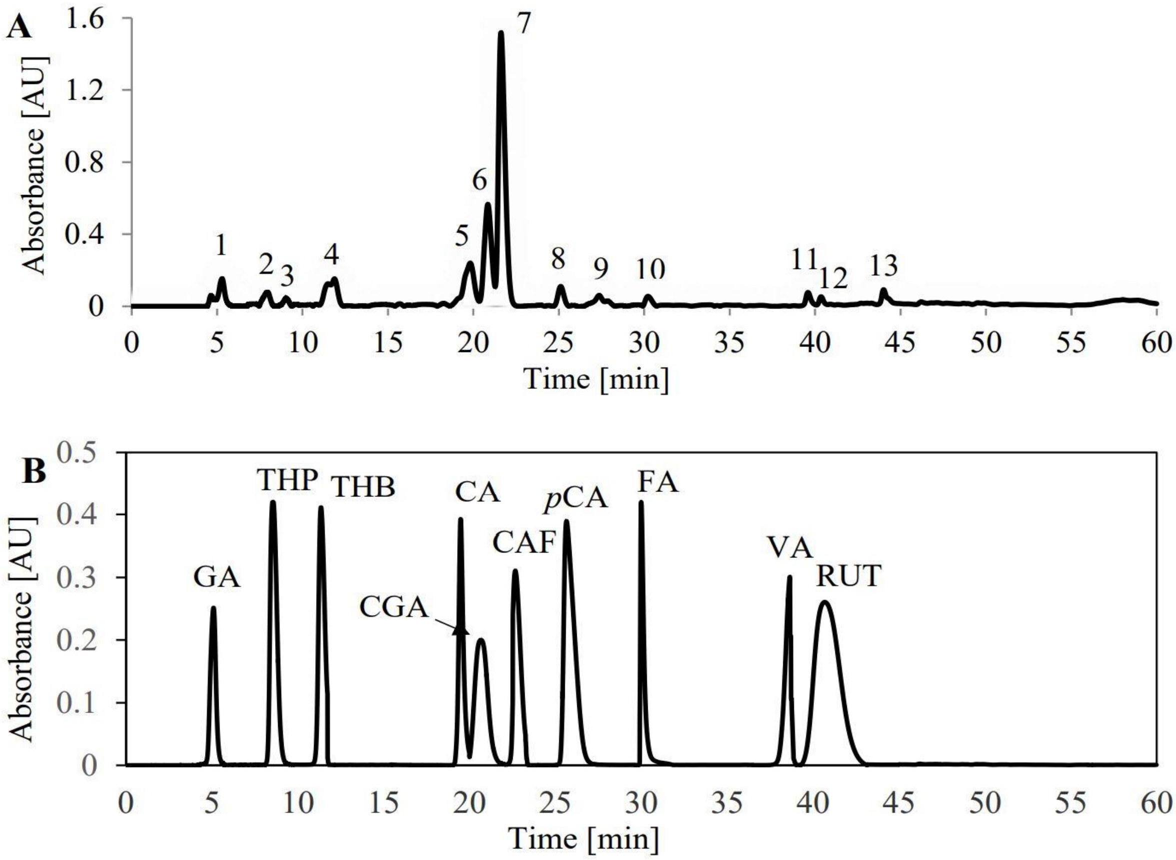

Figure S3. PCA loading plot describing the contribution of the target metabolites to the variation of data matrix.