

Comparative Analysis of Chemical Composition and Antioxidant Activity in Conventional, Civet, and Elephant Coffees: An Exploratory Study on Authentication Challenges

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Supplementary Tables and Figures

Table S1

Table S2

Figure S1

Figure S2

Figure S3

Figure S4

Figure S5

Supplementary Table S1 Validation parameters for HPLC-UV method

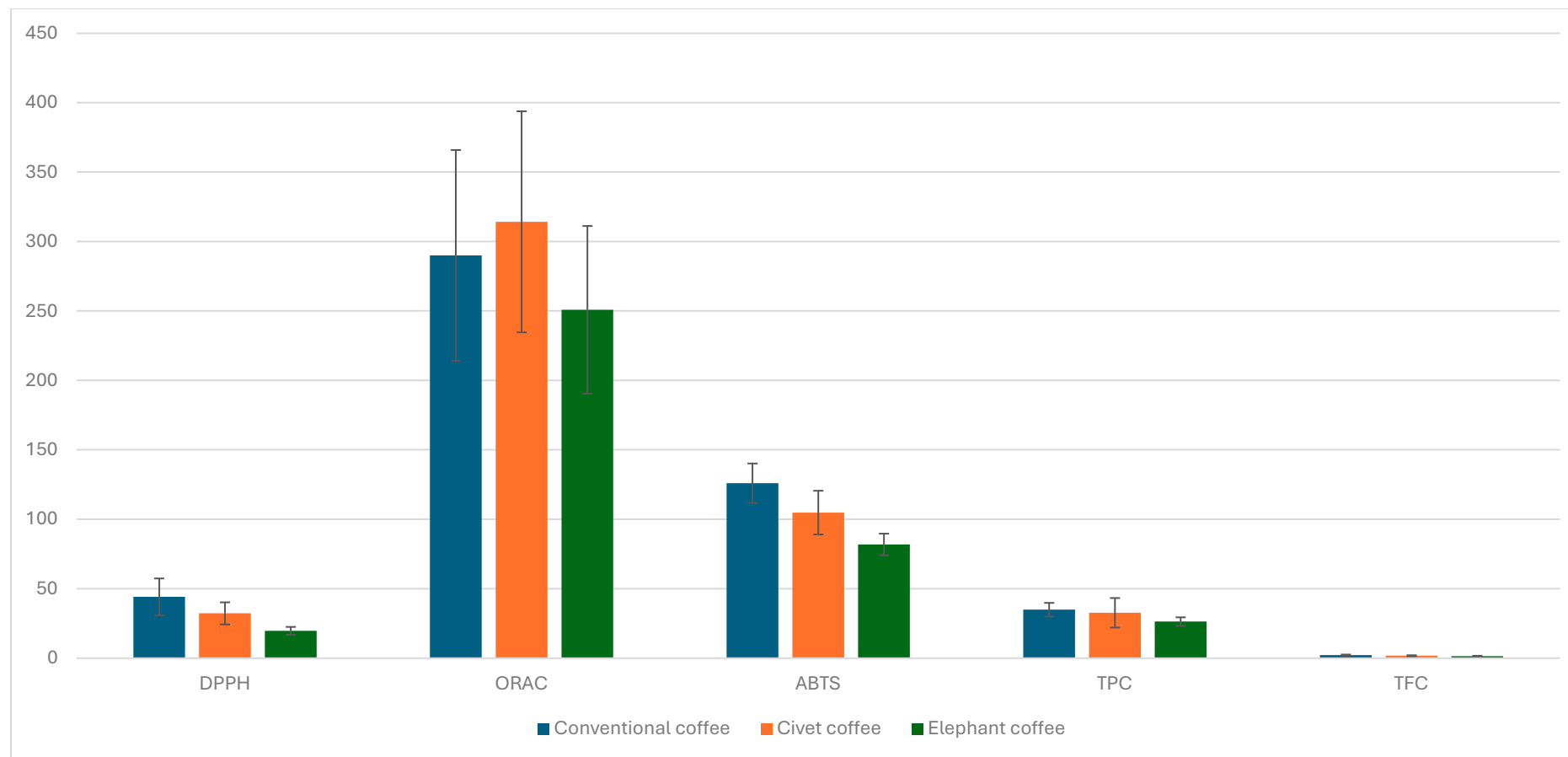
Compound	RT	Linear equation	Coefficient of determination	Limit of detection	Limit of quantification	Precision (for standards) ^a	Precision (for samples) ^b	Accuracy	Recovery
	min		R ²	µg/mL	µg/mL	%	%	%	%
Gallic acid	5.743	y = 0.2864x - 0.3575	0.9975	0.70	2.33	4.82	5.65	6.76	91.40
Caffeine	9.99	y = 0.1814x - 0.3326	0.9984	0.67	2.23	1.52	0.76	9.99	95.10
Chlorogenic acid	13.233	y = 0.3462x - 0.2033	0.9993	0.51	1.71	12.93	2.16	11.04	92.17
Caffeic acid	15.263	y = 0.4845x - 0.1006	0.9998	0.17	0.55	12.50	1.56	13.78	103.03
Cinnamic acid	31.59	y = 0.6725x - 0.5791	0.9993	0.57	1.91	15.60	3.31	7.73	102.92

^a Acquired data are related to 50 µg/mL standard concentration (average of three interday injections); ^b average of three interday injections

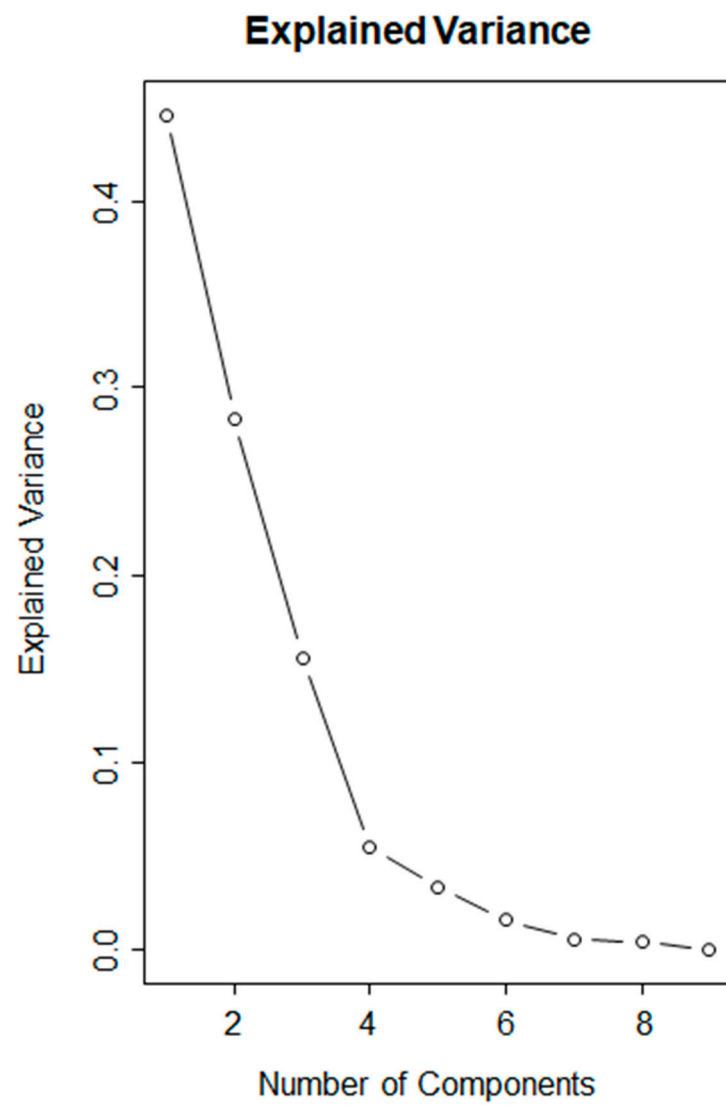
NOTE: methodology of validation parameters calculations is given in Tauchen, J.; Marsik, P.; Kvasnicova, M.; Maghradze, D.; Kokoska, L.; Vanek, T.; Landa, P. In Vitro Antioxidant Activity and Phenolic Composition of Georgian, Central and West European Wines. *Journal of Food Composition and Analysis* **2015**, *41*, 113–121, doi:10.1016/j.jfca.2014.12.029.

Supplementary Table S2 Retention times, retention indices and CAS number of volatile compounds detected by GC-MS-SPME in coffee samples

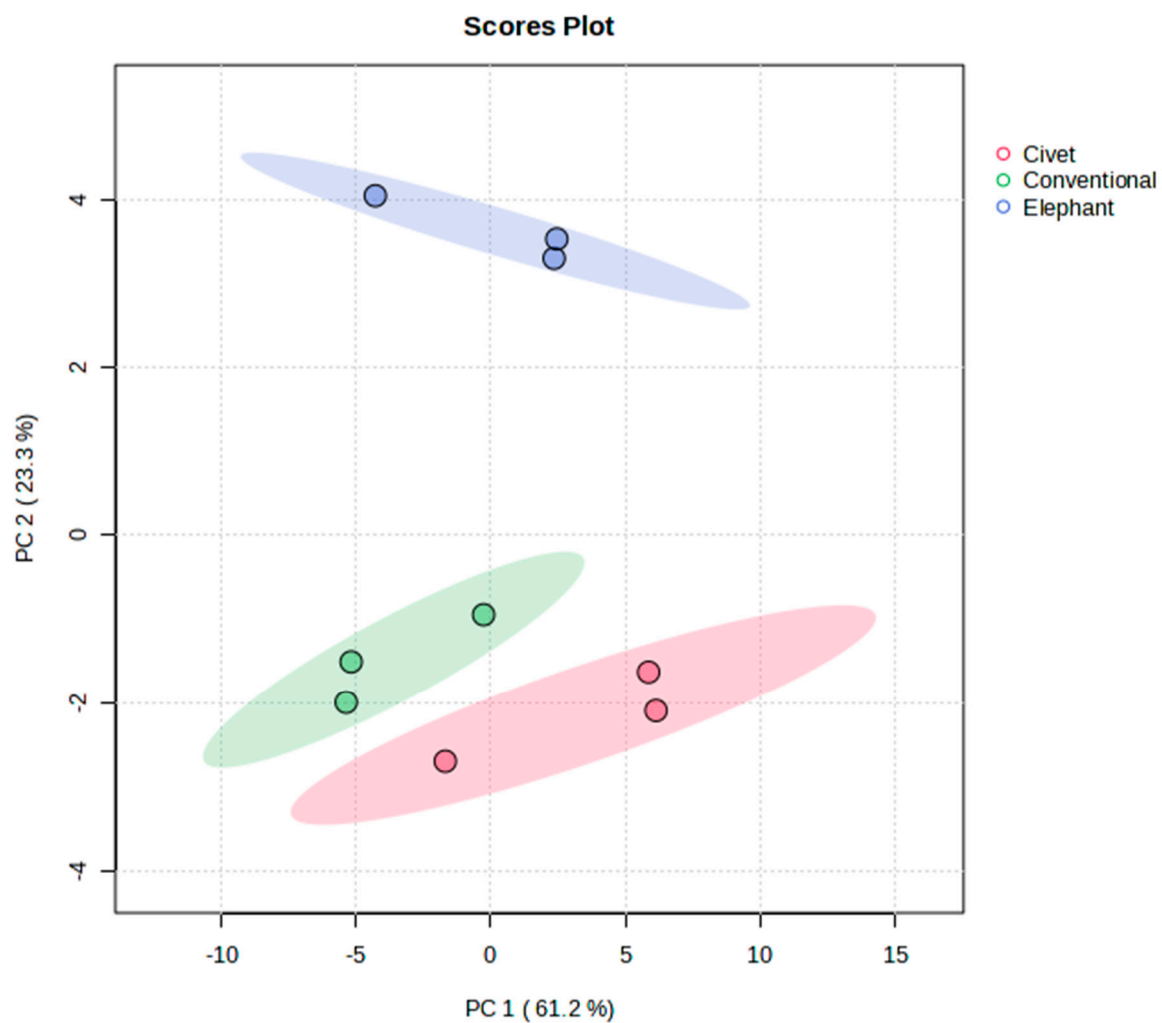
Compound	RT (min)	RI (measured)	RI (literature) ^b	CAS number
2-methylfuran	6,342	611	603	534-22-5
acetic acid	6,608	618	602	64-19-7
3-methylbutanal	7,411	647	649	590-86-3
2-methylbutanal	7,641	654	656	96-17-3
pentan-2,3-dione	8,536	684	696	600-14-6
propanoic acid	8,994	701	702	79-09-4
pyrazine	9,746	724	672	290-37-9
pyridine	10,140	737	740	110-86-1
methylpyrazine	12,956	824	824	109-08-0
furfural	13,395	839	829	98-01-1
furfuryl alcohol	14,390	862	866	98-00-0
1-acetoxy-propan-2-one	14,746	872	876	592-20-1
2-methylbutanoic acid	15,029	878	886.2	116-53-0
furfuryl formate,	16,121	909	902	13493-97-5
5-methylfurfural	18,206	968	969	620-02-0
furfuryl acetate	19,224	996	998	623-17-6
2-ethyl-5-methylpyrazine	19,459	1003	1004	13360-64-0
<i>N</i> -acetyl-4 <i>H</i> -pyridine	20,135	1024	1038	67402-83-9
(+)-limonene	20,429	1033	1035	5989-27-5
2,3-dimethyl-2-cyclopenten-1-one	20,764	1044	1052	1121-05-7
1-(1 <i>H</i> -pyrrol-2-yl)-ethanone	21,532	1067	1063	1072-83-9
3-ethyl-2,5-dimethylpyrazine	21,994	1081	1083	13360-65-1
3-ethyl-2-hydroxy-2-cyclopenten-1-one	22,546	1098	1082	21835-01-8



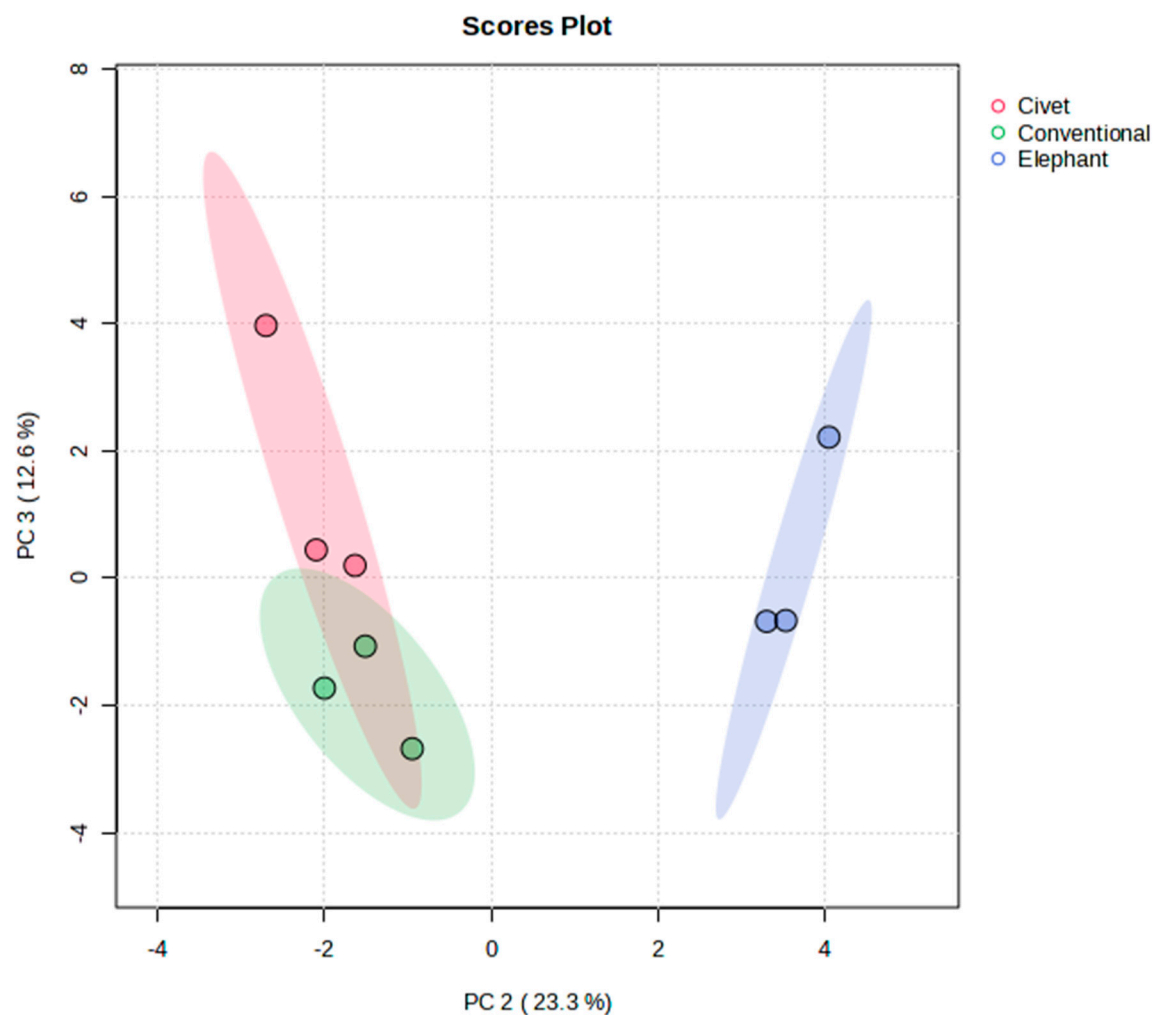
Supplementary Figure S1 Antioxidant activity and total phenolic and flavonoid contents (mean $\pm$ SD) of the tested coffee samples. Each coffee type was represented by a single commercial batch (N = 1). Results are obtained from three technical replicates each performed in three independent tests (9 repetitions).



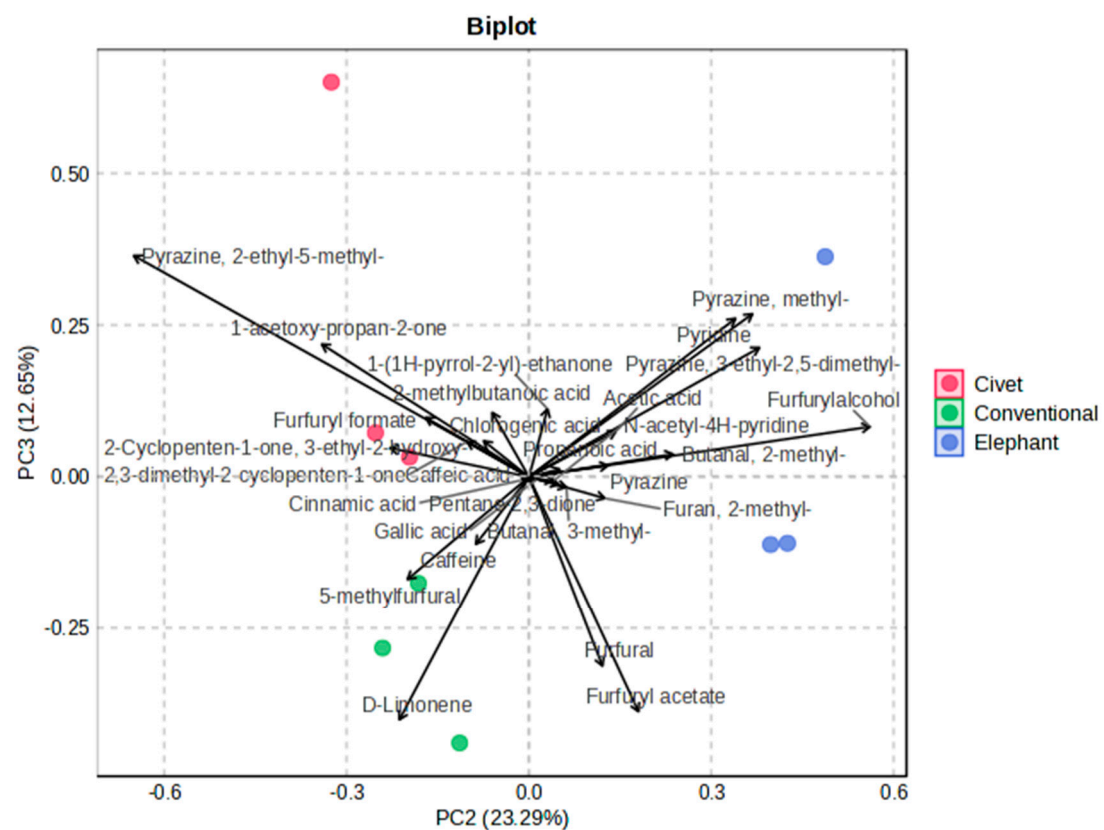
Supplementary Figure S2 Mean reconstruction error vs. number of principal components (cross-validation of PCA)



Supplementary Figure S3 PCA score plot of three sample groups—civet coffee (red), conventional coffee (green), and elephant coffee (blue)—based on a total of 9 samples. The 28 variables used were concentrations [mg/g DW], scaled using Pareto scaling. The significance of group separation was assessed using PERMANOVA ($F = 4.6045$, $R^2 = 0.6055$, $p = 0.035$; based on 999 permutations).



Supplementary Figure S4 PCA score plot of three sample groups—civet coffee (red), conventional coffee (green), and elephant coffee (blue)—based on a total of 9 samples. The 28 variables used were concentrations [mg/g DW], scaled using Pareto scaling. The significance of group separation was assessed using PERMANOVA ($F = 13.432$; $R^2 = 0.81743$; $p = 0.003$; based on 999 permutations).



Supplementary Figure S5 A PCA (PCA 2 and 3) score plot of three sample groups—civet coffee (red), conventional coffee (green), and elephant coffee (blue)—based on a total of 9 samples. All 28 measured variables, expressed as concentrations [mg/g DW] and Pareto-scaled, were used.