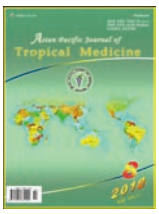

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Comparative phytochemical analysis of *Coffea benghalensis* Roxb Ex Schult, *Coffea arabica* L. and *Coffea liberica* Hiern

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ABSTRACT

Objective: To make phytochemical studies of the leaf, pericarp and seed of *Coffea benghalensis* (*C. Benghalensis*) compared with those of the widely known *Coffea arabica* and *Coffea liberica*.
Methods: The sample extracts were prepared by Soxhlet-extraction. Polyphenol content was analyzed by HPLC-ESI-MS/MS, the identification was carried out based on the retention time, UV and mass spectra of standards and literature data of the detected compounds.
Results: Phenolic acids like caffeoylquinic acids, dicaffeoylquinic acids, feruloylquinic acids and coumaroylquinic acid, as well as mangiferin were detected as main constituents in all extracts. Procyanidin trimers were present exclusively in the leaves. In *C. benghalensis*, main constituents were 5-caffeoylquinic acid and 4-caffeoylquinic acid. Flavan-3-ols were described in all immature and mature pericarp and leaf extracts. Even though 4-feruloylquinic acid was described in both immature and mature seed, dicaffeoylquinic acids were identified only in the mature seed extracts. Mangiferin was present in the leaf, mature pericarp and seed.
Conclusions: These analyses provide new chemotaxonomical data for the selected coffees, especially for *C. benghalensis*. Due to its high polyphenol content, our results indicate its significance of providing new data as a possible source for industry.

1. Introduction

Coffea species are well-known and wide-spread all over the world in tropical and subtropical areas, especially in the Equatorial region. The most important coffee producing continents are South America (Brazil, Colombia, Venezuela, Bolivia, Peru, Ecuador), Central America (Mexico, El Salvador, Cuba, Haiti, Dominica, Nicaragua, Guatemala), Africa (Angola, Liberia, Ethiopia, Congo, Kenya, Tanzania, Uganda, Nigeria, Malawi), and Asia (India, Sri Lanka, Malaysia, Indonesia, Java, Sumatra, New Guinea)[1,2] .

They also have an important role in science because of their pharmacological role. In addition, they are one of the most sought products after petrol and they also provide an income for more than 20 million families in more than 50 countries every year[3,4]. Because of the great impact on the quality of coffee beverages, many biochemical studies were reported about the chemical composition of the green and roasted coffee beans. By contrast, only few phytochemical and microbiological analyses are available about the leaf and pericarp of wild and cultivated coffee species.

Chlorogenic acids as characteristic components of coffee beans and commercial coffee products compose a group of esters formed between quinic acid and *trans*-hydroxycinnamic acids (*e.g.* caffeic, ferulic and *p*-coumaric acid), and sometimes from dimethoxycinnamic, trimethoxycinnamic and sinapic acid[5,6]. Among chlorogenic acids, caffeoylquinic, *p*-coumaroylquinic, feruloylquinic, dicaffeoylquinic and caffeoylferuloyl quinic acids have been reported in coffees[7]. Chlorogenic acids usually form a compound with caffeine which occurs in 1:1 ratio in plants[8].

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Many studies focused on cultivated species like *Coffea arabica* (*C. arabica*) L. (Arabic coffee), *Coffea canephora* Pierre ex A. Froehner (Robusta coffee) and *Coffea liberica* (*C. liberica*) Hiern. (Liberian coffee) nowadays, but only few data are available about wild coffees like *Coffea benghalensis* (*C. benghalensis*) Roxb. ex Schult. (Bengal coffee). Bengal coffee was studied for its histological and phytochemical characters; in our previous work, caffeic acid, sinapic acid and rutin were identified in the immature fruits[4]. Furthermore, caffeine and terpenoids (cafesterol, bengalensol) have been also detected in the leaf and the fruit earlier[9-11]. In addition, Trevisan *et al*.[12] denoted higher total mangiferin content in the leaf of plants growing under natural full-sun conditions compared to other ones living in management used organic treatment. The immature pericarp of Bengal and Liberian coffees showed higher amount of tannin and polyphenol components compared with Arabic species[13].

The aim of this study was to investigate and compare the phenolic content of the leaf, pericarp and seed of *C. benghalensis*, *C. arabica* and *C. liberica*. A further purpose was to evaluate the phenolic composition of their immature and mature pericarp, which has not been analyzed up to the present. The phytochemical analysis aimed at the qualitative comparison of phenolic compounds to provide new chemotaxonomical data for the selected coffee species.

2. Materials and methods

2.1. Plant materials

The leaf, immature and mature pericarp and seed of *C. benghalensis*, *C. arabica* and *C. liberica* were collected at the Botanical Garden, University of Pécs in the spring of 2015. The samples were air-dried at room temperature in the shade. Voucher specimens were deposited and labeled with unique codes at the Department of Pharmacognosy, University of Pécs.

2.2. Chemicals

The following chemicals were used for all analyses: petroleum ether (Molar Chemicals Ltd., Budapest, Hungary), methanol of analytical grade (Reanal, Budapest, Hungary), acetic acid and methanol of HPLC supergradient grade (Sigma-Aldrich, Steinheim, Germany). All aqueous eluents for LC-MS were filtered through MF-Millipore membrane filters (0.45 μm, mixed cellulose esters) (Billerica, MA, USA).

2.3. HPLC–ESI–MS/MS analysis

2.3.1.Sample preparation

All plant samples were powdered and extracted using Soxhlet-extraction method (70:30 v/v % methanol: distilled water). After the evaporation of the solvent, the residues were redissolved in 5 mL 70:30 v/v % methanol-water mixture. Apolar compounds were removed by liquid-liquid extraction with petroleum ether if needed. Prior to evaluation, all samples were submitted to SPE purification (500 mg/3 mL Supelco Supelclean LC-18 SPE cartridges, Sigma-Aldrich, Steinheim, Germany), and they were filtered through Sartorius (Goettingen, Germany) Minisart RC15 (0.2 μm) syringe filters.

2.3.2. HPLC–ESI–MS/MS

Chromatographic analyses were performed on an Agilent 1100 HPLC Series system coupled with an Agilent 6410 Triple Quadrupole mass spectrometer using an electrospray ion source in negative ionization mode. A ZORBAX SB-C18 3.0 mm × 150 mm, 3.5 μm column was used for separation. A total of 0.3 v/v % acetic acid in water and methanol was used as mobile phase A and B respectively with a gradient method as follows: 0 min 10 v/v % B, 30 min 100 v/v % B, 35 min 100 v/v % B. The temperature of the column was kept at 25 ℃. The flow rate of the mobile phase was 0.3 mL/min and the injection volume was 5 μL. ESI conditions were as follows: temperature: 350 ℃, nebulizer pressure: 40 psi (N2), drying gas flow rate: 9 L/min (N2), capillary voltage: 3 500 V, fragmentor voltage: 100 V. Collision energy was changed between 10-50 eV, according to structural differences. High purity nitrogen was used as collision gas. Full mass scan spectra were recorded over the range *m/z* 70-1 000 (1 scan/sec). The Masshunter B.01.03 software was used for data acquisition and qualitative analysis. For unambiguous identification, retention times, UV and mass spectra were compared with literature data and with those of authentic standards.

3. Results

3.1. HPLC analysis

Altogether 25 phenolic components were identified in the extracts of the studied parts of the selected *Coffea* species (Table 1) and 22 compounds were detected in Bengal coffee (Figure 1). Among them, 16 phenolic acid derivatives (*e.g.* caffeoylquinic acids), 2 flavan-3-ols, 2 procyanidin dimers and 2 procyanidin trimers, a xanthonoid, and 2 aliphatic tricarboxylic acids were qualitatively characterized by comparison of their LC-ESI-MS/MS data with the literature and mass spectral data of reference compounds (Table 1).

Phenolic compositions of the studied coffee species were similar with minor differences. Chlorogenic acids were observed as the main components in each extract. 4-caffeoylquinic acid (4-CQA) and 5-caffeoylquinic acid (5-CQA) were detected in each sample, except that the latter was absent in *C. arabica* leaf extract. The most complex composition including 17 compounds was detected in the immature pericarp of Arabic coffee, followed by the extract of the mature pericarp of Bengal coffee (16 compounds), and the leaf extract of Liberian coffee (16 compounds).

3.1.1. Leaf extracts

3-caffeoylquinic acid (3-CQA) was present in all leaf samples being the main compound in *C. arabica* (Table 2). 5-CQA was the main component in *C. benghalensis* and *C. liberica*. 5-coumaroylquinic acid (5-CoQA) and a further isomer of 5-CQA were present in Liberian coffee. 4-CQA, dicaffeoylquinic acids (diCQAs) and ferulic acid, as well as mangiferin were detected in all samples.

3.1.2.Immature pericarp extracts

5-CQA was characterized as the main component of all samples, additionally, 4-CQA and catechin/epicatechin also were abundant in all studied species (Table 3). Moreover, Arabic and Liberian coffee contained 3-CQA, a further isomer of 5-CQA, 4-feruloylquinic acid (4-FQA), 3,4-diCQA, 3,5-diCQA and 4,5-diCQA, as well.

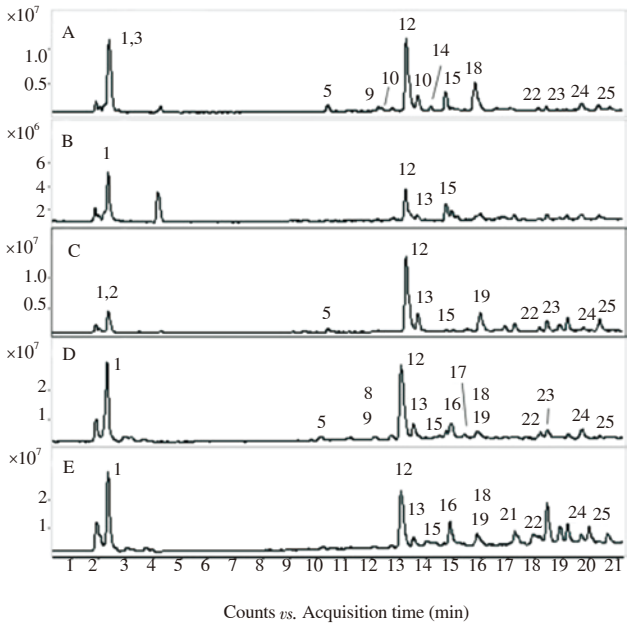

Figure 1. Total Ion chromatograms of the detected phenolic components of the studied parts of *C. benghalensis*.

A. Leaf, B. Immature pericarp, C. Immature seed, D. Mature pericarp, E. Mature seed.

3.1.3.Immature seed extracts

The main compound of all coffee immature seed samples was 5-CQA, followed by 4-FQA, 4-CQA and 3-CQA (Table 4). 5-CQA, 5-CoQA and 5-FQA were identified in Arabic and Liberian coffees. The latter was characterized by the presence of a procyanidin dimer and 5-CQA methyl ether that could be detected only in both immature and mature seed of Liberian coffee. The diCQA compounds were described for C. benghalensis and C. arabica, while Liberian coffee contained only 3,4-diCQA and 4,5-diCQA.

3.1.4. Mature pericarp extracts

Similar to the immature seed samples, the main compound of the mature pericarp extracts was 5-CQA, while 4-CQA was present in smaller amount in each species. For Bengal and Arabic coffees, 3-CQA, 5-CQA, 5-CoQA, 4-FQA and diCQA compounds were described, as well. Liberian coffee extract contained only 4,5-diCQA. Flavan-3-ols and a procyanidin compound were detected in Bengal coffee (Table 5).

3.1.5. Mature seed extracts

In the mature seed extracts, 5-CQA was identified as the main compound in Bengal and Arabic coffee, while 4-CQA, 5-CQA,4-FQA and the diCQAs were detected as minor components (Table 6). In addition, Bengal coffee was characterized by the presence of 5-FQA. The 5-CQA and 4-CQA isomers compounds, as well as the components 7 and 13, respectively, were detected with comparably high intensity in Liberian coffee extracts. Minor components of the mature seed samples were 3-CQA, 5-CoQA, 4-FQA and diCQAs. Citric acid was found only in *C. liberica*.

4. Discussion

Based on few literature data of phytochemical investigations of *C. benghalensis*, which could have formed the basis of the analyses, the comprehensive comparison of the plant with two well-known coffee species is essential to express its possible pharmacological

Table 1

Compounds detected in the studied parts of coffees by HPLC-ESI-MS/MS.

No.	Proposedcomponents	Rt(min)	Mature seed			Mature pericarp			Immature seed			Immature pericarp			Leaf		
			CB	CA	CL	CB	CA	CL	CB	CA	CL	CB	CA	CL	CB	CA	CL
1	Isocitric acid	2.3	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
2	Caffeoyl hexoside	2.3		+			+		+	+							
3	Quinic acid derivate	2.3					+								+		
4	Citric acid	3.0			+												
5	3-CQA	10.2		+	+	+	+		+	+	+		+	+	+	+	+
6	Procyanidin dimer	10.2											+				
7	4-CQA	11.6			+								+			+	
8	5-CQA derivative	12.1			+	+				+	+						
9	Catechin/ epicatechin	12.1				+							+	+	+		+
10	Procyanidin trimer	12.4													+	+	+
11	Procyanidin dimer	12.7				+					+			+			+
12	5-CQA	13.1	+	+	+	+	+	+	+	+	+	+	+	+	+		+
13	4-CQA	13.6	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
14	Procyanidin trimer	14.2													+	+	+
15	Catechin/epicatechin	14.7	+			+	+	+	+		+	+	+	+	+		+
16	5-CQA	14.9	+	+	+	+	+			+	+		+	+			+
17	5-CoQA	15.4		+	+	+	+			+	+		+				+
18	Mangiferin	15.8	+			+	+						+	+	+	+	+
19	4-FQA	15.9	+	+	+	+	+		+	+	+		+	+			
20	5-CQA methyl ether	16.8			+						+						
21	5-FQA	17.4	+							+	+		+				
22	3,4-diCQA	18.2	+	+	+	+	+		+	+	+		+	+	+	+	+
23	3,5-diCQA	18.4			+	+	+		+	+			+	+	+	+	+
24	4,5-diCQA	19.7	+	+	+	+	+	+	+	+	+		+	+	+	+	+
25	Ferulic acid	20.3	+		+	+	+		+	+	+		+		+	+	+

CA: *C. arabica*, CB: *C. benghalensis*, CL: *C.liberica*, Rt: Retention time.

Table 2

Detected compounds in the leaf of the studied coffees.

No.	Proposed components of the leaf	Rt(min)	[M-H]-(*m/z*)	MS/MS (*m/z*)	*C. benghalensis*	*C. arabica*	*C. liberica*
1	Isocitric acid	2.3	191	127 109 85	+	+	+
3	Quinic acid derivate	2.3	533	191	+		
5	3-CQA	10.2	353	191 179 135	+	+	+
9	Catechin/epicatechin	12.1	289	245 203	+		+
10	Procyanidin trimer	12.4	863	711 451 441	+	+	+
11	Procyanidin dimer	12.7	577	451 425 407 287			+
12	5-CQA	13.1	353	191	+		+
13	4-CQA	13.6	353	191 175 173	+	+	+
14	Procyanidin trimer	14.2	863	711 693 559 411 290	+	+	+
15	Catechin/epicatechin	14.7	289	245 203 179 151 125	+		+
16	5-CQA	14.9	353	191			+
17	5-CoQA	15.4	337	191			+
18	Mangiferin	15.8	421	403 331 313 301 259	+	+	+
22	3,4-diCQA	18.2	515	353 335 191 179 161	+	+	+
23	3,5-diCQA	18.4	515	353 191	+	+	+
24	4,5-diCQA	19.7	515	353 191 179	+	+	+
25	Ferulic acid	20.3	193	161 134 109	+	+	+

Rt: Retention time.

Table 3

Detected compounds in the immature pericarp of the studied coffee species.

No.	Proposed components of the immature pericarp	Rt(min)	[M-H]-(*m/z*)	MS/MS (*m/z*)	*C. benghalensis*	*C. arabica*	*C. liberica*
1	Isocitric acid	2.3	191	109 93 85 81 71	+	+	+
5	3-CQA	10.2	353	191 179 135		+	+
6	Procyanidin dimer	10.2	577	451 425 407 289 245		+	
7	4-CQA	11.6	353	191 179 173		+	
9	Catechin/epicatechin	12.1	289	245 203 179 175 151		+	+
11	Procyanidin dimer	12.7	577	425 407 289 245 161			+
12	5-CQA	13.1	353	191	+	+	+
13	4-CQA	13.6	353	191 179 175 173 135	+	+	+
15	Catechin/epicatechin	14.7	289	245 203 179 175 151	+	+	+
16	5-CQA	14.9	353	191		+	+
17	5-CoQA	15.4	337	191 173		+	
18	Mangiferin	15.8	421	403 331 313 301 259		+	+
19	4-FQA	15.9	367	191 173 134		+	+
21	5-FQA	17.4	367	191		+	
22	3,4-diCQA	18.2	515	353 191 179 173 161		+	+
23	3,5-diCQA	18.4	515	353 191 179 173		+	+
24	4,5-diCQA	19.7	515	353 191 179 173 135		+	+
25	Ferulic acid	20.3	193	161 133		+	

Rt: Retention time.

Table 4

Detected compounds in the immature seed of the studied coffee species.

No.	Proposed components of the immature seed	tR(min)	[M-H]-(*m/z*)	MS/MS (*m/z*)	*C. benghalensis*	*C. arabica*	*C. liberica*
1	Isocitric acid	2.3	191	109 93 85 81 71	+	+	+
2	Caffeoyl hexoside	2.3	341	179 119 113	+	+	
5	3-CQA	10.2	353	191 179 135	+	+	+
8	5-CQA derivative	12.1	385	353 191		+	+
11	Procyanidin dimer	12.7	577	191 179 173			+
12	5-CQA	13.1	353	191 173 135	+	+	+
13	4-CQA	13.6	353	191	+	+	+
15	Catechin/epicatechin	14.7	289	191 175 173 135	+		+
16	5-CQA	14.9	353	245 203 179 125		+	+
17	5-CoQA	15.4	337	191		+	+
19	4-FQA	15.9	367	191 173	+	+	+
20	5-CQA methyl ether	16.8	367	179 161 135			+
21	5-FQA	17.4	367	191		+	+
22	3,4-diCQA	18.2	515	353 335 179 173	+	+	+
23	3,5-diCQA	18.4	515	353 191 179 173	+	+	
24	4,5-diCQA	19.7	515	353 191 179 173	+	+	+
25	Ferulic acid	20.3	193	161 133 131	+	+	+

Rt: Retention time.

significance.

The identification of mangiferin in the leaf extracts was in concordance with earlier works[12]. They showed that the release of mangiferins depends on the temperature which most effective at 100 ℃, and it releases less in 50% methanol extraction because of lower temperature[12]. In contrast to Conéjéro *et al*. 2014 findings, we did not identify 5-caffeoylquinic acid in *C. arabica* leaves, but procyanidin trimers were described in each leaf sample[14].

DiCQAs were characteristic for most extracts, and 4,5-diCQA (24) was present in all samples, except Bengal coffee's immature pericarp; while 3,4-diCQA (22) was detected in each sample excluding the mature pericarp of Liberian coffee and the immature pericarp of Bengal coffee[5,7]. In addition, isocitric acid was described in all samples[15].

The results underline the presence of chlorogenic acids detected in our previous LC/MS studies which analysed their quantity in the non-hydrolysed extracts of the leaf and the immature fruit in Bengal coffee[16]. In our previous and actual investigations, ferulic acid was identified in immature pericarp extracts though the quantified ferulic acid concentration was insignificant in the immature pericarp of Bengal coffee[16]. The ferulic acid concentration was the same in the non-hydrolysed extract made of the immature seed and the leaf of Bengal coffee. The results overlap with the findings of an earlier comprehensive work mentioned the presence of chlorogenic acids[17,18]. Even though these phenolic compounds were described earlier in the immature seed of Arabic coffee[17], we identified in plus isocitric acid, caffeoyl hexoside, and catechin/epicatechin in the plant. However, this review includes 5-CQA, this compound was not identified in the green seed of Arabic coffee using our methods. Babova *et al*. underlined the importance of the geographical origine of plants which could influence the caffeine and phenolic content of the cultivated *C. arabica* and *C. canephora*[18]. The environmental

Table 5

Detected compounds in the mature pericarp of the studied coffee species.

No.	Proposed components of the mature pericarp	Rt(min)	[M-H]-(*m/z*)	MS/MS (*m/z*)	*C. benghalensis*	*C. arabica*	*C. liberica*
1	Isocitric acid	2.3	191	-	+	+	+
2	Caffeoyl hexoside	2.3	341	179 119 89		+	
3	Quinic acid derivate	2.3	533	191 127		+	
5	3-CQA	10.2	353	191 179 135	+	+	
8	5-CQA derivative	12.1	385	353 191	+		
9	Catechin/epicatechin	12.1	289	245 203 221 109	+		
11	Procyanidin dimer	12.7	577	425 407 289 125	+		
12	5-CQA	13.1	353	191	+	+	+
13	4-CQA	13.6	353	191 175 173 135	+	+	+
15	Catechin/epicatechin	14.7	289	245 203 179 151 125	+	+	+
16	5-CQA	14.9	353	191	+	+	
17	5-CoQA	15.4	337	191	+	+	
18	Mangiferin	15.8	421	331 301 259	+	+	
19	4-FQA	15.9	367	191 173	+	+	
22	3,4-diCQA	18.2	515	353 335 179 173	+	+	
23	3,5-diCQA	18.4	515	353 191 179	+	+	
24	4,5-diCQA	19.7	515	353 179 173	+	+	+
25	Ferulic acid	20.3	193	161 133	+	+	

Rt: Retention time.

Table 6

Detected compounds in the mature seed of the studied *Coffea* species.

No.	Proposed components of the mature seed	tR(min)	[M-H]-(*m/z*)	MS/MS (*m/z*)	*C. benghalensis*	*C. arabica*	*C. liberica*
1	Isocitric acid	2.3	191	127 109 93 85	+	+	+
2	Caffeoyl hexoside	2.3	341	179 119		+	
4	Citric acid	3.0	191	111 87 67			+
5	3-CQA	10.2	353	191 179 135		+	+
7	4-CQA	11.6	353	191 179 173			+
8	5-CQA derivative	12.1	385	353 191			+
12	5-CQA	13.1	353	191	+	+	+
13	4-CQA	13.6	353	191 179 173	+	+	+
15	Catechin/epicatechin	14.7	289	245 203 125	+		
16	5-CQA	14.9	353	191	+	+	+
17	5-CoQA	15.4	337	191 173		+	+
18	Mangiferin	15.8	421	331 301 259	+		
19	4-FQA	15.9	367	191 173	+	+	+
20	5-CQA methyl ether	16.8	367	179 135			+
21	5-FQA	17.4	367	191	+		
22	3,4-diCQA	18.2	515	353 335 191 179 173	+	+	+
23	3,5-diCQA	18.4	515	353 191			+
24	4,5-diCQA	19.7	515	353 191 179 173 135	+	+	+
25	Ferulic acid	20.3	193	161 133	+		+

Rt: Retention time.

attributes of the growing area, geographical origine and the growing practice can positively or negatively influence the chemical composition of the coffee plants[19]. In addition, chlorogenic acids content of green coffee beans can depend among others on genes, species, climate and nutrient state of soil[20].

Our results overlap with our earlier work, as the quantity of chlorogenic acids were three times higher in the non-hydrolised extract made of the immature seed of Arabic coffee than that of Bengal coffee[16]. Ky *et al*.[21]. and Campa *et al*.[22] also identified and quantified chlorogenic acids in the fruit of Liberian coffee, which confirms our detailed studies. Catechin, epicatechin, flavonols, anthocyanidins, flavan-3-ols and hydroxycinnamic acids like caffeoylquinic acid, its derivatives and p-coumaroylquinic acid detected by Ramirez-Coronel *et al*.[23] support our investigations and underline that the constitutive units of Arabic coffee fruit were mainly epicatechin, representing more than 90% of the proanthocyanidin units.

In conclusion, the cultivated Coffea species have been evaluated comprehensively; however, the wild Bengal coffee is less investigated, thus our results may contribute to the knowledge regarding its phytochemical composition. Our findings provide relevant new information about less investigated wild coffee taxa that may be of similar significance as Arabic and Liberian coffee. We could summarize that our findings completed the scientific literature of Bengal coffee presenting new opportunities and challenges for further phytochemical and medical studies of the species.

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Conflict of interest statement

The authors declare that there was no conflict of interest.

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