Original Article

Comparison of antioxidant, antimicrobial activities and chemical profiles of three coffee (*Coffea arabica* L.) pulp aqueous extracts

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ABSTRACT

Background: This study explored the bioactivities and nutrient compositions of coffee (*Coffea Arabica* L.) pulp which was prepared in three different ways [Coffee Pulp Extracts (CPE) 1–3].
Methods: The coffee pulp was prepared in three different ways by distinct selecting and freezing processes. The nutritional values, polyphenol contents, antioxidant activity, and antibacterial properties of the coffee pulp as well as the characterization of the active ingredients by liquid chromatography-electrospray ionization-quadrupole-time-of-flight mass spectrometry (LC-ESI-Q-TOF-MS) were evaluated.
Results: The chemical profiles of three aqueous extracts were compared and characterized using LC-ESI-QTOF-MS. They showed slightly different nutrient compositions. The total phenolic content was highest in CPE1, and decreased in the following order: CPE1 > CPE2 > CPE3. Among the CPEs tested, CPE1 showed the most potent antioxidant activity with IC$_{50}$ 18 µg/mL and 82 µg/mL by 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) and 1,1-diphenyl-2-picryl-hydrazyl assay, respectively. Chlorogenic acid and caffeine were the most prominent in CPE1 and it contained more compounds than the others. Moreover, CPE1 demonstrated antibacterial activity against both gram-positive (*Staphylococcus aureus* and *Staphylococcus epidermidis*) and gram-negative bacteria (*Pseudomonas aeruginosa* and *Escherichia coli*).

Conclusion: These findings indicated that CPE1 has powerful nutrients with antioxidant and antibacterial properties—the potency of which is impacted by the preparation process.

1. Introduction

Coffee (*Coffea arabica* L.) belongs to the Rubiaceae family, and is a popular beverage worldwide. During coffee processing, a residue or by-product (pulp, silver skin, and parchment) is generated. Ripe coffee fruits (cherries; Fig. 1A) are harvested, and the skin and pulp are removed from the coffee beans (Fig. 1B, 1C). Every 2 tons of coffee produces 1 ton of coffee pulp (CP),[1] leading to a serious environmental problem. Therefore, the development and added values of residual parts are of interest. Several studies have focused on developing and using the waste products for fermentation by cultivation of edible fungus, active ingredient extraction, animal feed, or compost[2–4] due to the presence of enriched nutrients, minerals, amino acids, polyphenol, and caffeine.[4,5] Murthy and Naidu[6] suggested that CP, a red color, is a source of anthocyanins for potential applications as a natural food colorant.[2] Furthermore, hydroxycinnamic acids (chlorogenic, caffeic, and ferulic acid) found in CP have antioxidant properties.[4] It has been noted that antioxidants neutralize excess free radicals to prevent cells against free radical damage and to contribute to disease prevention.[7] In addition, CP has been revealed to have antimicrobial activity against bacteria such as *Staphylococcus aureus*, *Enterococcus faecalis*, *Bacillus subtilis*, *Pseudomonas aeruginosa*, and *Escherichia coli*, etc.[8–10] Previous studies revealed different compositions of coffee by-products,[6,11] including CP,[12] in fresh and dehydrated conditions.[11] However, the nutrient composition may vary depending on the processing, preparation, and storage. Even though there have been reports on the composition and utilization of CP, and antioxidant properties of different parts of coffee, there are limited reports on the nutritional values and biochemical properties of CP by different processing preparations. The aim of this study was to determine the nutritional values, polyphenol contents, antioxidant activity, and antibacterial properties of CP, as well as to characterize the active ingredients by liquid chromatography-electrospray ionization-quadrupole-time-of-flight mass spectrometry (LC-ESI-Q-TOF-MS) in three different processing preparations. A preferred processing method might thus be illuminated and contribute to the development of CP as a food additive or preservative in the food industry.

2. Methods

2.1. Chemical and reagents

Standard D-(-)-quinic acid, L-(-)-malic acid, citric acid, chlorogenic acid, Folin–Ciocalteu's phenol reagent, 1,1-diphenyl-2-picrylhydrazyl (DPPH), 2,2′-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS) were obtained from Sigma-Aldrich (Saint Louis, MO, USA). Methanol (high performance liquid chromatography grade) acetonitrile (LC-MS reagent) and water (LC-MS grade) were purchased from RCI Labscan Limited (Bangkok, Thailand). Formic acid (analytical grade) was purchased from JT Baker (Philipsburg, NJ, USA). A nylon syringe filter 0.45 μm was bought from Lubitech Technologies Ltd. (Shanghai, China). Muller-Hinton agar (MHA) and Muller-Hinton broth (MHB) were purchased from Difco Laboratories, Inc. (Difco, Franklin Lakes, NJ, USA).

2.2. Plant materials

The pulp of *C. arabica* L. was obtained from Chao-Thai-Pukao Factory (Chiang Mai, Thailand), that collected samples from Baan Khun Lao, Wieng Pa Pao, and Chiang Rai, Thailand. The plant was taxonomically authenticated and a voucher specimen was deposited in the herbarium of the Faculty of Biology (NU herbarium), Naresuan University, Phitsanulok, Thailand.

2.3. Sample preparation

To prepare CP, ripe coffee cherries were harvested, washed (washing process), and coffee fruits were selected by removing the floating fruit (selecting process). The coffee bean was removed from the pulp using machine separates (removing process) as shown in Fig. 1C. Pulp was frozen at –20 °C (freezing process) and then dried using far-infrared rays, and blended using blender machine as shown in Fig. 1D (drying and blending process). In the present study, CP was prepared three different ways. CP extract 1 (CPE1) was prepared as follows: washing → removing → drying and blending; CPE2: washing → selecting → removing → drying and blending; CPE3: washing → selecting → removing → freezing → drying and blending.

The dried CP powder resulting from each of these process preparations was extracted with hot water (92± 3 °C) for 2 minutes. The ratio of CP and hot water was 1:5. The filtered solution was further lyophilized to give three aqueous extracts labeled as CPE1, CPE2, and CPE3. The CP aqueous extracts were kept at –20 °C before use. For the chemical characterization, 2 mg/mL of each was dissolved in methanol and water [1:1 volume/volume (v/v)] and then further filtered through a 0.45-μm nylon syringe filter prior to injection into the LC-MS system.

2.4. ESI-Q-TOF-MS conditions

A 6540 ultrahigh definition accurate mass Q-TOF (Agilent Technologies, Palo Alto, CA, USA) was converted into an Agilent 1260 infinity high performance liquid chromatography instrument (Agilent, Waldbonn, Germany) via an ESI interface. Analysis parameters were set using both negative and positive ion modes with spectra acquired over a mass range of *m/z* 100–1,000 amu. The ESI-MS condition parameters were
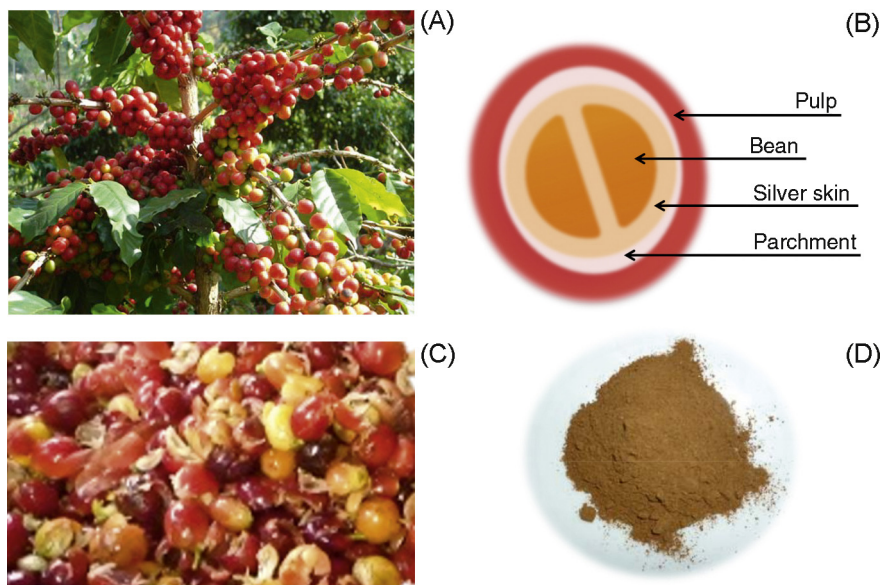
Fig. 1 – *Coffea arabica* L. tree. (A) Coffee cherries. (B) Cross-section of coffee cherry. (C) Coffee pulp after removal from the bean. (D) Characteristics of coffee pulp powder after drying and blending processes.

as follows: capillary voltage, +3,500 V; dry gas temperature, 350 °C; dry gas flow, 10 L/min; nebulizer pressure, 30 psig; and spectra rate, 4 Hz. Fragmentations were performed using auto MS/MS experiments with collision energies at 10 V, 20 V, and 40 V. Nitrogen was used as a collision gas.

Chromatographic separation was performed using a phenomenex Luna C-18(2) column (5 μm, 150 × 4.6 mm internal diameter) (Phenomenex Inc., Torrance, CA, USA). The mobile phase consisted of 0.1% formic acid in water v/v (Solvent A) and 0.1% formic acid in acetonitrile v/v (Solvent B). The linear gradient started from 10% to 90% of Solvent B for 30 minutes. The injection volume was 5 μL. The mobile phase flow rate was 0.5 mL/min.

2.5. Peak identification

Peak identification was performed by comparing the retention time, mass spectra, and fragmentation patterns with standard compounds, reported data, and a library search of the Mass Hunter METLIN metabolite database (Agilent Technologies).

2.6. Proximate analysis

Moisture, crude proteins, fat, fiber, and ash were analyzed using the Association of Official Analytical Chemists procedures.[13] The crude protein content (N × 6.25) of CP was estimated using the macro Kjeldahl method; the crude fat was determined by extraction using Soxhlet apparatus, and the ash content was analyzed by weight before and after incineration at 600 ± 15 °C for 24 hours.

2.7. Determination of total phenolic contents

The total phenolic content of the CPEs were assessed using the Folin–Ciocalteu procedure as modified by Kahkonen and coworkers.[14] Briefly, 200 μL of crude extract solution was

mixed with 1 mL of Folin–Ciocalteu reagent, and then 0.8 mL sodium carbonate (7.5%) was added. The mixture was incubated at room temperature for 30 minutes, and was measured at 750 nm using a spectrophotometer. The results were expressed as mg/L of gallic acid.

2.8. Determination of antioxidant activity

2.8.1. ABTS assay
The ABTS free radical cation scavenging activity was measured according to the method of Re et al[15] with a slight modification. Briefly, ABTS+ was made with 7 mM ABTS and 2.45 mM potassium persulfate in 100 mM phosphate buffer solution (pH 7.4) stored in the dark for 12–16 hours at room temperature. A working solution was diluted to absorbance values 0.7 ± 0.02 at 734 nm with 100 mM phosphate buffer solution (pH 7.4). Then, 990 μL of working ABTS solution was added to different concentrations of the extracts (2 μL). After 6 minutes of incubation at room temperature, absorbance was measured at 734 nm and the percentage of inhibition was calculated as follows:

$$\% \text{ inhibition} = [(A_{734} \text{ control} - A_{734} \text{ test sample})/A_{734} \text{ control}] \times 100.$$

2.8.2. DPPH assay
Scavenging activity on DPPH was assessed according to the method reported by Blois[16] with a slight modification. Briefly, an action mixture of 10 μL of different concentrations of the extracts and 190 μL of 80 μM DPPH in methanol was shaken and incubated at room temperature in the dark for 30 minutes. The control was prepared without extract. Absorbance at 517 nm was measured with a spectrophotometer. The experiment was performed three times, each time in triplicate. The

percent inhibition was calculated from control using the following equation:

% inhibition = [(A_{517} control – A_{517} test sample)/A_{517} control)] × 100.

2.9. Antibacterial activity

2.9.1. Inoculum preparation
Inoculums were prepared by transferring colonies of S. *aureus*, S. *epidermidis*, P. *aeruginosa*, and E. *coli* growth on MHA into individual tubes of MHB. All strains were incubated at 37 °C for 24 hours and then adjusted either growing inoculums to the turbidity of the McFarland standard number 0.5.

2.9.2. Determination of antibacterial activity
Lyophilized CPE1 was chosen to determine antibacterial activity, as CPE1 produced the highest lyophilized dried weight using the agar well diffusion method. Briefly, the inoculums [10^7 colony forming units (CFU)/mL] were swabbed on MHA and followed by punching the agar with a 6-mm sterile cork borer. The sample, at a concentration of 300 mg/mL, was applied to each well. Gentamicin (Oxoid, Basingstoke, UK) and sterile distilled water was used as a positive control and negative control. The plates were incubated at 37 °C for 24 hours and the diameter (mm) of the inhibition zone was measured.

2.9.3. Determination of the minimum inhibitory concentration and minimal bactericidal concentration
Minimum inhibitory concentrations (MICs) were determined using a broth microdilution method recommended by the Clinical and Laboratory Standards Institute.[17] Twofold serial dilutions of the samples were carried out using MHB and then were added to a sterile 96-well plate (final concentration ranging from 0.59 mg/mL to 300 mg/mL). A bacterial suspension was subsequently added to obtain a final concentration at 5 × 10^5 CFU/mL. All plates were incubated at 37 °C for 24 hours and MIC was defined as the lowest concentration of CPE1 sample that inhibited the growth of bacteria. Next, the suspension in the well of MIC and higher concentrations were spotted on MHA and incubated. The lowest concentration that inhibited growth of bacteria was defined as the minimal bactericidal concentration.

2.10. Statistical analysis

Data are reported as means ± standard error. Data were analyzed by one-way analysis of variance and *post hoc* Duncan's multiple range test. Statistical significance was considered at a *p* value of < 0.05.

3. Results

In the present study, dried powder of three CPEs, prepared by different processes, was extracted with hot water (92 ± 3 °C) for 2 minutes and lyophilized. The percent yield of lyophilized dried weight of CPE1, CPE2, and CPE3 were 28.74%, 16.59%, and 13.89%, respectively. The preliminary chemical constituents in these samples were studied using thin layer chromatography

with ethyl acetate:methanol:water (7.0:2.5:0.5 v/v/v) as a mobile phase. Similarities in band patterns for all extracts were found (data not shown). The identity of chemical constituents from the aqueous extracts was further investigated with high performance liquid chromatography LC-ESI-Q-TOF-MS.

3.1. Phytochemical characterization

The chromatographic profiling of three aqueous CPEs were studied and identified using LC-ESI-QTOF-MS. The total ion current (TIC) chromatograms were compared in both ESI positive mode and negative modes (Fig. 2). The three extracts displayed similar chromatographic profiles; however, slightly different components were observed in two ranges–retention time (t_R) at 3–6 minutes and 10 minutes. The results revealed that CPE1 contained the highest number of chemical compounds and was used for compound identification.

Twenty-nine compounds were detected and 20 compounds were tentatively identified and compared with the results of standard compounds, published data, and the pubic database. Table 1 provides a summary of the identified compounds, including retention times, ionization mode, measured mass, and MS/MS fragmentation ions. The compounds found in CP were proposed as sugar, small organic acid, alkaloid, hydroxy-cinnamic acid, fatty amide, and sterol.

3.2. Nutritional values of CP

The nutritional composition of the CPEs is shown in Table 2. All CPEs contained moisture, ash, fat, and protein at ∼5.28–5.93%, 8.32–8.88%, 1.40–2.51%, and 7%, respectively. Additionally, caffeine was found in CPE1, CPE2, and CPE3 at 0.69%, 0.77%, and 0.68%, respectively.

3.3. Polyphenol contents and antioxidant activity

Total phenolic content (TPC) of the three CPEs are presented in Table 2. CPE1 had the highest TPC. CPE1 and CPE2 were significantly different among groups. TPC extracts decreased in following order: CPE1 > CPE2 > CPE3, likely due to the different processing methods used. Based on the DPPH and ABTS radical scavenging assay, the antioxidant activity of the CPEs were confirmed with IC_{50} values (the concentration required to inhibit radical formation by 50%). CPE1 showed a highly significant difference antioxidant activity with an IC_{50} of 18 μg/mL and 82 μg/mL by ABTS and DPPH, respectively, compared with CPE2 and CPE3. Different antioxidant activities were observed depending on the assay.

3.4. Antibacterial activity

Because of the highest TPC and lyophilized dried yield of CPE1, it was chosen to subsequently determine the antibacterial activity using the agar well diffusion method. The results, zone of inhibition, revealed that the CPE1 showed inhibitory action against all tested bacteria as shown in Table 3. The inhibition zone of CPE1 against gram-positive bacteria, S. *aureus* and S. *epidermidis*, was significantly more than against gram-negative bacteria, P. *aeruginosa* and E.
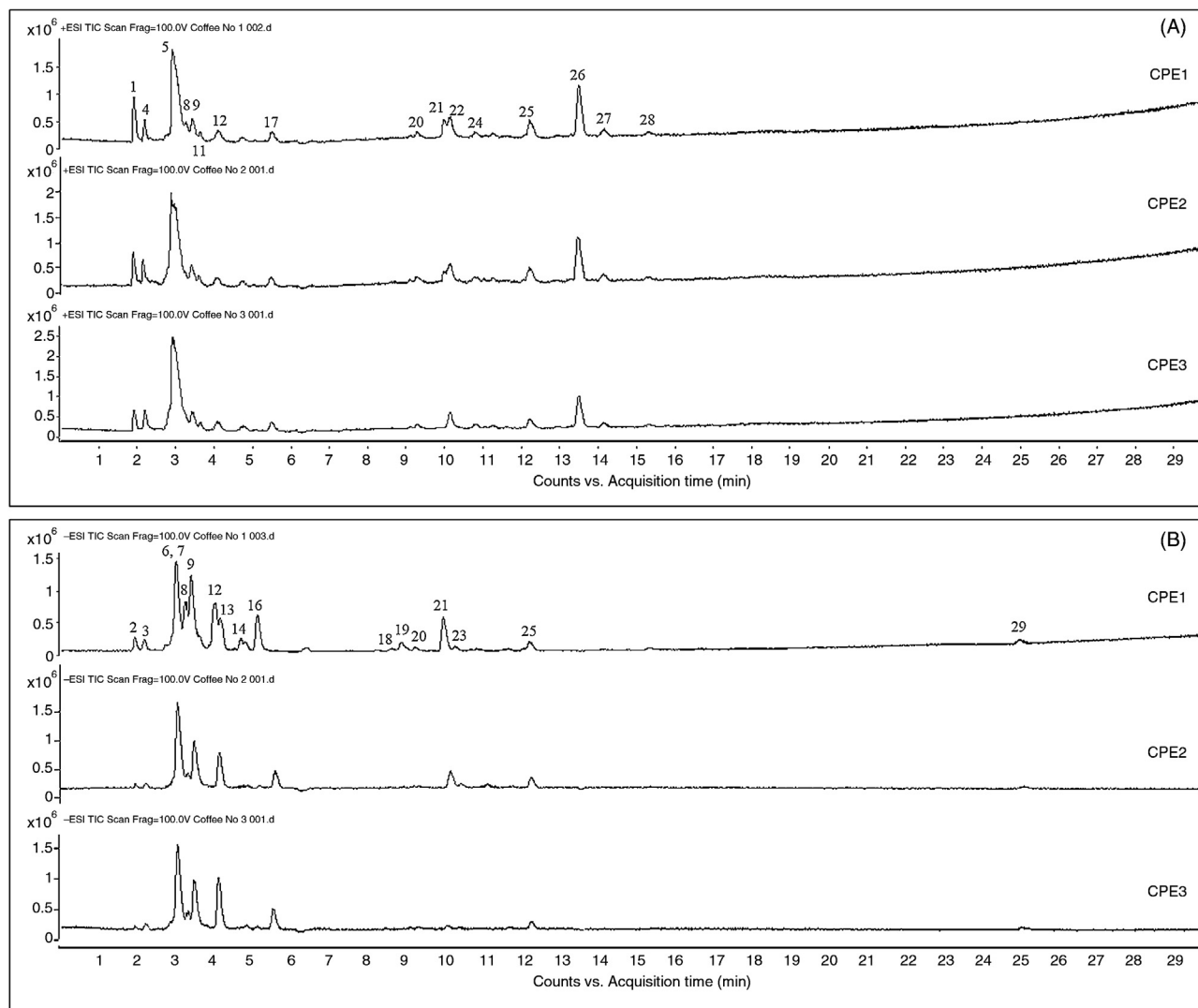
Fig. 2 – Total ion current (TIC) chromatograms of 2 mg/mL coffee pulp CPE1–3. Monitored in (A) electrospray ionization (ESI) positive mode and (B) ESI negative mode. The peak numbers and compounds identification were summarized in Table 1. CPE, coffee pulp extract.

coli. The results of MIC were according to those obtained in the agar diffusion method. The concentration of CPE1 inhibiting the growth of gram-positive bacteria was lower than the concentration that inhibited gram-negative bacteria. Noticeably, CPE1 was effective against *S. epidermidis* and had the lowest MIC, only 4.69 mg/mL. Amounts in the range of 4.69–75 mg/mL of CPE1 had the potential to inhibit the growth of gram-positive and gram-negative bacteria (5 x 10^5 CFU/mL). The minimal bactericidal concentration results showed that CPE1, at a concentration of 300 mg/mL, was not antibacterial. CPE1 extract may act as a bacteriostatic agent only.

4. Discussion

This study explored the bioactivities and nutrient compositions of CPs in three different preparation processes. The TIC chromatograms of three CPEs displayed similar

chromatographic profiles. The *m/z* 181.0506 [M–H]$^-$ (Peak 6) and 179.0518 [M–H]$^-$ (Peak 7) looked like dihydrocaffeic acid and caffeic acid but the fragmentation pattern and mass accuracy are related to D-manitol and hexose.

Trigonelline (Peak 5), an alkaloid, was detected with *m/z* 138.0555 [M+H]$^+$ corresponding with reported data on a compound identified in aqueous extract of pure arabica and robusta coffees.[18,19] Caffeine (Peak 22), an alkaloid was identified at t_R 10.15 minutes with a positively charged molecular ion [M+H]$^+$ at 195.0873, and producing MS^2 fragment ions at *m/z* 138.0663, 110.0711, and 83.0607 corresponding to [M+H-CH$_3$-N=C=O]$^+$, –CO and –CHN, respectively.[20,21]

Peak 8, Peak 9, Peak 12, Peak 13, Peak 14, and Peak 16 with *m/z* [M–H]$^-$ at 195.0577, 191.0517, 133.0102, 193.0420, 191.0153, and 147.0356 were tentatively identified as gluconic acid, quinic acid, malic acid, ferulic acid, citric acid, and citramalic acid, respectively, based on comparison with a database search and authenticated compounds.

Table 1 – Mass spectrometry (MS) data of (+/−) LC-ESI-QTOF-MS spectra and the identification of the coffee pulp aqueous extract method 1 (CPE1)

Peak	t_R (min)	Ion mode	Measured mass	MS/MS	Molecular formula	Error (ppm)	Compound identified
1	1.87	+	125.9682 [M+H]$^+$	—			Unidentified
2	1.94	−	174.9623 [M−H]$^-$	—			Unidentified
3	2.13	−	128.8650 [M−H]$^-$	84.9606			Unidentified
4	2.19	+	104.1069 [M+H]$^+$	58.6055	$C_5H_{13}NO$	0.88	2-amino-3-methyl-1-butanol
5	2.91	+	138.0555 [M+H]$^+$	92.0496	$C_7H_7NO_2$	−3.95	Trigonelline
6	3.01	−	181.0506 [M−H]$^-$	89.0209, 59.0160	$C_6H_{14}O_6$	23.40	D-manitol
7	3.06	−	179,0518 [M−H]$^-$	89.0209, 59.0109	$C_6H_{12}O_6$	24.08	Hexose
8	3.23	−	195.0577 [M−H]$^-$	129.0513, 75.0088	$C_6H_{12}O_7$	−34.4	Gluconic acid
		+	219.0470 [M+Na]+	132.9535, 84.9534		2.39	
9	3.36	−	191.0517 [M−H]$^-$	127.0351, 85.0261	$C_7H_{12}O_6$	22.97	Quinic acid*
		+	215.0527 [M+Na]+	136.0241, 100.8086		−0.42	
10	3.64	+	407.1159 [M+H]$^+$	215.0506, 110.9952			Unidentified
11	3.66	+	167.0786 [M+H]$^+$	119.0835			Unidentified
12	3.98	−	133.0102 [M−H]$^-$	114.9995, 71.0105	$C_4H_6O_5$	30.43	Malic acid*
13	4.12	−	193.0420 [M−H]$^-$	103.0038, 59.1040	$C_{10}H_{10}O_4$	44.72	Ferulic acid
14	4.67	−	191.0153 [M−H]$^-$	111.0045, 87.0053	$C_6H_8O_7$	23.17	Citric acid*
15	4.75	+	275.1096 [M+H]$^+$	—			Unidentified
16	5.11	−	147.0356 [M−H]$^-$	115.0035, 71.0139	$C_5H_8O_5$	−38.53	Citramalic acid
17	5.50	+	171.0261 [M+Na]$^+$	111.0412			Unidentified
18	8.63	−	353.0966 [M−H]$^-$	191.0563, 179.0347, 135.0453	$C_{16}H_{18}O_9$	−24.91	3-Caffeoyl quinic acid
19	8.87	−	153.0250 [M−H]$^-$	109.0298	$C_7H_6O_4$	−37.04	Protocatechuic acid
20	9.25	−	415.2655 [M+HCOO]$^-$	—	$C_{24}H_{34}O_3$	−39.74	3-Oxo-5β- chola-7,9(11)-
		+	393.2461 [M+Na]+	179.0814		−15.47	dien-24-oic Acid*
21	9.94	−	353.0826 [M−H]$^-$	191.0509, 85.0262	$C_{16}H_{18}O_9$	14.74	Chlorogenic acid*
		+	355.1011 [M+H]$^+$	-		3.54	
22	10.15	+	195.0873 [M+H]$^+$	138.0663, 110.0711, 83.0607	$C_8H_{10}N_4O_2$	1.80	Caffeine
23	10.26	−	353.0962 [M−H]$^-$	191.0566, 173.0453, 135.0452	$C_{16}H_{18}O_9$	−23.77	4- Caffeoyl quinic acid
24	10.82	+	518.8894 [M+H]$^+$	—			Unidentified
25	12.26	−	497.3455 [M+HCOO]$^-$	—	$C_{27}H_{48}O_5$	−5.75	Cholestane-3α,7α,12α,24R,
		+	453.3444 [M+H]$^+$	100.112, 55.0543		28.79	25-pentol
26	13.51	+	340.2611 [M+Na]$^+$	100.1120, 55.0544	$C_{21}H_{35}NO$	−0.04	N-methyl arachidonoyl amine
27	14.17	+	453.3447 [M+Na]$^+$	387.7959, 228.1591, 114.0911, 69.0702	$C_{28}H_{46}O_3$	16.89	3β-Hydroxy-5α-cholest-7-ene-4 α-carboxylate
28	15.32	+	463.3241 [M+Na]$^+$	—	$C_{29}H_{44}O_3$	−37.4	5α,8α-epidioxy-stigmasta-6,9(11),22E-trien-3β-ol
	15.36	−	485.3437 [M+HCOO]$^-$	—		−33.9	
29	25.00	−	293.1845 [M−H]$^-$	236.1047, 221.1549			Unidentified

* Compared with standard compound.
LC-ESI-Q-TOF-MS, liquid chromatography-electrospray ionization-quadrupole-time-of-flight mass spectrometry.

Table 2 – Nutrient, total polyphenol contents, and antioxidant activity of coffee pulp extracts

	Nutrients					Antioxidant activity	
	Moisture (%)	Ash (%)	Fat (%)	Protein (%)	Total polyphenolic content (mg/L of gallic acid)	ABTS (IC$_{50}$ μg/mL)	DPPH (IC$_{50}$ μg/mL)
CPE1	5.63	8.78	1.40	7.06	17.40 ± 0.74	18 ± 1.9	82 ± 7.8
CPE2	5.28	8.88	1.76	7.00	10.47 ± 0.77	27 ± 0.6	153 ± 9.3
CPE3	5.93	8.32	2.51	7.00	7.61 ± 0.42	27 ± 1.2	140 ± 9.2

Mean ± standard deviation.
ABTS, 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid); CPE, coffee pulp extract; DPPH, 2,2-diphenyl-1-picrylhydrazyl.

Table 3 – *In-vitro* antibacterial activity of coffee pulp extract 1 (CPE1)

Bacteria	Inhibition zone (mm)	MIC (mg/mL)	MBC (mg/mL)
Staphylococcus aureus	12*	37.5	> 300
Staphylococcus epidermidis	16*	4.69	> 300
Pseudomonas aeruginosa	10†	75	> 300
Escherichia coli	10†	37.5	> 300
Negative control	6	—	—

Significant differences are as follows:
* $p < 0.05$ compared among groups ($n = 3$).
† $p < 0.05$ compared with negative control.
MBC, minimum bactericidal concentration; MIC, minimum inhibitory concentration.

Peak 18, Peak 21, and Peak 23 showed the same molecular ion $[M–H]^-$ at m/z 353. The MS^2 fragmentation pattern at m/z 191 corresponded to the loss of the caffeoyl acid moiety (162 Da). With the aid of available commercial standards, it was possible to identify Peak 21 as chlorogenic acid. Other compounds (Peak 18 and Peak 23), with the same molecular ion, were identified as the isomers, 3-caffeoylquinic acid and 4-caffeoylquinic acid, by comparing the fragmentation pattern with spectrometric characteristics previously reported in literature.[22,23]

A signal of dihydroxybenzoic acid; protocatechuic acid was detected and identified at t_R 8.87 minutes with m/z 153.0250 $[M–H]^-$. The fragment ion at m/z 109.0298 was observed, corresponding to loss of CO_2.[24]

Peak 25, Peak 27, and Peak 28 were identified as sterol compounds, while Peak 26 was a fatty amide. As mentioned above, phenolic compounds such as hydroxycinnamic acid and derivatives, hydroxylbenzoic acids, were tentatively identified. Additionally, alkaloid compound, trigonelline, and caffeine, and some organic acids were identified in aqueous extract of CP. These results correspond to those previously reported.[25]

In addition, CPEs showed powerful of nutrients and antioxidant activities. The moisture, ash, fat, and protein content of all CPEs possessed similar levels. The antioxidant results of ABTS+ were higher than the results of DPPH free radicals. The DPPH had a stable free radical 2,2-diphenyl-1-picrylhydrazyl reaction with H-donors, while ABTS$^+$ had poor selectivity in the reaction with H-atom donors.[26] Several studies report that CP contains antioxidant compounds.[4–6] Arellano-González and coworkers[4] reported CP contains hydroxycinnamic acids (chlorogenic, caffeic, and ferulic acid). These compounds donate a hydrogen atom to an oxidized molecule. In support of Arellano-González et al's[4] study, hydroxycinnamic acids—quinic acid, chlorogenic acid, and caffeic acid—were present in the CP in the present investigation. The results point to a relationship between antioxidant activities of CP and phenolic content. This study showed that CPE1 has the potential to be developed as a natural antioxidant supplement.

Moreover, CPE1 revealed antibacterial activity. Gram-positive bacteria, *S. aureus*, and *S. epidermidis*, were more susceptible than gram-negative bacteria, *P. aeruginosa* and *E. coli*. The results correlate with the results of other reports.[9,10] The efficiency of CPE1 against each bacteria was different because of many factors, such as the structure of the bacterial envelope and the active compounds of the extract. Normally, hydrophobic compounds (phenols and tannins) are difficult to uptake the outer membrane of gram-negative bacteria, which is composed of phospholipids. Our data indicated that gram-positive bacteria (particularly *S. epidermidis*) were susceptible to CPE1, which contains phenolic compounds, more than gram-negative bacteria. These results corresponded to the results of those previously reported.[9] TIC chromatograms of CPE1 illustrated quinic acid, malic acid, chlorogenic acid, and caffeine were the primary compounds present, which may indicate their effectiveness against the growth of microorganisms. A number of other reports suggest that phenolic acids, malic acid, tannin, caffeine, and hydroxycinnamic acid (particularly the hydroxyl groups on chlorogenic acid) are responsible for the antimicrobial activity.[27,28] A possible mechanism is disruption of cell membrane permeability by these compounds.[27] Some studies suggested that caffeine and malanoidins respond to antibacterial activity against gram-negative bacteria.[8,29,30] Furthermore, some authors propose that melanoidins inhibit the growth of bacteria through metal chelating mechanisms.[31,32] Consequently, crude extracts of CP, specifically CPE1, are effective as antibacterial agents due to the mixture of active compounds.

In conclusion, the nutritional value, polyphenol content, antioxidant activity, and chemical profiles of aqueous CPEs by LC-ESI-Q-TOF-MS—in three different processing preparations—were determined. CPE1 had the highest TPC and antioxidant activity. The antimicrobial activity of CPE1 was significant against many bacteria and showed that it is potential compound for further application. CPE1 is active against *S. epidermidis* and *P. aeruginosa* found as nosocomial pathogens. Inhibition of *S. aureus* and *E. coli* (responsible for food poisoning) indicated that CPE1 was also a promising extract to develop as a food additive or preservative for use in the food industry. For the comparison of chemical profiles, the three extracts showed slightly different nutrient compositions. Chlorogenic acid and caffeine were the most prominent in active aqueous extract (CPE1) and it contained numerous compounds, more than the others. Further purification and structure elucidation of marker compounds are still needed. Finally, the pulp preparation process is significant to its potency and potential viability. CP contains several ingredients which may have use in the food industry.

Conflicts of interest

The authors declare no conflicts of interest.

Acknowledgments

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