International Journal of Phytocosmetics and Natural Ingredients 2020;7:5

doi 10.15171/ijpni.2020.05

Original Article

Alternative use of coffee beans and leaves from seven regions of Guatemala for their antioxidant activity and chemical composition

Armando Caceres[1,2], Silvia A. Pinales-Tobar[1], Mónica M. Ramos-Medina[1], María N. Marroquín[1], Sully M. Cruz[1*]

[1]Facultad de Ciencias Químicas y Farmacia, Universidad de San Carlos de Guatemala
[2]Laboratorio de Productos Naturales Farmaya

***Correspondence to**
Sully M. Cruz
Email:
smargotcv@gmail.com

Received 21 Dec. 2019
Revised 10 Feb. 2020
Accepted 10 Apr. 2020
ePublished 25 Apr. 2020

Abstract

Background: Coffee is a Rubiaceae with great economic importance for many countries. Guatemala is the largest producer from Central America, cultivating basically Arabic (*Coffea arabica* L.) type, which is sell internationally as high quality coffee and the sale of beans has been the main national revenue for two centuries. Nevertheless the recent coffee rust crisis evidenced the need for alternative management, as well as innovative utilization of beans and leaves.

Materials and Methods: Beans and leaves from four varieties of coffee were collected from seven regions of Guatemala with the aim to characterize the chemical composition and evaluate the antioxidant activity. Ethanol extracts were prepared by percolation and concentrated by rotaevaporator. It was determined by spectrophotometry the concentration of total phenolics, caffeine, and chlorogenic acid as chemical markers, as well as total sugars.

Results: Extraction yields were high (beans: 29.8, leaves: 48.3%). Both organs showed antioxidant activity by DPPH (IC_{50} 0.81, 0.63 mg/mL), ABTS (IC_{50} 1.48, 0.92 mg/mL), ferric reducing antioxidant power (FRAP) (7.37, 7.94 Fe^{+2}/g), and total phenolics (90.40, 87.50 µg gallic acid/mg), as well as chlorogenic acid (4.91, 1.04%), caffeine (1.19, 0.72%) and total sugars (3.20, 2.98%), respectively.

Conclusions: The results confirmed the good quality of the beans for its chlorogenic acid and caffeine content. It was also demonstrated the potential of the leaves for its phenolic content and antioxidant activity, considered an underutilized resource that might be used for industrial diversification of coffee by-products. The antioxidant activity of both organs suggests a potential application as functional food and for phytocosmetic application.

Keywords: Coffee, Chlorogenic acid, Total phenolic, Total sugars, Caffeine, Antioxidants

Background

Coffea species are perennial shrubs from the Rubiaceae family which grow in tropical and subtropical regions, particularly in Equatorial regions at 200-1600 masl and temperature range 18-22°C.[1,2] Coffee beans is one of the most consumed beverages in the world.[3,4] The most cultivated varieties are *Coffea arabica* L. (Arabica) and *C. canephora* L. (Robusta), which are used for commercial production, representing 60 and 39% respectively of the world market, and *C. liberica* Hiern is <1% of the market.[5]

The chemical composition of coffee leaves contain alkaloids (caffeine, trigonelline, adenine-7-glycosil, theobromine and methylxanthine), flavonoids (anthocyanins, quercetin glycosides, quercetin, isoquercetin, rutin and kaempferol), terpenoids (kahweol, cafestol and 16-O-methyl cafestol), amino acids (histidine and pipecolic acid), sugars, tannins, xanthonoids (mangiferin and isomangiferin), phenolic acids (caffeic, chlorogenic, p-coumaric, ferulic and synaptic), tannins (catechin and epicatechin).[6-8]

Coffee as functional food has demonstrated antioxidant activity, reduce cancer, diabetes, and hepatic disease incidence, protect against Parkinson's disease and reduce mortality risks.[9] Green coffee beans extract show hypotensive effect in spontaneously hypertensive rats,[10] reduce visceral fat and body weight in relation with its bioactive compunds.[11]

Chlorogenic acid (CA) is the main phenolic compound of green coffee, which has anti-inflammatory, antibacterial and anticancer activities by DNA protection.[12,13] Phenolic compounds and hydroxycinnamate has been identified and quantitated in coffee beans from China, India and México.[14,15] Jang et al[16] reported that CA can help in preventing retinal degeneration.

Guatemala is the largest coffee producer from Central

Please cite this paper as: Caceres A, Pinales-Tobar SA, Ramos-Medina MM, Marroquín MN, Cruz SM. Alternative use of coffee beans and leaves from seven regions of Guatemala for their antioxidant activity and chemical composition. Int J Phytocos Nat Ingred. 2020;7:05. doi:10.15171/ijpni.2020.05.

America, cultivating basically *C. arabica* type, which is sell internationally as high quality coffee and it has been the main national revenue for two centuries. Nevertheless the recent coffee rust crisis evidenced the need for alternative management, as well as innovative utilization of beans and leaves. Due to geographic, edaphic and microclimate variables, at least seven well characterized regions has been recognized for coffee growing.

In this study leaves and beans extracts were chemically characterized and antioxidant activity evaluated in order to select the most promising regions for the proposal of new ingredients for the food, cosmetic, pharmaceutical and agroindustrial industries.

Materials and Methods

Plant material and extraction

Representative organic farms were selected from the seven coffee producing regions of Guatemala belonging to the Association of Private Natural Reserves of Guatemala (APNRG). From each farm, 1 kg of leaves and beans were collected at fruiting time (Dry season) dried (<10% humidity) in a forced-air convection oven at 40°C and ground.

Total solids were determined in triplicate by ethanol (30%, 50%, 70% and 95%) extraction and dried, to determine the best ethanol concentration for metabolites extraction. With the best solvent, extracts were obtained by percolation, followed by rotavapor concentration at <45°C. From green beans, fixed oil was obtained by cold mechanical press, and oleoresin by hexane extraction and concentration.[17]

Chemical characterization

Caffeine was determined by official methods with some modifications.[18] To 10 mL of coffee extract, 5 mL of HCl 1N and 1 mL of lead acetate were added, diluted to 100 mL with distilled water and filtered in Whatman No. 1 paper. Filtrate (25 mL) with 0.3 mL of sulfuric acid solution was diluted to 50 mL and filtered. Absorbance was measured at 270 nm, and caffeine content estimated form a standard curve (0-250 mg/L).

CA was determined by standard method according to Solís and Herrera.[19] Ground coffee (1 g) was diluted to 100 mL in hot water and CA concentration estimated from a standard curve with CA (10-70 mg/mL) determined from the second derivative spectrum in UV region (200-400 nm) for each dilution.

Total sugars concentration. Was determined by a calibration curve prepared with several sugars standards (10-70 mg/mL), using the Dubois method (phenol-sulphuric), water as a blank and absorbance at 40 nm.[20]

Antioxidant activity

Total phenolic compounds (TPC) were determined by a standard macrometric methods using the Folin-Ciocalteu reagent according to Phipps et al[21] read in a Thermo Genesys 10 spectrophotometer at 765 nm, and the concentration was estimated by a regression curve expressed in µg of gallic acid equivalent/mg of dry extract.[3]

1,1-diphenyl-2-picrylhidrazyl (DPPH). Qualitative discoloration was evaluated by a standard TLC method in $60F_{254}$ silica gel plates and sprayed with DPPH using tert-butyl-hydroquinone (TBHQ) as antioxidant standard. Macrometric method was performed in tubes using acetate buffer, methanol, DPPH, and extract; after agitation and incubation for 30 minutes at room temperature, it was read in a Thermo Genesys 10 spectrophotometer at 517 nm against blank, and the IC_{50} was calculated. Micrometric determination was done in a similar setting, taking into consideration the scaling down needed to maintain the system in a 96-well plate, and evaluated in an Elisa reader (Bio-Tek ELx-800) at 490 nm, followed by mean inhibitory concentration (IC_{50}) calculation in mg/mL of dry extract from the regression line or TDAC.[22,23]

2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid (ABTS). Discoloration of ABTS was evaluated according to Re et al.[23,24] The ABTS radical cation was produced by mixing ABTS solution (7 mM) with potassium persulfate (2.45 mM), kept in the dark at room temperature for 16-18 hours. For analysis, the reagent was diluted in ethanol until the absorbance at 734 nm was 0.70 ± 0.02 at 30°C. Extract dilutions were added to the diluted reagent and read at 1, 4 and 6 minutes. For each dilution, a curve was prepared in 60%-70% inhibition, and the IC_{50} was calculated in mg/mL as Trolox dilution.

Ferric reducing antioxidant power (FRAP)

The methanol extract (50-500 µg of dry extract/mL) was mixed with phosphate buffer (0.2 M, pH 6.6) and potassium ferricyanide 1%, and incubated at 50°C for 20 min. Trichloroacetic acid was added to the mixture and centrifuged at 3000 rpm for 10 minutes. The upper layer was mixed with water and ferric chloride (0.1%), the absorbance measured at 700 nm and compare with a standard curve of $FeSO_4$ (02-10 nM). The same procedure was used with standards (gallic and ascorbic acids) and expressed as µg Fe (II)/g of extract. Increased absorbance of the reaction mixture indicates increased reducing power.[25]

Statistical analysis

The average of 5 repetitions of each assay were tabulated and the standard deviation calculated in Office Excel 2010. The calculation of antioxidant activity of DPPH and ABTS as IC_{50} was done by a linear regression using five dilutions, blank and standard. The statistically significant differences were evaluated by Tukey test using the free R Studio 351 program.

Results

During February-April 2019, leaves and beans samples of *C. arabica* organically produced were collected by

convenience from four varieties in seven regions of Guatemala with the support of associates of APNRG. The main variety correspond to Caturra (Table 1, Figure 1).

All the ethanol extraction yields from leaves were above 28% (28.80%-48.32%). Beans ethanol yields were lower (26.25%-29.83%) than leaves; fixed oil yields varied from 1.30%-4.18% and oleoresin from 1.31%-7.01% (Table 2).

Caffeine and CA were chosen as markers, the first one because it is the most representative alkaloid from coffee and the second for being the most active biologically. The highest amount of caffeine in the leaves were from region VI (0.72%) and from beans it was region I (1.19%); while for CA, the highest content in leaves were from region I (1.04%) and in beans from region IV (6.67%) (Table 3). The total sugars content was highest in leaves from region III (2.98%) and in beans of region I (3.29%) (Table 3).

As seen in Table 4, both organs demonstrated some antioxidant activity, showing the better activity for DPPH and ABTS for the leaves from region VI (IC_{50} 0.63 ± 0.01 mg/mL and 0.92 ± 0.02 mg/mL, respectively), for TPC the leaves from region VII (87.50 ± 2.39 µg of gallic/mg of extract), and for FRAP the leaves from region II (3.97 ± 0.11 g Fe^{+2}/g of extract), beans demonstrated the better activity region I for DPPH (IC_{50} 0.81 ±0.01 mg/mL), region V for ABTS (IC_{50} 1.48 ±0.01 mg/mL), TPC region IV (90.40 ±2.42 µg of gallic/mg of extract) and FRAP region III (7.37±0.06 g Fe^{+2}/g of extract).

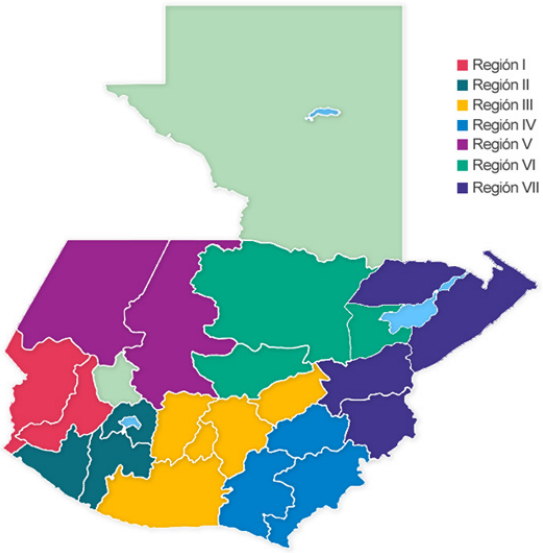

Figure 1. Map of Coffee Growing Regions From Guatemala.

Discussion

Extraction yields are quite promising in all leaves samples, particularly from regions VI (48.32%) and VII (41.36%); beans showed a lesser yield, demonstrating the highest amount of samples from regions III and VII (29.83%); these results indicate a potential cost effectiveness for industrial extraction.

The fixed oil content of beans is moderate (1.3-4.18 %), the highest yield was demonstrated in beans from region IV (4.18%). Previous studies reported green coffee bean oil yield of 0.2-0.3% and in roasted beans up to 12-13% in beans of *C. canephora*,[17] and 10-16% in beans of *C. arabica*.[8] Total lipids in green coffee reported in others studies 11.7-14%, beans of *C. arabica* reported 13.5-17.4% and *C. canephora* 9.8-10.7%; this differences are due to different variables, particularly geographic origin, soil, light, humidity and altitude, cultivation method, roasted coffee, extraction procedures, and solvents used.[26]

The content of caffeine and CA in coffee leaves are being proposed as chemical markers to evaluate coffee quality. In this study the leaves from region I presented the highest amount of CA (1.04%) and in the beans from region IV (6.67%). The content of CA has been studied in various species, varieties, geographical origin, development state of the fruit, post-harvest and roasted process, and ways of preparation of the beverage. The highest differences has been described in wild African strains, with an average of CA content of 1.4-14.4% (sum of the tree isomers),[27,28] indicating that CA concentrations are less in leaves than beans.

In commercial coffee beans CA content is very variable, in *C. arabica* it varies from 5-8%,[29,30] in *C. canephora* content goes from 6.6-12.3%, and in *C. liberica* from 7.6-14%.[28,31,32] CA is an important factor which determines coffee flavor, contributes to final acidity and bittnerness,[33] and confers astringency.[34]

There are few studies on the CA content in coffee leaves. One study reported total CA in aqueous extracts of eight coffee species cultivated in greenhouse in Belgium, in two different seasons (January and July), in

Table 1. Provenance and Varieties of Coffee Samples Collected

Region	Private Natural Reserve	Place of Collection	Varieties
I	CAT Tajumulco	Tajumulco, San Marcos	Caturra
II	Alianza S.A.	El Palmar, Quetzaltenango	Sarchimor
III	Guardabarranca	Villa Canales, Guatemala	Caturra
IV	Santa Isabel	Pueblo Nuevo Viñas, Santa Rosa	Catisique
V	Santa Elena	La Democracia, Huehuetenango	Bourbon
VI	Rincón Grande	Salamá, Baja Verapaz	Caturra
VII	Quetzal Juyú	Usumatlán, Zacapa	Caturra

Table 2. Extraction yield (%) Obtained From Coffee Materials of Seven Regions

Region	Leaves		Beans			
	Ethanol Used	Ethanol Extract (%)	Ethanol Used	Ethanol Extract (%)	Fixed Oil (%)	Oleoresin (%)
I	30	28.80	50	27.73	3.08 (0.42)	7.01
II	70	30.44	50	26.25	1.30 (0.53)	1.31
III	70	34.45	30	29.83	3.98 (0.81)	5.36
IV	50	32.31	50	27.47	4.18 (0.44)	4.19
V	50	37.79	50	26.46	2.70 (0.36)	4.60
VI	70	48.32	50	26.36	1.57 (0.48)	2.70
VII	50	41.36	50	29.83	3.18 (0.39)	6.05

Table 3. Chemical Coffee Markers Concentrations in Leaves and Beans

Region	Leaves			Beans		
	CA (%)*	Caffeine (%)	Sugars (%)	CA (%)	Caffeine (%)	Sugars (%)
I	1.045 (0.002)^{a}	0.453 (0.003)^{a}	2.12 (0.03)^{a}	4.916 (0.015)^{a}	1.193 (0.004)^{a}	3.29 (0.07)^{a}
II	0.108 (0.001)^{b}	0.588 (0.004)^{b}	2.22 (0.03)^{b}	0.412 (0.001)^{b}	0.520 (0.002)^{b}	2.44 (0.03)^{b}
III	0.435 (0.003)^{c}	0.494 (0.004)^{c}	2.98 (0.02)^{c}	1.373 (0.002)^{c}	1.177 (0.008)^{c}	2.98 (0.05)^{c}
IV	0.293 (0.002)^{d}	0.494 (0.003)^{c}	1.61 (0.02)^{d}	6.675 (0.007)^{d}	1.142 (0.011)^{d}	2.79 (0.05)^{d}
V	0.325 (0.002)^{e}	0.648 (0.004)^{d}	1.33 (0.04)^{e}	0.677 (0.001)^{e}	1.134 (0.003)^{d}	1.35 (0.02)^{e}
VI	0.487 (0.002)^{f}	0.725 (0.003)^{e}	1.57 (0.02)^{d}	1.075 (0.002)^{f}	0.568 (0.003)^{e}	2.28 (0.06)^{f}
VII	0.580 (0.004)^{g}	0.577 (0.003)^{f}	2.55 (0.08)^{f}	0.747 (0.003)^{g}	1.136 (0.008)^{d}	2.87 (0.03)^{d}

CA: Chlorogenic acid.
Different letters means $P < 0.05$ (Tukey test).

Table 4. Total Phenolics Concentration and Antioxidant Activity of Ethanol Extracts of *C. arabica* Leaves and Beans by Different Methods

Regions	Leaves				Beans			
	TPC	DPPH	ABTS	FRAP	TPC	DPPH	ABTS	FRAP
	µg gallic acid/ mg of extract**	IC_{50} [CI 95%]	IC_{50} [CI 95%]	g Fe^{+2}/g of extract**	µg gallic acid/ mg of extract **	IC_{50} [CI 95%]	IC_{50} [CI 95%]	g Fe^{+2}/g of extract**
I	33.99 (0.92)^{a}	1.92 (0.05) [1.88, 1.96]	2.05 (0.02) [2.04, 2.07]	4.10 (0.03)^{a}	78.23 (2.01)^{a}	0.816 (0.001) [0.814, 0.817]	1.51 (0.03) [1.48, .154]	4.39 (0.04)^{a}
II	44.94 (1.20)^{b}	1.47 (0.04) [1.43, 1.50]	2.03 (0.02) [2.01, 2.05]	3.97 (0.11)^{a}	85.68 (2.37)^{b,c}	1.03 (0.05) [0.97, 1.09]	1.75 (0.01) [1.74, 1.75]	4.91 (0.04)^{b}
III	53.27 (1.48)^{c}	1.24 (0.06) [1.18, 1.29]	1.44 (0.12) [1.33, 1.54]	6.37 (0.03)^{b}	86.15 (2.36)^{c}	1.69 (0.01) [1.68, 1.69]	2.28 (0.08) [2.21, 2.35]	7.37 (0.06)^{c}
IV	50.05 (1.31)^{c}	1.44 (0.06) [1.39, 1.49]	1.97 (0.03) [1.96, 2.00]	5.23 (0.09)^{c}	90.40 (2.42)^{d}	2.03 (0.10) [1.94, 2.11]	2.03 (0.10) [1.94, 2.11]	6.91 (0.04)^{d}
V	61.16 (1.71)^{d}	1.16 (0.02) [1.14, 1.18]	1.75 (0.09) [1.67, 1.83]	7.20 (0.06)^{d}	61.31 (1.57)^{e}	1.03 (0.02) [1.00, 1.06]	1.48 (0.01) [1.47, 1.50]	7.04 (0.05)^{e}
VI	81.05 (2.09)^{e}	0.63 (0.01) [0.62, 0.64]	0.92 (0.02) [0.90, 0.94]	7.94 (0.03)^{e}	81.88 (2.22)^{a,b}	1.03 (0.01) [1.02, 1.04]	1.83 (0.14) [1.70, 1.95]	5.56 (0.04)^{f}
VII	87.50 (2.39)^{f}	1.03 (0.04) [0.98, 1.08]	2.01 (0.08) [1.94, 2.08]	6.30 (0.06)^{c}	60.85 (1.65)^{e}	1.03 (0.04) [0.98, 1.08]	1.86 (0.05) [1.81, 1.91]	6.74 (0.04)^{g}
Quercetin	-	0.0749 (0.0004) [0.0745, 0.0752]	0.1136 (0.0008) [0.1129, 0.1143]	66.03 (7.08)	-	0.0749 (0.0004) [0.0745, 0.0752]	0.1136 (0.0008) [0.1129, 0.1143]	66.03 (7.08)
Trolox	-	0.1145 (0.0008) [0.1140, 0.1154]	0.2726 (0.0006) [0.2721, 0.2731]	23.19 (1.58)	-	0.1145 (0.0008) [0.1140, 0.1154]	0.2726 (0.0006) [0.2721, 0.2731]	23.19 (1.58)
TBHQ	-	0.1147 (0.0007) [0.1141, 0.1153]	0.1992 (0.0008) [0.1985, 0.1999]	36.57 (1.74)	-	0.1147 (0.0007) [0.1141, 0.1153]	0.1992 (0.0008) [0.1985, 0.1999]	36.57 (1.74)
Vitamin C	-	0.0876 (0.0105) [0.0783, 0.0969]	0.0201 (0.0002) [0.1999, 0.2008]	30.75 (1.88)	-	0.0876 (0.0105) [0.0783, 0.0969]	0.0201 (0.0002) [0.1999, 0.2008]	30.75 (1.88)

DPPH = 1,1-diphenyl-2-picrylhidrazyl; ABTS = 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid; TPC = Total phenolic compounds; FRAP = Ferric reducing antioxidant power; IC_{50} = Mean inhibitory concentration.
** Different letters indicate $P < 0.05$ (Tukey test).

which higher contents in *C. arabica* leaves were found in January (41.0 mg/L, 0.0004%), while *C. liberica* showed the highest concentration in July (127.0 mg/L, 0.0127%),[35] demonstrating that time of collection is very important in the metabolites concentration. De Almeda et al[36] reported variable CA content (0.2-1.75%) in leaves from three regions of Brazil, confirming that coffee leaves could be used as a functional drink for its polyphenolic content, that might provide biological activity including antioxidant effects or other.[37] Health benefits of CA has been demonstrated in control of oxidative stress, inflammation, aging and cancer.[38] Dicaffeoylquinic and feruloylquinic acids have also being detected in *C. canephora* leaves.[39]

Leaves from region VI demonstrated the highest amount of TPC (81.05 µg gallic acid/mg of extract), which correlated with antioxidant activity. According to the statistical analysis there are differences among the samples, with exception of samples from regions I and II that showed no differences.

Patay et al[8] compared ethanol extracts of *C. benghalensis* leaves at 5%, demonstrating CA as main compound (428 µg/mL) against an hydrolyzed extract with HCl 2N, which demonstrated caffeic acid as main compound (125.55 µg/mL); while *C. arabica* presented CA (605.65 µg/mL) as main compound, and the hydrolyzed extract presented ferulic acid (103.90 µg/mL) as main compound.

Another study demonstrated that the age of the leaves and the process affects the phytochemical profile, influencing the biological activity; it was observed that young leaves might have industrial potential, due to the production of natural compounds for its antioxidant and anti-inflammatory properties, while older leaves might mitigate high blood pressure, reducing cardiovascular damage or protect against microbial agents.[38]

For coffee beans the caffeine content is reported between 0.1-3.3%[31,40]; Farah et al[30] reported caffeine contents in green coffee beans (0.96-1.23%), Belay et al[41] reported samples from four regions of southern Ethiopia contents of 0.90-1.10%. According to the results of this study, all the samples from the seven regions contain interesting concentrations of caffeine, in leaves it was 0.453-0.725%, and in beans it was 0.520%-1.193%.

Caffeine and trigonelline has been reported from green coffee beans from Ethiopia, showing 0.84%-1.15% (w/w) and 0.83%-1.13 % (w/w),[42] 0.87%-1.38% and 0.98%-1.32%,[43] and 0.90%-1.10% and 0.62%-1.16% respectively.[44] Correlation of contents and altitude demonstrated that caffeine content are lower in green coffee beans cultivated in highlands, and contents are higher in beans cultivated in lowands.[45] As average content it has been proposed 0.8%-1.4% for caffeine and 0.6%-1.2% for trigonelline.[46]

In the different Guatemala regions it was demonstrated that higher altitudes showed higher caffeine content. According to the statistical analysis it was demonstrated significant difference in CA content in leaves than beans, while the caffeine content in leaves is the same in regions III and IV, but different from the other regions; in the bean no difference was demonstrated in regions IV, V and VII and in regions II and VI caffeine content was lower in beans than in leaves.

All samples presented moderate antioxidant activity in comparison with the standards. A high concentration of CA is considered as a constituent that brings antioxidant activity in the leaves, since this can trap free radicals and metals, and regulate the expression of antioxidant activity.[47]

Conclusions

This study demonstrated that coffee beans and leaves samples of all the regions has important antioxidant activity as well as chemical constituents (caffeine, TPC and AC), although there are differences among them.

It is suggested to strengthen the research activities in the sub-utilized coffee by-products (pulp, silver skin, flower, rejected beans, and coffee ground) that could offer interesting activities (antimicrobial, photoprotective, healing, anti-inflammatory) of ingredients for the cosmetic industry within an alternative, sustainable and ecological approach.

Competing Interests

None.

Acknowledgments

The authors wish to thank the General Directorate of Research (DIGI) of USAC for financial support (grant No. 4.8..63.4.13 of 2018), as well as the Association of Private Natural Reserves of Guatemala (ARNPG) and Abraham Elias for providing the coffee materials.

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