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Effect of extraction solvent on metabolites content, antioxidant, and antibacterial activity of coffee bagasse

Frida Lourdes García-Larez[1], Juan Luis Murillo-Hernández[2], Rey David Vargas-Sánchez[3], Gastón Ramón Torrescano-Urrutia[3], Brisa del Mar Torres-Martínez[3] and Armida Sánchez-Escalante[3]*

[1]Departamento de Ciencias Químicas Biológicas, División de Ciencias Biológicas y de la Salud, Universidad de Sonora (USON), Hermosillo 83000, Sonora, México. [2]Departamento de Ingeniería en Industrias Alimentarias, Instituto Tecnológico de Estudios Superiores de Zamora, Zamora de Hidalgo 59720, Michoacán, México. [3]Coordinación de Tecnología de Alimentos de Origen Animal (CTAOA), Laboratorio de Investigación en Carne y Productos Cárnicos, Centro de Investigación en Alimentación y Desarrollo, A. C. (CIAD). Carretera Gustavo Enrique Astiazarán Rosas #46, Hermosillo 83304, Sonora, México. E-mail: *armida-sanchez@ciad.mx

Abstract

Bagasse, one of the residues obtained from coffee processing, is an important source of metabolites, such as polysaccharides and phenolic compounds, with functional properties derived from its antioxidant and antimicrobial activities. The objective of this study was to evaluate the effect of the extraction solvent on metabolites content, as well as on antioxidant and antibacterial activity of the aqueous (T1), ethanolic (T2) and aqueous-ethanolic (T3) extracts of coffee bagasse. The results demonstrated that T1 showed the highest total carbohydrates, flavanones and dihydroflavonols content, T2 presented the highest total flavonoids and caffeoylquinic acid content, while T3 showed the highest total phenolic, flavones and flavonols content ($p < 0.05$). Furthermore, the greatest inhibitions of free-radicals and ferric reducing antioxidant power were detected in T1 and T3, while T2 showed higher reducing power ability depending on the concentration ($p < 0.05$). Greater inhibitory effects were observed in T3 against Gram-positive bacteria (*Staphylococcus aureus* and *Listeria monocytogenes*), T2 against *Echerichia coli*, T2 and T3 against *Pseudomonas aeruginosa* depending upon the concentration ($p < 0.05$). In conclusion, these results indicate that composition and properties of extract of coffee bagasse depend of the solvent used during the extraction.
Keywords: coffee residues, extraction technologies, bioactive compounds, antioxidant, antibacterial.

Efecto del disolvente de extracción sobre el contenido de metabolitos, actividad antioxidante y antibacteriana del bagazo de café

Resumen

El bagazo, es uno de los residuos que se obtienen del procesamiento del café, es una fuente importante de metabolitos, como polisacáridos y compuestos fenólicos, con propiedades funcionales derivadas de su actividad antioxidante y antimicrobiana. El objetivo de este estudio fue evaluar el efecto del solvente de extracción sobre el contenido de metabolitos, así como sobre la actividad antioxidante y antibacteriana del extracto acuoso (T1), etanólico (T2) y acuoso-etanólico (T3) del bagazo del café. Los resultados demostraron que T1 presentó el mayor contenido total de carbohidratos, flavanonas y dihidroflavonoles, T2 presentó el mayor contenido total de flavonoides y ácido cafeoilquínico mientras que T3 presentó el mayor contenido de fenoles totales, flavonas y flavonoles ($p < 0.05$). Además, la mayor inhibición de radicales libres y poder de reducción antioxidante del ión férrico se detectaron en T1 y T3, mientras que T2 mostró mayor capacidad de poder reductor dependiendo de la concentración ($p < 0.05$). Se observaron mayores efectos inhibidores en T3 frente a las bacterias Gram-positivas (*Staphylococcus aureus* y *Listeria monocytogenes*), T2 frente a *Echerichia coli*, T2 y T3 frente a *Pseudomonas aeruginosa* según la concentración ($p < 0.05$). En conclusión, estos resultados indican que la composición y propiedades del extracto de bagazo del café dependen del solvente utilizado durante la extracción.
Palabras clave: residuos de café, tecnologías de extracción, compuestos bioactivos antioxidante, antibacteriano.

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Introduction

Coffee is a strategic product in the Mexican economy; per capita consumption is 1.3 kg per year and develops more than 50,000 producers (SAGARPA, 2016). Chemical composition of the coffee bean is very varied, complex, and including carbohydrates (mono-, oli- and polysaccharides), insoluble polysaccharides (cellulose), lipids (wax and oils), nitrogen compounds (free amino acids, proteins, caffeine, among others), alkaloids (caffeine), and minerals like potassium, calcium, magnesium, iron, copper, sodium, manganese, zinc, among others. Also, some organic acids such as citric, malic and quinic, as well as phenolic acids like mono-, dicaffeoyl-, and feruloylquinic acid have been identified (Mussatto, Machado, Martins & Teixeira, 2011a; Ballesteros, Teixeira & Mussatto, 2014).

The great demand of coffee ground produces large amounts of residues, due more than 90% of the matter remains in by-products (Mussatto *et al*., 2011a). Furthermore, several investigations demonstrated that by-products obtained from the spend coffee (bagasse) are an important source of bioactive compounds, mainly phenolic compounds (phenolic acids and flavonoids). These compounds have great potential as antiproliferative, antifungal, antioxidant, and antibacterial agents (Monente *et al*., 2015; Balzano *et al*., 2020). Metabolites, including polysaccharides and phenolic compounds, present in many agro-industrial residues received much attention in the last decade due to their functional properties. For this reason, there is interest in the recovery of these compounds through different extraction methods and conditions (Santana-Méridas, González-Coloma & Sánchez-Vioque, 2012). In addition, solvent extraction is the most frequently used technique to recover antioxidant and antibacterial phytochemicals from plants; however, the extraction yields and bioactivity are highly dependent on the polarity of the solvent used, due to the presence of different phytochemicals in the source material, which depends on the chemical characteristics and polarity (Chemat *et al*., 2019).

Currently, there are different methods available for phenolic compounds extraction including conventional (agitated solvent extraction, maceration, and exhaustive extraction in Soxhlet equipment) and non-conventional procedures (ultrasound, microwave, supercritical fluid, pressurized liquid, subcritical water, high hydrostatic pressure, enzyme-assisted extraction, and pulsed electric field system) (Oreopoulou, Tsimogiannis & Oreopoulou, 2019). In addition, it has been demonstrated that several parameters such as pH, temperature, extraction time, solvent-solid ratio and solvent polarity used for extraction have an influence in the presence of some phenolic compounds (Chemat *et al*., 2019; Oreopoulou *et al*., 2019). Therefore, the objective of this study was to evaluate the effects of three solvents on the extraction of bioactive compounds from coffee bagasse and the resulting antioxidant and antibacterial properties of the extracts.

Materials and methods

Chemical and reagents

All utilized chemicals were of analytical grade. The following materials (≥ 99%) were purchased from Sigma-Aldrich (St. Louis, MO, USA): Folin-Ciocalteu's phenol reagent 2 M; sodium carbonate (Na_2CO_3); potassium persulfate ($K_2S_2O_8$); urea (NH_2CONH_2); 2,2-diphenyl1-picrylhydrazyl (DPPH); 2,2′-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid ≥ (ABTS); iron(II) sulfate 7-hydrate ($FeSO_4•7H_2O$); glacial acetic acid; trichloroacetic acid; potassium ferricyanide; and gentamicin sulfate salt. The next materials (≥95-98%) were also acquired: sodium hydroxide (NaOH); sodium nitrite ($NaNO_2$); phenol solution (C_6H_6O); gallic acid; 2,4,6-tri(2-pyridyl)-s-triazine (TPTZ); 2,4-dinitrophenylhydrazine (DNP); and iron (III) chloride hexahydrate ($FeCl_3•6H_2O$). In addition, potassium hydroxide ≥ 85% (KOH), ethanol and methanol (HPLC purity), and BD Brain Heart Infusion Agar were procured. The following compounds (≥ 99%) were obtained: ascorbic acid catechin, chlorogenic acid, glucose, and quercetin. Moreover, aluminum chloride hexahydrate 98% ($AlCl_3•6H_2O$), hydrochloric acid 37% (HCl), sulfuric acid 95-98% (H_2SO_4) were obtained from Merck (Darmstadt, Germany). Also, naringenin ≥ 99% was purchased from INDOFINE Chemical Company, Inc. (Hillsborough, NJ).

Extracts of coffee bagasse

A commercial supplier (CAFFENIO®) located in Hermosillo Mexico, donated coffee bagasse from dark *Coffea arabica* L. Metabolites compounds from coffee bagasse were extracted with water (T1), ethanol (T2), and a mixture of water-ethanol as solvents 1:1 w/v (T3). Also, the extraction was enhanced by ultrasound-assisted method (42 KHz/ 30 min/ avoiding temperatures above 35 °C), using a 1:10 bagasse-solvent ratio and an ultrasound bath (Bransonic 3800, Ultrasonics Corp., Jeju, Korea). The resultant mixture was filtered (Whatman No. 4 filter paper) under vacuum (vacuum pump MVP 6, Jeju, Korea), concentrated under reduced pressure at 60 °C (rotary evaporator Yamato RE301BW, Tokyo, Japan) and lyophilized (freeze dryer Yamato DC401, Tokyo, Japan). The obtained aqueous coffee bagasse extract (T1), ethanol coffee bagasse extract (T2), and aqueous-ethanol coffee bagasse extract (T3) were stored at −20 °C in the dark, until analysis (Ramírez-Rojo *et al*., 2019).

Quantitative phytochemical screening

Total carbohydrates content (TCC) of each extract was determined by the UV-sulfuric acid method (Albalasmeh, Berhe & Ghezzehei, 2013). An aliquot of extract (0.25 mL, 5 mg/mL) was homogenized for 1 min with 0.125 mL of aqueous phenol solution (5%, v/v) with 0.625 mL of concentrated H_2SO_4. Subsequently, the resultant mixture was cooled on ice for 5 min and absorbance was measured at 315 nm using a UV-Vis spectrophotometer (Multiskan FC UV-Vis, Thermo Scientific, Tokyo, Japan). TCC values were calculated from a standard curve of glucose (62.5 – 1,000 µg/mL; y = 0.6534x;

$R^2 = 0.9988$) and expressed as mg of glucose equivalent (GE) per 100 g of dried coffee bagasse (mg GE/100g).

Total phenolic content (TPC) was determined by the Folin-Ciocalteu method (Ainsworth & Gillespie, 2007). An aliquot of extract (10 µL, 5 mg/mL) was homogenized for 1 min with 80 µL of distilled water, 60 µL of Na_2CO_3 (7%, w/v), and 40 µL of Folin-Ciocalteu's reagent (0.25 *N*). The resultant mixture was mixed with 80 µL of distilled water and incubated during 1 h at 25 °C, in the dark. The absorbance was measured at 750 nm. TPC values were calculated from a standard curve of gallic (62.5 – 1,000 µg/mL; y = 0.4934x; $R^2 = 0.9996$) and caffeic acid (y = 0.3467x; $R^2 = 0.9969$), and expressed as mg of gallic acid equivalent (GAE) or caffeic acid equivalent (CAE) per 100 g of dried coffee bagasse (mg GAE/100g and mg CAE/100g, respectively).

Total flavonoids content (TFvC) was determined by the $NaNO_2$-$Al(NO_3)_3$-NaOH method (Zhishen, Mengcheng & Jianming, 1999). An aliquot of extract (500 µL, 5 mg/mL) was homogenized for 1 min with 1 mL of $NaNO_2$ (5%, w/v), 1 mL of $AlCl_3$ (10%, w/v) and 10 mL of NaOH. After, the resultant solution was adjusted to 25 mL with ethanol (70%), incubated (15 min at 25 °C, in the dark), and the absorbance was measured at 510 nm. TFvC values were calculated from a standard curve of catechin (62.5 – 1,000 µg/mL; y = 0.0298x; $R^2 = 0.9994$), and expressed as mg of catechin equivalent (CE) per 100 g of dried coffee bagasse (mg CE/100g).

Flavone and flavonol contents (FFC) were evaluated using the aluminum chloride-complex formation method (Popova *et al*., 2004). An aliquot of extract (10 µL, 5 mg/mL) was homogenized for 1 min with 130 µL of methanol and 10 µL of $AlCl_3$ (5%, w/v). The resultant mixture was incubated for 30 min at 25 °C, and absorbance was measured at 415 nm. FFC values were calculated from a standard curve of quercetin (62.5 – 1,000 µg/mL; y = 2.4965x; $R^2 = 0.9987$), and expressed as mg of quercetin equivalent (QE) per 100 g of dried coffee bagasse (mg QE/100g).

Flavanone and dihydroflavonol contents (FDC) were determined using by the 2,4-dinitrophenylhydrazine (DNP) method (Popova *et al*., 2004). An aliquot of extract (40 µL, 5 mg/mL) was homogenized for 1 min with 80 µL of DNP solution (50 mg DNP in 100 µL of 96% sulfuric acid diluted to 10 mL with methanol) and heated during 50 min at 50 °C. The resultant mixture was diluted with 280 µL of KOH in methanol (10%, w/v), and 30 µL of the resulting solution was mixed with 250 µL of methanol. The absorbance was measured at 490 nm. FDC values were calculated from a standard curve of naringenin (62.5 – 1,000 µg/mL; y = 0.2935x; $R^2 = 0.9997$), and expressed as mg of naringenin equivalent (NE) per 100 g of dried coffee bagasse (mg NE/100g).

Caffeoylquinic acid content (CAC) of each extract was determined (Griffiths, Bain & Dale, 1992). An aliquot of extract (100 µL, mg/mL) was homogenized for 1 min with 200 µL of urea (0.17 M), 200 µL of glacial acetic acid (0.1 M) and 500 µL of distilled water. The resultant mixture was mixed for 1 min with 500 µL of $NaNO_2$ (0.14 M) and 500 µL of NaOH, centrifuged (2,250 g for 10 min at 4 °C), and absorbance was measured at 510 nm. CAC values were calculated from a standard curve of chlorogenic acid (62.5 – 1,000 µg/mL; y = 0.1203x; $R^2 = 0.9991$), and expressed as mg equivalents of chlorogenic acid (CLAE) per 100 g of dried coffee bagasse (mg CLAE/100g).

Antioxidant activity

The $DPPH^•$ radical inhibition of each extract was determined by the 1,1-diphenyl-2-picrylhydrazyl method (Molyneux, 2004). An aliquot of extract (100 µL, at 62.5 – 500 µg/mL) was homogenized for 1 min with 100 µL of DPPH solution (300 µmol), and incubated during 30 min at 25 °C, in the dark. Ascorbic acid (25 µg/mL) was used as a control for $DPPH^•$ radical inhibition, while the solvent without extract was used as negative control (NC). The absorbance was measured at 520 nm and results were calculated as follows, $DPPH^•$ radical inhibition (%) = [1 − absorbance of antioxidant + $DPPH^•$ solution at 30 min/absorbance of $DPPH^•$ solution at 0 min] × 100.

ABTS-radical cation ($ABTS^{•+}$) inhibition was evaluated by the 2,2′-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) radical cation method (Re *et al*., 1999). Prior to analysis, equal parts of ABTS 7 mM solution of 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) 7 mM solution and potassium persulfate 2.45 mM were homogenized for 1 min and allowed to stand in darkness for 16 h at 25 °C. Afterwards, this radical was diluted with ethanol to obtain an absorbance of 0.8, and mixed for 1 min with an aliquot of extract (62.5 – 500 µg/mL) in a ratio 99:1. Ascorbic acid (25 µg/mL) was used as control for $ABTS^{•+}$ radical inhibition, while the solvent without extract was used as NC. The absorbance was read at 734 nm after 6 min of rest in the dark at 25 °C, and results were calculated as follows, $ABTS^{•+}$ radical inhibition (%) = [(absorbance of $ABTS^{•+}$ solution at 0 min) – (absorbance of antioxidant + $ABTS^{•+}$ solution after incubation period/absorbance) / (absorbance of $ABTS^{•+}$ solution at 0 min)] × 100.

Ferric reducing antioxidant power (FRAP) was determined (Benzie & Strain, 1999). An aliquot of extract (5 µL, at 62.5 – 500 µg/mL) was homogenized for 1 min with 150 µL of FRAP solution [10:1:1, 300 mM buffer sodium acetate in glacial acetic acid at pH 3.6 and 10 mM 4,4,6-tripyridyl-S-triazine (TPZ) in 40 nM HCl and 20 mM $FeCl_3$] and incubated during 8 min at 25 °C, in the dark. Ascorbic acid (25 µg/mL) was used as a positive control for FRAP, while the solvent without extract was used as negative control (NC). The absorbance was measured at

595 nm, and results were expressed as mg of Fe (II) equivalent per g (mg Fe^{2+}/g).

Reducing power ability (RPA) was determined by the Prussian blue method (Berker, Güclü, Tor, Demirata & Apak, 2010). An aliquot of extract (100 µL, at 62.5 – 500 µg/mL) was mixed for 1 min with 300 µL of phosphate buffer (0.2 M, pH 6.6), and 300 µL of potassium ferricyanide (1%, w/v), and incubated in a water bath during 20 min at 50 °C. The resultant mixture was mixed for 1 min with 300 µL of trichloroacetic acid (10%, w/v) and centrifuged at 4,200 x g/10 min (Sorvall ST18R, Thermo Fisher Scientific, Waltham, USA). Thereafter, the supernatant was mixed for 1 min with 100 µL of distilled water and 250 µL of ferric chloride (0.1%, w/v). Ascorbic acid (25 µg/mL) was used as a positive control for RPA, while the solvent without extract was used as negative control (NC). The absorbance was measured at 700 nm, and results were expressed as absorbance increase at the same wavelength.

Antibacterial activity

The antibacterial activity of each extract was performed according to the broth microdilution method (CLSI, 2012), with slight modifications. *Staphylococcus aureus* (ATCC 29213B), *Listeria monocytogenes* (ATCC 33090), *Escherichia coli* (ATCC 25922), and *Pseudomonas aeruginosa* (ATCC 15442) were donated by the culture collection of the Dairy Laboratory (Food Research and Development Center, A.C.), and initially reactivated in liquid nutrient broth (BHI, brain hearth infusion) for 24 - 48 h at 37 °C. Afterwards, the strain's suspension were diluted with saline solution until reach the turbidity of 0.5 McFarland standard, barium sulfate - $BaSO_4$ (ca. 1.5 x 10^8 CFU/mL). The accuracy of the method was verified by using a spectrophotometer with a 1 cm light path, i.e., for the 0.5 McFarland standard, the absorbance at 620 nm should be 0.08 to 0.13. Then, the diluted solution was mixed with an aliquot of extract (50 µL, at 62.5 – 500 µg/mL). Gentamicin (25 µg/mL) was used as a positive control for bacterial growth inhibition, and BHI was used as bacterial growth blank or negative control. The plates were incubated during 24 h at 37 °C, and absorbance was read at 620 nm (OD, optical density). The results were expressed as inhibition (%) = (OD620 untreated bacteria−OD620 nm treated bacteria) / (OD620 nm untreated bacteria) × 100.

Statistical analysis

All variables were conducted in triplicate for at least three independent experiments, and the results are given as mean ± standard deviation (n = 9). Data of metabolites contents were subjected to analyses of variance (ANOVA), while data of antioxidant and antibacterial properties were subjected to a two-way-factorial ANOVA in which the solvent (water, ethanol, and 1:1 water-ethanol) and concentration level (62.5 – 500 µg/mL) were the fixed effects in the model. A Tukey-Kramer multiple comparison test was performed for mean separation ($p < 0.05$). In addition, the association between metabolites and properties of coffee bagasse extract were determined by using Pearson's correlation analysis (SPSS, version 19).

Results and discussion

Quantitative phytochemical screening

Table I reports the results of metabolites extracted from coffee bagasse with different extraction solvents. The results indicated that T1 showed the highest total ($p < 0.05$) carbohydrate (TCC) and flavanones and dihydroflavonols contents (FDC); while T2 showed the highest ($p < 0.05$) total flavonoids (TFvC) and caffeoylquinic acid contents (CAC). In addition, T3 showed the highest ($p < 0.05$) total phenolic (TPC) and flavones and flavonols contents (FFC). The carbohydrate content measured by the sulfuric acid-UV method depends on the UV absorbance of furfural derivates produced by reaction with concentrated sulfuric acid, which are determined spectroscopically at 315 nm. The advantages of the sulfuric acid-UV method in comparison to Phenol-Sulfuric acid method involve accuracy of the measurements and avoid the health and environmental hazards due phenol use (Albalasmeh *et al*., 2013).

Phenolic acids contents are based on the transfer of electrons in alkaline medium from phenolic component to phosphomolybdic/phosphotungstic acid complexes, which are determined at 765 nm (Ainsworth & Gillespie, 2007). In addition, total flavones and flavonols contents method relies on the formation of a complex between the aluminum ion and the carbonyl and hydroxyl groups of phenolic compounds, which is measured at 415 nm (Popova *et al*., 2014); while total flavonoids method uses $AlCl_3$ and $NaNO_2$ to form a colorful complex whose absorbance is measured at 510 nm (Zhishen *et al*., 1999). Additionally, flavanones and dihydroflavonols determination involve the interaction of phenolic compounds with 2,4-dinitrophenylhydrazine in acidic media to form colored

Table I. Metabolites content of coffee bagasse extracts (mg/100g).

Item/Solvent	Water	Ethanol	1:1
TCC (GE)	3,504 ± 12[c]	1,372 ± 13[a]	3,283 ± 25[b]
TPC (GAE)	1,297 ± 17[b]	760 ± 12[a]	3,104 ± 21[c]
TPC (CAE)	1,778 ± 25[b]	1,300 ± 19[a]	4,086 ± 44[c]
TFvC (CE)	2,316 ± 21[a]	3,246 ± 19[c]	2,918 ± 38[b]
FFC (QE)	143 ± 17[a]	168 ± 20[b]	438± 25[c]
FDC (NE)	2,302 ± 12[c]	845 ± 17[a]	1,580 ± 39[b]
CAC (CLAE)	460 ± 11[a]	1,175 ± 60[c]	1,048 ± 27[b]

Results are given as mean ± SD (n = 9). 1:1, aqueous-ethanol solvent; TCC, total carbohydrate content; TPC, total phenolic content; TFvC, total flavonoids content; FFC, flavones and flavonols content; FDC, flavanones and dihydroflavonols content; CAC; caffeoylquinic acids. Means with different superscripts (a–c) among samples in each row indicate significant differences ($p < 0.05$).

phenylhyrazones whose absorbance is measured at 490 nm (Popova *et al*., 2014); while caffeoylquinic acid determination relies the interaction of nitrous acid with ortho-dihydoxy phenols (catechol group), which are determined at 510 nm (Griffiths *et al*., 1992).

In agreement with our work, Bravo, Monente, Juániz, De Peña & Cid (2013) evaluated the effect of solvent extraction on phenolic compounds of spent coffee grounds, and results showed that solvent had an effect on TPC extraction efficiency in the order aqueous-ethanol (40:60) > aqueous > methanol > ethanol (at 90 °C). Also, Panusa, Zuorro, Lavecchia, Marrosu & Petrucci (2013) reported a high TPC, TFvC, and CAC extraction efficiency in extracts from coffee spent grounds (*Coffea arabica*), obtained when a mixture of solvent was used [aqueous-ethanol (40:60) > water (at 60 °C)]. While, Mussatto, Ballesteros, Martins & Teixeira (2011b) reported a high TFvC and CAC recovery in extracts from coffee spent grounds, obtained by solid-liquid extraction when a mixture of solvent was used [methanol 80% > methanol 20% > aqueous (at 60 °C)].

Moreover, it has been reported the presence of carbohydrates on spent aqueous coffee extract (Ballesteros *et al*., 2014). However, information about the extraction of carbohydrates coffee residues using as solvent water in combination with other solvents are still limited. Also, Choi & Koh (2017) reported a TPC < 30 mg GAE/g in spent coffee water-methanol extract (40:60, at 60 °C) from *Coffea arabica*, which was obtained by solid-liquid extraction. In another study, Kim, Ahn, Eun & Moon (2016) determined the effect of solvent extraction on phenolic compound extraction from coffee spent grounds, and the results demonstrated that high TPC extraction efficiency was obtained in the order ethanol at 80 °C > ethanol at room temperature > aqueous at 80 °C). In the same order, Mussatto *et al*. (2011b) reported high TPC recovery in the extract with methanol 80% > methanol 20% > aqueous at 60 °C. In this regard, it has been reported that carbohydrates are polar like the solvent water, while phenolic compounds are considered medium-polarity molecules, i.e., depending on their chemical structure they can be soluble in polar and lower polarity solvents (Marcović *et al*., 2012; Ballesteros *et al*., 2014).

Based on the above, it is important to emphasize the need to carry out more studies on the characterization of each group of bioactive compounds extracted from natural sources, using spectroscopic and spectrometric techniques (Lozada-Ramírez, Ortega-Regules, Hernández & Anaya de Parrodi, 2021).

Antioxidant activity

Antioxidants are considered substances that a low concentration can reduce the oxidation of macromolecules, including proteins and lipids, through free-radicals inhibition mechanism or by chelating metal ions (Liu, 2010; Pisoschi & Pop, 2015). In order to evaluate the antioxidant effectiveness of natural extracts several methods have been used (Liu, 2010). Antioxidants can be classified into three groups such as primary antioxidants (free-radical scavengers), secondary antioxidants (reducers of chain-initiation reactions) and tertiary antioxidants (repairers of damaged biomolecules) (Pisoschi & Pop, 2015).

The $DPPH^{\bullet}$ is a stable radical used to evaluate the radical-scavenging properties of natural antioxidants, including phenolic acids and flavonoids. In addition, the H atom from OH-group may transfer from ArOH (phenolic compound) to $DPPH^{\bullet}$ to neutralize it, i.e. by the mechanism of hydrogen atom transfer-HAT (Molyneux, 2004; Liu, 2010). While, $ABTS^{\bullet+}$ measure the overall reductive ability of the whole molecule or antioxidant-ArOH (Liu, 2010). In addition, it has been reported that $DPPH^{\bullet}$ and $ABTS^{\bullet+}$ procedures measured the antioxidant activity of hydrophobic and water-soluble compounds (Molyneux, 2004; Liu, 2010; Pisoschi & Pop, 2015). Furthermore, the ferricyanide/Prussian blue assay or RPA measurement, involving the reduction by antioxidants (ArOH) of Fe(III)-L complex to Fe(II)-L complex, where L represents the iron-binding ligand, while FRAP assay utilizing tripyridyltriazine (TPTZ) ligand (Berker *et al*., 2010). However, it has been extensively demonstrated that solvent affect the metabolites recovery and antioxidant activity of natural extracts (Leopoldini, Marino, Russo & Toscano, 2004; Sultana, Anwar, & Ashraf, 2009).

Table II reports the antioxidant activity of each extract, and results obtained indicated that antioxidant activity was significantly affected by the type of extraction solvent and tested extract concentration ($p < 0.05$). The results showed that T1 and T3 exhibits the highest ($p < 0.05$) $DPPH^{\bullet}$ radical inhibition (> 85%) than T2 (< 60%), while T3 presented the highest ($p < 0.05$) $ABTS^{\bullet+}$ radical inhibition (> 70%) and FRAP (> 80%) values in concentration-dependence. Also, a high RPA values (> 0.1 absorbance) was obtained for T2 in concentration-dependence ($p < 0.05$). In addition, the positive control (ascorbic acid) showed the highest antioxidant effect on $DPPH^{\bullet}$ (95% of inhibition), $ABTS^{\bullet+}$ (89.1% of inhibition), FRAP (1.4 mg Fe^{2+}/g), and RPA (1.2 absorbance).

In agreement with our study, Choi & Koh (2017) reported a high $DPPH^{\bullet}$ radical inhibition (> 80%) of spent coffee extract obtained by solid-liquid extraction from *Coffea arabica*, when a mixture of solvent was used (water-methanol, 40:60; at 60 °C). Also, Ballesteros *et al*. (2014) demonstrated the antiradical inhibition and RPA of spent coffee grounds extract, which was produced by solid-liquid extraction and using ethanol as solvent extraction. In another study, Murthy & Naidu (2010) evaluated the recovery and antioxidant effectivity of compounds from spent coffee through the Soxhlet extraction method, and results reported that extract exert high $DPPH^{\bullet}$ radical inhibition (> 50%, at 500 µg/mL) in concentration-dependence, when a mixture of solvents was used (aqueous:isopropanol, 40:60). In addition, Bravo *et al*. (2013) reported a high $DPPH^{\bullet}$ radical

Table II. Antioxidant activity of coffee bagasse extracts.

Item	μg/mL	Solvents		
		Water	Ethanol	1:1
DPPH• (%)	500	85.3 ± 1.4^{bD}	58.4 ± 3.0^{aD}	86.2 ± 1.2^{bD}
	250	76.3 ± 2.5^{cC}	29.0 ± 0.9^{aC}	52.5 ± 1.8^{bC}
	125	27.9 ± 2.2^{bB}	15.8 ± 2.9^{aB}	37.7 ± 1.4^{cB}
	62.5	4.9 ± 1.5^{bA}	1.8 ± 0.3^{aA}	13.3 ± 2.3^{cA}
	NC	--	--	--
ABTS•+ (%)	500	63.7 ± 1.2^{bC}	56.9 ± 2.1^{aD}	70.5 ± 1.7^{cD}
	250	39.3 ± 1.7^{aB}	37.2 ± 2.6^{aC}	47.9 ± 1.9^{bC}
	125	25.6 ± 3.0^{aA}	28.8 ± 3.0^{aB}	33.9 ± 2.1^{bB}
	62.6	24.9 ± 3.3^{bA}	14.8 ± 2.7^{aA}	24.7 ± 1.7^{bA}
	NC	--	--	--
FRAP (mg Fe^{2+}/g)	500	0.78 ± 0.10^{bD}	0.57 ± 0.06^{aD}	0.85 ± 0.10^{cD}
	250	0.51 ± 0.03^{bC}	0.35 ± 0.04^{aC}	0.52 ± 0.05^{bC}
	125	0.34 ± 0.02^{bB}	0.25 ± 0.04^{aB}	0.34 ± 0.04^{bB}
	62.5	0.23 ± 0.01^{bA}	0.18 ± 0.01^{aA}	0.24 ± 0.02^{bA}
	NC	--	--	--
RPA (Absorbance)	500	0.03 ± 0.00^{a}	0.15 ± 0.01^{b}	0.02 ± 0.00^{a}
	250	--	--	--
	125	--	--	--
	62.5	--	--	--
	NC	--	--	--

Results are given as mean ± SD (n = 9). 1:1, aqueous-ethanol solvent; NC, negative control; --, unobserved activity. Means with different superscripts indicate significant differences among solvent extraction (a–c) x concentration (A–D) ($p < 0.05$).

inhibition of spent coffee extract obtained in the order aqueous > aqueous-ethanol (40:60) > methanol > ethanol at 90 °C); while, ABTS•+ radical inhibition effect was exerted in the order aqueous-ethanol (40:60) > aqueous > methanol > ethanol.

Moreover, Kim *et al*. (2016) determined the effect of extraction solvent on antiradical activity from spent coffee grounds, and results demonstrated a high DPPH• radical inhibition in the order ethanol at 80 °C > ethanol at room temperature > water at 80 °C, in concentration-dependence. In another study, Mussatto *et al*. (2011b) reported high FRAP values of spent coffee grounds extract in the order methanol 80% > methanol 20% > aqueous. Furthermore, it has been reported that aqueous coffee extract from *Coffea arabica* obtained by solid-liquid extraction method, at 500 µg/mL exerted < 50% of DPPH• radical inhibition, and > 1.0 absorbance of RPA (Yen, Wang, Chang & Duh, 2005).

Antibacterial activity

Table III reports the antibacterial activity of each extract, and results indicate that bacterial growth was significantly affected by solvent extraction used and tested extract concentration ($p < 0.05$). The results showed that T3 exhibited the highest ($p < 0.05$) antibacterial activity against *S. aureus* and *L. monocytogenes* (> 35% of inhibition by both strains). A high *E. coli* inhibition was also observed (> 28%) with T2 ($p < 0.05$). While the highest *P. aeruginosa* inhibition was observed (> 20%) by T2 and T3 ($p < 0.05$). In addition, the positive control (gentamicin) showed the highest antibacterial effect on the tested bacteria (> 90%).

In agreement with our work, Klangpetch (2017) reported a higher antibacterial effect of coffee extract obtained with a mixture of solvents (aqueous:ethanol, 40:60) than ethanol, in concentration-dependence, against *S. aureus* > *E. coli*. Similarly, Monente *et al*. (2015) reported an antibacterial effect (> 15 mm of inhibition) of spent coffee extract of *C. arabica* and *C. robusta* obtained with water, against Gram-positive bacteria (*L. monocytogenes* > *S. aureus*), which was associated with the presence of some metabolites like caffeine, caffeoylquinic acid and dicaffeoylquinic acid. However, in the same study no

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Table III. Antibacterial activity of coffee bagasse extracts.

Bacteria	µg/mL	Solvents		
		Water	Ethanol	1:1
S. aureus	500	18.8 ± 0.7^{aB}	21.6 ± 2.2^{bC}	36.8 ± 0.6^{cC}
	250	8.6 ± 2.4^{aA}	12.2 ± 1.7^{aB}	12.7 ± 3.3^{aB}
	125	4.8 ± 2.2^{aA}	7.4 ± 1.4^{aA}	7.3 ± 1.2^{aA}
	62.5	--	--	--
	NC	--	--	--
L. monocytogenes	500	28.3 ± 0.3^{aC}	30.5 ± 0.6^{bC}	35.4 ± 0.7^{cB}
	250	24.6 ± 1.5^{bB}	26.5 ± 0.9^{bB}	20.1 ± 0.7^{aA}
	125	13.7 ± 0.2^{aA}	16.0 ± 0.8^{bA}	21.8 ± 1.2^{cA}
	62.6	--	--	--
	NC	--	--	--
E. coli	500	26.2 ± 1.1^{aB}	29.4 ± 1.1^{bB}	27.9 ± 0.3^{aB}
	250	8.2 ± 1.0^{aA}	14.7 ± 1.2^{bA}	20.6 ± 2.2^{cA}
	125	--	--	--
	62.5	--	--	--
	NC	--	--	--
P. aeruginosa	500	16.0 ± 1.5^{aB}	21.2 ± 2.0^{bB}	20.0 ± 0.7^{bB}
	250	11.1 ± 2.2^{bA}	13.3 ± 2.6^{bA}	6.2 ± 1.5^{aA}
	125	--	--	--
	62.5	--	--	--
	NC	--	--	--

Results are given as mean ± SD (n = 9). 1:1, aqueous-ethanol solvent; NC, negative control; --, unobserved activity. Means with different superscripts indicate significant differences among solvent extraction (a–c) x concentration (A–C) ($p < 0.05$).

significant effect was observed for both extracts against Gram-negative bacteria (*E. coli* and *P. aeruginosa*). In another study, Sant'Anna *et al*. (2017) reported absence of antibacterial effect of aqueous extract from spent coffee, against Gram-positive (*S. aureus*, *B. cereus*, *L. monocytogenes*, and *L. innocua*) and Gram-negative bacteria tested (*E. coli*, *S. enteritidis*, and *P. aeruginosa*). In previous studies, it has also been reported the relationship of total phenolic and flavonoids contents, and the chemical phenolic components like chlorogenic and protocatechuic acids with the antibacterial activity of coffee residues (Esquivel & Jiménez, 2012). Also, it has been demonstrated that during coffee roasting polysaccharides are degraded to low molecular weight carbohydrates, which exert an antibacterial effect on the growth of some enterobacterial strains including *Clostridum perfringens* and *E. coli* (Asano *et al*., 2001).

Correlation between phytochemical and biological properties

Table IV reports the association of individual phytochemicals with the antioxidant and antibacterial activities of coffee bagasse extracts. The results of Pearson's correlation coefficient showed that DPPH$^{\bullet}$ radical inhibition showed a high positive correlation with TCC and TPC 0.992 and 0.979, respectively), and ABTS$^{\bullet+}$ radical inhibition showed a positive correlation with TCC (0.815), TPC and FFC (0.954 and 0.825, respectively). In contrast, DPPH$^{\bullet}$ and ABTS$^{\bullet+}$ radical inhibition were negative correlated with TFvC (-0.751 and -0.348) and CAC (-0.615 and -0.166, respectively). Regarding to reducing power, FRAP values showed a positive correlation with TCC and TPC (0.91 and 0.881, respectively), and RPA values demonstrated a positive correlation with TFvC and CAC (0.938 and 0.986, respectively). In addition, FRAP values showed a

Table IV. Correlation between metabolites of coffee bagasse extract and their biological properties.

	TCC	TPC1	TPC2	TFvC	FFC	FDC	CAC	DPPH•	ABTS•+	FRAP	RPA	SA	LM	EC	PA
TCC	1.000	0.605	0.557	-0.827	0.345	0.911	-0.707	0.992	0.815	0.910	-0.579	0.280	0.121	-0.894	-0.745
TPC1		1.000	0.998	-0.520	0.956	0.224	0.136	0.979	0.954	0.881	0.299	0.934	0.864	-0.183	0.081
TPC2			1.000	0.007	0.972	0.166	0.194	0.954	0.935	0.851	0.355	0.953	0.892	-0.125	0.140
TFvC				1.000	0.243	-0.985	0.982	-0.751	-0.348	-0.519	0.938	0.308	0.458	0.991	0.991
FFC					1.000	-0.071	0.419	0.458	0.825	0.704	0.565	0.998	0.973	0.112	0.369
FDC						1.000	-0.935	0.854	0.504	0.659	-0.863	-0.139	-0.298	-0.999	-0.953
CAC							1.000	-0.615	-0.166	-0.350	0.986	0.481	0.616	0.949	0.998
DPPH•								1.000	0.880	0.954	-0.475	0.396	0.242	-0.832	-0.657
ABTS•+									1.000	0.982	0.000	0.785	0.674	-0.468	-0.220
FRAP										1.000	-0.189	0.653	0.522	-0.627	-0.401
RPA											1.000	0.620	0.739	0.883	0.975
SA												1.000	0.987	0.180	0.432
LM													1.000	0.337	0.572
EC														1.000	0.965
PA															1.000

SA, *S. aureus*; LM, *L. monocytogenes*; EC, *E. coli*; PA, *P. aeruginosa*.

negative correlation with TFvC and CAC (-0.519 and -0.350, respectively), while RPA was negative correlated with TCC and FDC (-0.579 and -0.863).

Furthermore, antibacterial results revealed a positive correlation of *S. aureus* with TPC and FFC (0.934 and 0.998, respectively), *L. monocytogenes* with TPC and FFC (0.864 and 0.973, respectively), *E. coli* with TFvC and CAC (0.991 and 0.949, respectively) and *P. aeruginosa* with TFvC and CAC (0.991 and 0.998, respectively). In contrast, a negative correlation was showed between *S. aureus* and *L. monocytogenes* with FDC (-0.139 and -0.298, respectively), *E. coli* with TCC, TPC and FDC (-0.894, -0.183 and -0.999, respectively). These findings demonstrated that phytochemicals are contributing to the antiradical and reducing power activities of coffee bagasse extracts, as well as to its antibacterial properties. Polyphenols are the major plant compounds associated with their biological properties, however, differences in phenolic composition and the presence of some non-phenolic compound could exert interferences with the correlation of polyphenols and their activities. In addition, structural properties, synergistic and antagonistic interactions, presence of another type of bioactive compounds, method used to determine the bioactivity, might also be responsible for enhancing or reduced the correlation (Terpinc, Čeh, Ulrih & Abramovič, 2012).

Conclusions

In conclusion, this investigation demonstrated that the extraction solvent significantly affected metabolites content, as well as the antioxidant and antibacterial activity of coffee bagasse extracts. Aqueous extracts showed the highest carbohydrate and flavanones and dihydroflavonols contents, while ethanol extracts showed the highest flavonoids and caffeoylquinic acids contents. In addition, aqueous-ethanolic extract showed the highest phenolic and flavone and flavonol contents. Furthermore, the aqueous-ethanolic extract showed the highest antiradical and reducing power values, as well as the highest antibacterial effect against *S. aureus*, *L. monocytogenes* and *P. aeruginosa*. In this regard, ethanol extract exerted the highest antibacterial activity against *E. coli*. Finally, there was a high correlation between metabolites content and biological properties.

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