Optimal Aqueous Extraction Conditions as A Green Technique for Recovery of Phenolic Antioxidants from Robusta Dried Coffee Pulp

Thy Minh Kieu Tran, Taiwo Akanbi, Timothy Kirkman, Minh Huu Nguyen, and Quan Van Vuong

***Abstract*—Coffee pulp, a by-product of coffee processing, contains high level of flavonoids and other phenolic compounds. This by-product also contains high levels of other bioactive compounds such as chlorogenic acids and caffeine, which can be potentially recovered for further applications. This study used water as an inexpensive green solvent, for the maximum recovery of phenolics, major bioactive compounds and antioxidant capacity from coffee pulp. Recovery yield from optimal aqueous extraction was compared with organic solvent extraction. The results showed that temperature, extraction time and solid/solvent ratio significantly affected recovery yields from coffee pulp ($P<0.05$). Optimal aqueous extraction conditions were 100 °C, 60 min and the ratio of sample to solvent 1:100 g/mL. Under these optimal conditions, recovery yields were similar to those of 50% aqueous acetone extraction. Recovery yields were significantly higher than pure acetone, methanol and ethanol as well as methanol and ethanol in combination with water (50% v/v). Therefore, these optimal aqueous conditions are recommended for recovery of bioactive compounds from coffee pulp for further applications.**

***Index Terms*—Dried Coffee Pulp, Coffea Canephora, Robusta, Bioactive Compounds, Antioxidant Capacity, Phenolics, Hot Water, Solvent, Extraction, Conventional Extraction.**

I. INTRODUCTION

There are about 70 species of coffee, however only two of them are grown commercially in large quantities around the world; Arabica (Coffea arabica) and Robusta (Coffea canephora) [1]. Coffee pulp is the major by-product of the coffee industry because it occupies 40% of coffee fruit weight [2]. A large quantity coffee pulp is generated annually and is mainly used for mulching or ends up in landfill [3].

Coffee pulp is a source of several phytochemicals, including caffeine, chlorogenic acid, neochlorogen acid and feruloylquinic acid, which have been reported to have health benefits [4], hence it could be utilized as valuable ingredients for foods, cosmetics and pharmaceuticals industries [5].

Extraction is an essential step for recovery of bioactive constituents from plant materials. Conventional extraction techniques such as solvent extraction, maceration, infusion, percolation, and decoction have been applied to extract bioactive compounds from food materials [6]. Methanol, acetone [7], isopropanol, water [8] and formic acid [9] have been used to extract tannins, phenols and caffeine from coffee by-products. Advanced techniques, such as ultrasound-assisted extraction, microwave-assisted extraction, and supercritical fluid extraction have also been applied for improvement of extraction yields [10,11].

Conventional extraction techniques are simple, do not require advanced equipment, and are easy to scale up; however, they often give low recovery yields [12]. Therefore, factors affecting recovery yield need to be investigated to improve recovery yields if conventional techniques are applied. Several factors have been reported to significantly affect recovery yields of bioactive constituents, including solvent, temperature, extraction time, solid/ solvent ratio and particle size. Among the solvents used, water is considered as a preferred solvent because it is accessible, inexpensive and safe [13]. A previous study used a combination of solvents, including water, for extraction of phenolics from coffee pulp, but found methanol to be the best solvent for extraction [14]. However, this study did not investigate optimal aqueous conditions for recovery of bioactive constituents from coffee pulp. Also, there are few studies done in this area.

This study therefore aims to investigate the impact of aqueous extraction conditions including temperature, extraction time, and solid/solvent ratio on extraction yield of total phenolics, caffeine, chlorogenic acid, and antioxidant properties from coffee pulp. The optimal extraction conditions for recovery of these bioactive components will be established. Extraction efficiency under these optimal conditions is further compared with those of other organic solvents, such as acetone, methanol, ethanol, and their combination with water (50:50 v/v).

Published on September 10, 2020.

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II. Material and Methods

A. Material

Coffee pulp (Robusta) was collected from Thang Loi Company, Krong Pak, DakLak, Vietnam. After collection, samples were transferred immediately to the laboratory and stored at -18 °C. The frozen sample was then thawed overnight at room temperature and dried at 90 °C for 6.5 h under vacuum pressure of 3.75 mmHg using a vacuum dryer (Memmert VO200). After drying, dried coffee pulp was ground using an electric grinder (Nutri Bullet Branch) and then screened by a sieve less than 1.4mm using an Endecotts sieve (London, England). The dried ground sample was packed and sealed in polyethylene bags then stored at -18 °C until used.

Chemicals used in the experiments were analytical and HPLC grades: Folin-Ciocalteu's reagent, ethanol, sodium nitrite, acetone, acetonitrile, formic acid, sodium hydroxide, hydrochloric acid, methanol, aluminium chloride and iron (III) chloride and anhydrous sodium carbonate were from Merck brand, Germany. 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS), trolox, 2,4,6-tris(2-pyridyl)-s-triazine (TPTZ), gallic acid, (+)-catechine, caffeine, chlorogenic acid, and 2,2-diphenyl-1-picrylhydrazyl (DPPH) were purchased from Sigma-Aldrich Pty Ltd.

B. Method

1) Experimental design

Determination of the impact of temperature on polyphenol extraction yield was implemented as following: 0.2 g of ground coffee pulp was extracted with 20 mL of water at different temperatures (40, 50, 60, 70, 80, 90 and 100 °C) for 30 min using a shaking water bath with a temperature controller. The optimal temperature (100 °C) was then used to identify the effect of the extraction time. To investigate the impact of extraction time, 0.2 g of ground coffee pulp was extracted with 20 mL of water at 100 °C for various length of time (10, 20, 30, 40, 50, 60, 70 and 80 min). The optimal time (60 min) and temperature (100 °C) were then used to determine the impact of the solid/solvent ratio; ground coffee pulp was extracted in 20 mL of water at various ratios of 0.5, 1, 2.5, 5, 7.5, 10: 100 g/mL. Finally, to compare extraction yield of the optimal aqueous extraction conditions with extraction yields of organic solvents, acetone, methanol and ethanol, 0.2 g of ground dried coffee pulp was extracted in 20 mL of water at 100 °C for 60 min or alternatively 0.2 g of ground coffee pulp in 20 mL of solvent (acetone, ethanol or methanol and their combination with water at 50:50 v/v) at room temperature (RT) for 72 h. All extracts were filtered using a Whatman No. 1 filter paper (Lomb Scientific, Australia) to remove the solids and then stored in a fridge at 4 °C within a day for further analysis.

2) Determination of total phenolic content (TPC)

In this study, total phenolic content (TPC) was determined as the method of Vuong et al. (2013) [24]. Briefly, 0.5 ml of diluted sample was added to 2.5 mL of diluted Folin Ciocalteau 10%, the mixture was left at room temperature for 8 min. 2 mL of sodium carbonate solution 7.5 % was then added to the mixture and shaken well. The solution was left at RT, in a dark room for 30 min. Finally, the solution was measured at an absorbance of 765 nm using a UV-Vis systems (Cary 60 UV-Vis Spectrometer, Agilent Branch). The results were figured out according to an external standard curve which was designed by using gallic acid and showed as mg of gallic acid equivalents per g of dry weight of sample (mg GAE/g DW).

3) Determination of total flavonoid content

Total flavonoid content (TFC) was measures using the method of Vuong et al. (2013) [24]. Briefly, 0.5mL of diluted sample was added to 2 mL of water, followed by 0.15 mL of 5% NaNO2 solution. The mixture was allowed to stand for 6 min at RT. Then, 0.15mL of AlCl3 (10% w/v) was added and left for 6 min. Ater that, the addition of 2 mL NaOH (4% w/v). 0.7 mL of DI water was added in the mixture to bring its final volume of 5 mL. The solution was well-mixed and allowed to stand at room temperature for another 15 min. The absorbance was determined at 510 nm using UV-Vis systems (Cary 60 UV-Vis Spectrometer, Agilent Branch). The external curve was created by using catechin and the results were showed as mg of catechin equivalents per gram of dry weight of sample (mg CE/g DW).

4) Determination of caffeine and chlorogenic acids content

Caffeine and chlorogenic acid were determined by using a HPLC system (Thermo Finnigan Corporation, USA). The standard curves were created by standard caffeine and chlorogenic acid in methanol at concentrations between 12.5 – 1000 µM. The standards and extracts were filtered through a Phenex syringe filters, and were then automatically injected into the HPLC system with a 30 µL injection volume. A Luna 5 µ Phenyl-hexyl column (Phenomenex, USA) was used for experiments. All samples and standards were measured at 210 nm. The mobile phase was 0.2% formic acid in distilled H2O (A) and 0.2% formic acid in methanol (B). The flow rate was set at 1 mL/min. Temperature of column oven was maintained at 35 °C. The contents of caffeine and chlorogenic acids were identified by matching retention times and areas with the standard curve values and presented as mg per gram of dried sample (mg/g DW).

5) Determination of antioxidant capacity

Antioxidants reduce the damage caused by free radicals which may otherwise contribute to cancers, tumors and cardiovascular diseases [15]. The measurement of antioxidants should be conducted with multiple assays to reduce their individual limitations [16]. In this study, three in-vitro antioxidant assays were applied to determine total antioxidants of the extracts of coffee pulp.

a) 2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) assay

ABTS radical scavenging capacity was figured out as the method of Vuong et al. (2013) [24]. 10mL of 7.4 mM ABTS was then added to 10mL of 2.6 mM K2S2O8 to obtain stock solutions. They were then mixed well and placed in a dark room for 15 h. For preparation of the working solution, 1 mL of stock solution was diluted with approximately 60 mL of methanol. The mixture was measured at 734 nm by employing a UV-Vis system (Cary 60 Bio, UV-Vis,

Malaysia) to obtain a value of 1.1 ± 0.02 unit. The ABTS assay was prepared by adding 0.15 mL of the sample to 2.85 mL of working solution and incubated in a dark room for 2 h. The absorbance of mixture was figured out at 734 nm. Trolox was used to create a standard curve and the results were expressed as mg of trolox equivalents per g of sample dry weight (mg TE/g dried sample).

b) 2,2-diphenyl-1-picrylhydracyl (DPPH) assay

DDPH radical scavenging capacity was determined as the method of Thaipong et al. (2006) [29]. Briefly, for preparation of stock solution, 24 mg of DPPH was dissolved in 100 mL of methanol and stored at -20 °C until required. A working solution was prepared by adding 10 mL of DPPH stock solution to 45 mL methanol to obtain a working solution, and it was then measured to obtain an absorbance of 1.1 ± 0.02 units at 515 nm. For the assay, 0.15 mL of the sample extract was added to 2.85 mL of working solution and was analyzed after incubating in 3 h. The measurement of mixture was done at 515 nm using a UV-Vis spectrophotometer (Cary 60 Bio, UV-Vis, Malaysia). Trolox was used to create a standard curve and the results were expressed as mg of trolox equivalents per g of sample dry weight (mg TE/g DW).

c) Ferric reducing antioxidant power (FRAP)

FRAP assay was implemented as the method of Benzie and Strain (1996) [30]. A working mixture was prepared by mixing in ratio 10:1:1 of 300 mM acetate buffer, 10mM tripyridil-s-triazine (TPTZ) in 40 mM HCl and 20 mM FeCl3. The mixture was warmed at 37 °C before using for experiments. For FRAP assay analysis, 2.85 mL of the mixture was added in 0.15 mL of sample, then left in a dark room at RT for 30 min before measuring the absorbance at 593 nm using a UV-Vis system (Cary 60 Bio, UV-Vis, Malaysia). The results of FRAP assay were showed as mg of trolox equivalents per g of dry weight of sample (mg TE/g DW).

6) Statistical analysis

All experiments were implemented in triplicate. The data analysis was conducted by using ANOVA in SPSS Statistics 25 software with a P value less than 0.05 (P<0.05) being considered significant. All mean comparisons of were presented with ± standard deviation (SD) and conducted with LSD Post Hoc Test.

III. Results and Discussion

A. Effect of extraction temperature on polyphenol yield and antioxidant activity

The impact of temperature on polyphenol yield is presented in the Fig. 1. The total phenolic and flavonoid compounds increased when the extraction temperature increased from 40 to 100 °C, and achieved the highest level at 100 °C (19.58 ± 0.59 GAE/g DW and 18.20 ± 0.35 mg CE/g DW, respectively). It could be explained that hot temperature is a dominant factor in enhancing the release of phenolic compounds inside the cells of samples [17,18]. While the caffeine content remained relatively unchanged from 90 to 100 °C, the chlorogenic acids slightly decreased. This may be because chlorogenic acids were degraded by the hot water [19]. The results indicated that phenolic compounds were retained more when higher temperature was applied. There was a similar result in the study of Andueza et al., more caffeine was obtained when the temperature of extraction was 98 °C while the level of chlorogenic acid was slightly decreased when the temperature increased from 88 to 98 °C [20].

Fig. 2 shows the effect of temperature on the radical scavenging capacity of pulp coffee extracts. As shown (Fig. 2), all the ABTS, DPPH radical scavenging activities and FRAP increased with the increasing temperatures. At 100 °C, highest scavenging activities were obtained with ABTS (37.34 ± 0.03 mgTE/g DW) and FRAP (35.75 ± 1.92mg TE/g DW), followed by DPPH at 90 °C (3.99 ± 0.02 mg TE/g DW). These results followed the same trend with the effect of temperature on polyphenols. Several previous studies have also shown that temperatures has a significant effect on the total phenolic compounds in extracts and their antioxidant activities [13, 21-23].

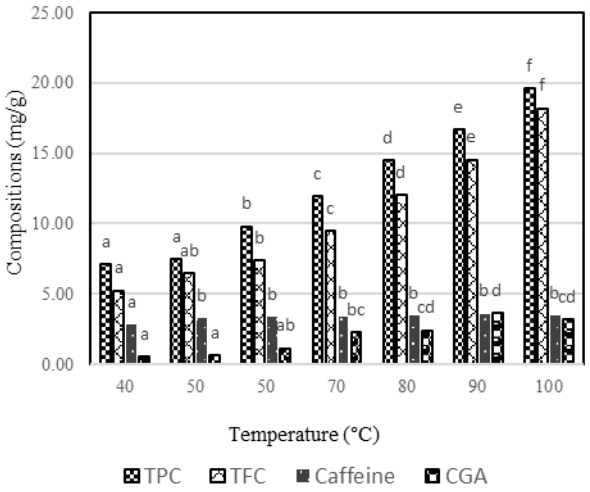

Fig. 1. The effect of temperature on TPC, TFC, caffeine and CGA of coffee pulp extraction.
The bars are presented as means (n = 3). Means with various letters (a to f) on top of the column show significant differences (P < 0.05)

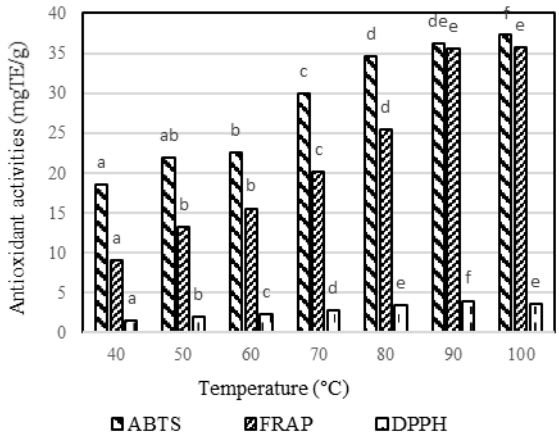

Fig. 2. The effect of temperature on antioxidant capacity of coffee pulp extraction.
The bars are presented as means (n = 3). Means with different letters (a to f) on top of the column show significant differences (P < 0.05).

B. Impact of extraction time on the total phenolic compounds and antioxidant capacity

Results presented in Fig. 3 and Fig. 4 show the influence

of extraction time on the total bioactive compounds from the coffee pulp. Highest levels of TPC (23.83 ± 1.07 mg GAE/g DW) and TFC 19.55 ± 2.83 mg CE/g DW) were achieved after 60 min. However, higher levels of caffeine (4.26 ± 0.49 mg) and chlorogenic acids (4.02 ± 0.13 mg) were obtained after 40 mins, beyond that (50 to 80 min), levels of these compounds reduced. Studies have shown that extraction time could affect the antioxidant levels of samples [17, 24, 25]. In this study, 60 min was selected as the ideal extraction time for the next experiments.

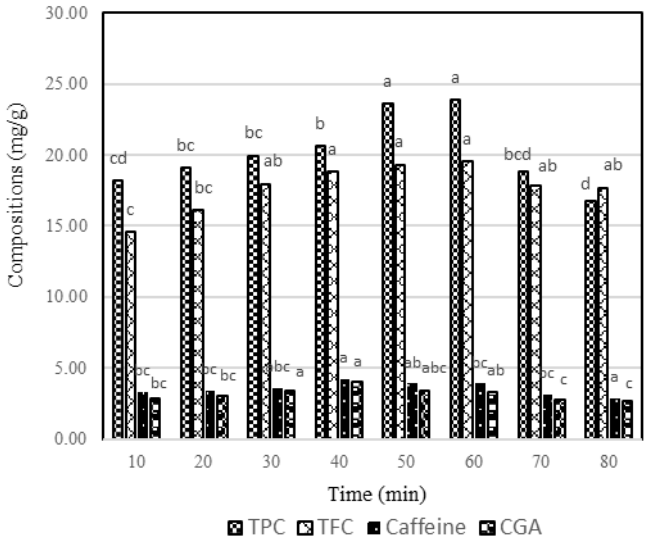

Fig. 3. Effect of time extraction to total phenolic compounds, TFC, caffeine and chlorogenic acids
The bars are presented as means (n = 3). Means with various letters (a to d) on top of the column show significant differences

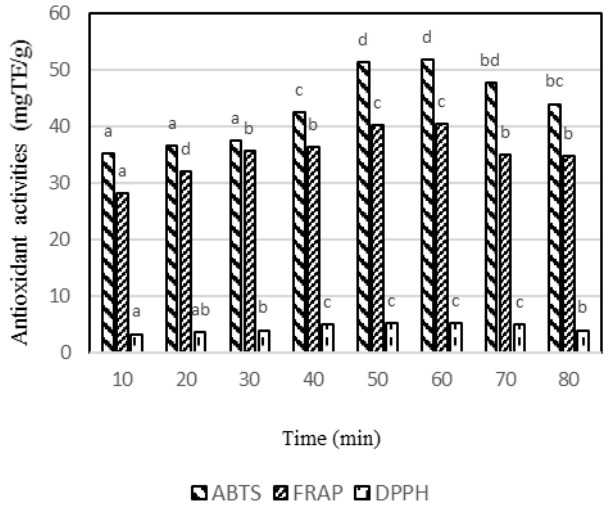

Fig 4. The effect of time extraction on antioxidant capacity of coffee pulp extraction.
The bars are presented as means (n = 3). Means with different letters (a to d) on top of the column show significant differences (P < 0.05).

C. Effect of the ratio of sample to water on bioactive compounds and antioxidants

The effect of ratio of dried coffee pulp to water is shown in Fig. 5 and Fig. 6. This factor affected the level of the total polyphenol concentration significantly. The maximum levels of bioactive compounds were extracted at a solid/solvent ratio of 1: 100 g/mL (dried coffee pulp to water), with extract levels decreasing with increasing amount of coffee pulp. The highest levels of TPC, TFC, caffeine and chlorogenic acids were also obtained when using a solid/solvent ratio of 1: 100 g/mL.

Antioxidant properties were also highest at the 1:100 g/mL solid/solvent ratio and reduced when the ratio of coffee pulp to water was increased. In the study of Pinelo et al., there was a decrease in yield when the material concentration was increasing [27]. Also, the same trend was observed in a previous study where catechins were extracted from green tea [13]. This was because the extraction yield based on mass transfer principle, when the polyphenol concentration gradient of samples inside and outside of cells are balanced, the extraction would be terminated. Therefore, the solid/solvent ratio 1: 100 g/mL was chosen as the optimal ratio for further study.

From the findings, this study recommends an optimal aqueous extraction conditions for recovery of phenolics or major bioactive compounds from coffee pulp were 100 °C, 60 min, and solid/solvent ratio 1: 100 g/mL. These optimal conditions were applied for further comparison with organic solvent conventional extraction.

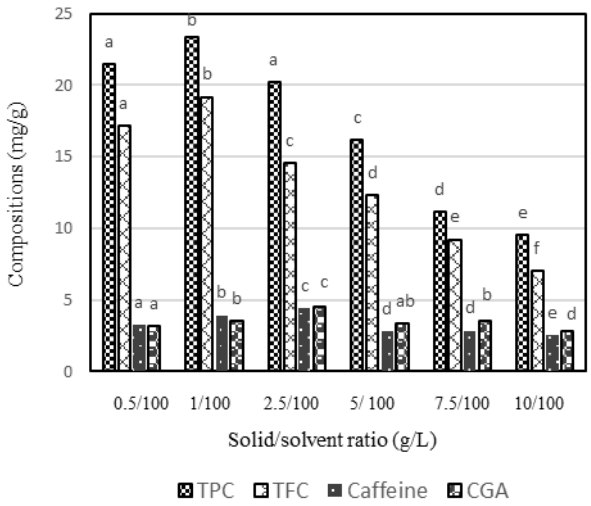

Fig. 5. Effect of solid/solvent ratio to total phenolic compounds, TFC, caffeine and chlorogenic acids.
The bars are presented as means (n = 3). Means with various letters (a to f) on top of the column show significant differences

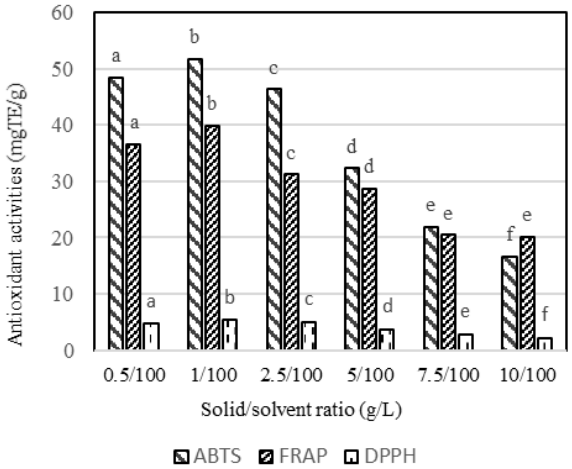

Fig. 6. The effect of solid/solvent ratio on the antioxidant capacity of dried coffee pulp.
The bars are presented as means (n = 3). Means with various letters (a to f) on top of the column show significant differences

TABLE I: COMPARISON OF OPTIMAL AQUEOUS EXTRACTION WITH ORGANIC SOLVENT EXTRACTIONS

Solvents	TPC (mg GAE/g DW)	TFC (mg CE/g DW)	Caffeine (mg/g DW)	CGA (mg/g DW)	ABTS (mg TE/g DW)	DPPH (mg TE/g DW)	FRAP (mg TE/g DW)
Methanol (100%)	6.74 ± 0.31[a]	4.80 ± 0.18[a]	1.71 ± 0.05[a]	1.14 ± 0.08[a]	15.05 ± 0.65[a]	1.26 ± 0.04[a]	9.73 ± 0.86[a]
Ethanol (100%)	2.94 ± 0.10[b]	2.49 ± 0.75[b]	0.41 ± 0.03[b]	0.15 ± 0.03[b]	4.20 ± 1.15[b]	0.36 ± 0.04[b]	1.61 ± 0.30[b]
Acetone (100%)	1.16 ± 0.40[b]	2.24 ± 0.43[b]	0.47 ± 0.14[b]	0.02 ± 0.02[b]	2.78 ± 0.34[b]	0.21 ± 0.02[b]	0.81 ± 0.17[b]
Methanol (50%)	16.88 ± 1.59[c]	12.21 ± 2.33[c]	3.33 ± 0.59[c]	1.58 ± 0.52[a]	43.61 ± 4.40[c]	4.11 ± 0.24[c]	20.52 ± 1.35[c]
Ethanol (50%)	17.75 ± 1.78[c]	12.85 ± 0.58[c]	4.10 ± 0.22[d]	2.48 ± 0.01[c]	50.68 ± 2.05[d]	4.58 ± 0.53[c]	26.07 ± 2.17[d]
Acetone (50%)	23.25 ± 3.21[d]	16.86 ± 1.92[d]	4.32 ± 0.24[d]	3.03 ± 0.18[d]	51.11 ± 0.49[d]	6.32 ± 0.42[d]	36.79 ± 4.99[e]
Water	6.68 ± 0.68[a]	4.78 ± 0.16[a]	1.16 ± 0.15[e]	0.04 ± 0.10[b]	24.71 ± 0.53[e]	1.56 ± 0.04[a]	1.57 ± 0.33[b]
Hot water	23.39 ± 1.06[d]	19.18 ± 0.48[e]	3.92 ± 0.11[d]	3.54 ± 0.11[e]	51.67± 0.60[d]	5.31 ± 0.06[e]	39.92 ± 0.81[f]

Data were presented as means ± SD (n = 3). Means with various letters in the same column show significant differences (P < 0.05).

D. Comparisons of optimal aqueous extraction with organic solvent extractions

To compare the TPC, TFC, caffeine and chlorogenic acids levels, dried coffee pulp was extracted with methanol, ethanol, acetone and their combination with water (50% v/v). The results are shown in Table I.

Results showed that optimal aqueous (hot water) extraction had similar levels of TPC, TFC, caffeine, and ABTS as compared to those of 50% acetone extract; and had significantly higher levels of all tested bioactive compounds and antioxidant properties in comparison with other tested organic solvent extracts. The higher recovery with hot water can be explained by the hydrophobicity of the majority of phenolic compounds in the samples [27]. These results are supported by a previous study by Vuong et. al, which found that water was more effective than acetone, ethanol and methanol for the extraction of phenolics in Carica papaya leaf [24]. Also, Pham et. al reported that water was the optimal solvent for extraction of phenolic compounds from H. hirsuta L. leaves [28]. The samples with higher level of TPC and TFC showed that their radical scavenging capacities were higher than those with lower TPC and TFC. From this study, it can be concluded that hot water is the most suitable solvent to extract phenolic and flavonoid compounds from dried coffee pulp.

IV. CONCLUSION

The study further confirmed that extraction parameters, including temperature, length of extraction, solid/solvent ratio and type of solvent significantly affected the extraction efficiency of bioactive compounds from samples, including coffee pulp. This study also indicated that extraction of coffee pulp using hot water under optimal conditions was more effective than other organic solvent extractions. Since water, an environmental friendly solvent, was effective for the extraction of phenolics and major bioactive compounds from dried coffee pulp, this study recommended the recovery of phenolics and major bioactive compounds under aqueous conditions of 100 °C, 60 min, and solid/solvent ratio 1: 100 g/mL for further applications.

ACKNOWLEDGMENT

The authors sincerely acknowledge the funding support from the Viet Nam International Education Development – Ministry of Education and Training (Project 911) and the University of Newcastle for awarding a VIED-TUIT to Thy Minh Kieu Tran.

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