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Submission date: 02-Sep-2019 07:56AM (UTC-0700)
Submission ID: 1166259193
File name: 2708_pdf.pdf (421.1K)
Word count: 4463
Character count: 23490

Journal of Applied Pharmaceutical Science Vol. 8(08), pp 138-143, August, 2018
Available online at http://www.japsonline.com
DOI: 10.7324/JAPS.2018.8819
ISSN 2231-3354
BY-NC-SA

Application of Natural Deep Eutectic Solvent-Based Ultrasonic Assisted Extraction of Total Polyphenolic and Caffeine Content from Coffe Beans (*Coffea Beans* L.) For Instant Food Products

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ARTICLE INFO

Article history:
Received on: 09/05/2018
Accepted on: 12/07/2018
Available online: 31/08/2018

Key words:
Caffeine content, *Coffea arabica* L, natural deep eutectic solvent, total polyphenolic content, ultrasonic-assisted extraction.

ABSTRACT

This study aims to determine and obtain the total polyphenolic and caffeine content from coffee beans (*Coffea arabica* L.) with some combination of factors. The extraction process was performed using natural deep eutectic solvent-based ultrasonic-assisted extraction (NADES-UAE) method with the different condition including extraction time, NADES ratio, and liquid-solid ratio. The total polyphenolic content was calculated using a microplate reader 96 well. The caffeine content was examined using high-performance liquid chromatography. Based on the results demonstrated the effect of different NADES components (namely lactic acid-sucrose and citric acid-glucose) on the total polyphenolic and caffeine content. The highest total polyphenolic content was 87.01 mg GAE/g sample (2 g/g lactic acid-sucrose ratio and 10 mL/g liquid-solid ratio for 15 minutes) and 62.91 mg GAE/g sample (5 g/g citric acid-glucose ratio and 15 mL/g liquid-solid ratio for 15 minutes). Whereas, the highest caffeine content was 4.45 mg/g (4 g/g lactic acid-sucrose and 15 mL/g liquid-solid ratio for 35 minutes) and 1.30 mg/g (2 g/g lactic acid-sucrose and 30 mL/g liquid-solid ratio for 5 minutes), respectively. These results were obtained from the green extraction method with rapid, easy, inexpensive, and environmentally friendly.

INTRODUCTION

Indonesia is one of the largest coffee producer countries in the world (Rahardjo, 2012). Coffee beans sold in the world market is usually a combination of two types of plants consisting of arabica and robusta (Rahardjo, 2012). The difference between both species mainly in the taste and level of caffeine content. Arabica coffee tends to be more expensive, softer, and lower caffeine levels compared to robusta types (Njoroge *et al.*, 2005).

Coffee beans (*Coffea arabica* L.) was used as a sample, and these species contain rich a polyphenolic compound mainly chlorogenic acid (Amorim *et al.*, 2009; Baeza *et al.*, 2016). Polyphenolic compounds in coffee beans have efficacy as weight loss with the modulation of glucose metabolism in humans body (Kozuma *et al.*, 2005; Blum *et al.*, 2007), it also has activity as antihypertension (Watanabe *et al.*, 2006). Coffee is also famous for its caffeine content (Zhang *et al.*, 2013). Caffeine is a derivative of the xanthine alkaloids present in coffee and has a stimulating effect on the body (Xia *et al.*, 2015). To be utilized more widely in the health field mainly for treatment, secondary metabolite compounds (especially polyphenols and caffeine groups) need to be separated using the appropriate extraction method. To extract polyphenols and caffeine from coffee beans requires the right solvent for the target compound to be optimally separated.

The application of green chemistry principles to the extraction of target secondary metabolite from plants continues to rise (Kerton, 2009). The use of a natural deep eutectic solvent (NADES) is one of the principles of green chemistry, the selection of the right solution is crucial to success in the extraction process (Kerton, 2009; Dai *et al.*, 2016). From the standpoint of biochemical and pharmaceutical study, the development of new and green methods for the extraction of bioactive compounds

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from a natural product is essential (Chemat and Vian, 2014). NADES has some advantages over conventional solvents that are inexpensive, environmentally friendly, and food grade, so it is safe to consume (Dai *et al.*, 2015). The use of NADES as a green solvent is expected that the obtained extracts are directly used to prepare the finished product and are not directed to the isolation and purification of the compound. In this process can be applied with the desired finished product is granule or instant granule and another type of food product. In addition, NADES has also been recognized as a new class as a sustainable solvent as it is made up of widely available, natural, non-toxic and biodegradable components (Fernández *et al.*, 2017; Pan *et al.*, 2017). Some studies has been reported that the use of NADES as a green solvent combined with nonconventional extraction methods (including ultrasonic, microwave, supercritical, and other) to extract the target secondary metabolite such as baicalin (Wang *et al.*, 2018), flavonoid (Wei *et al.*, 2015), phenolic (Wei *et al.*, 2015; Fernández *et al.*, 2017), holo-cellulose, α-cellulose, acid-insoluble-lignin (Pan *et al.*, 2017), anthocyanins (Dai *et al.*, 2016), and so on. However, extraction of polyphenolic and caffeine content from coffee beans has not been reported.

In this study, the extraction of polyphenolic and caffeine content from coffee beans was performed using NADES consisting of lactic acid-sucrose and citric acid-glucose components combined with ultrasonic-assisted extraction (NADES-UAE) method based on the parameter factors of extraction condition. This study aims to determine and obtain the total of polyphenolic and caffeine content with some combination factors of extraction conditions such as extraction time, NADES ratio, and liquid-solid ratio.

MATERIALS AND METHODS

Materials

The sample of coffee beans was obtained from Gayo Coffee shop at Takengon, Central Aceh, Indonesia. Caffeine standard was purchased from CSPC, China. Citric acid, lactic acid, sucrose, glucose, and aqua demineralization were purchased from Brataco, Indonesia. Methanol HPLC grade was obtained from PT. Merck, Germany (via PT. Elo Karsa Utama, Indonesia). Gallic acid standard, Folin-Ciocalteu reagent, and sodium carbonate were purchased from Sigma-Aldrich, USA (via PT. Elo Karsa Utama, Indonesia).

Equipment

The equipment was used in this study, such as ultrasonic bath type (Krisbow) modified for as ultrasonic assisted extraction, microplate reader 96 well spectrophotometer UV-VIS (VersaMax™ ELISA Microplate Reader, USA), micropipette 10-100 and 100-1000 µL (Thermo Scientific, USA), vortex mixer (WiseMix), centrifuge (Labsco, USA), high performance liquid chromatography (Shimadzu, Japan), column C_{18} 4.6 mm × 150 mm, 5 µm (Shimpack), pH meter (Eutech Instrument).

Preparation of Natural Deep Eutectic (NADES) solvent

In this study, NADES was used include lactic acid-sucrose with ratio 4:1, 5:1, and 6:1 g/g and citric acid-glucose with ratio 1:1, 2:1, and 3:1 g/g, respectively. The NADES components were weighed according to the predefined ratio and were added aqua demineralization, then was stirred using hotplate stirrer and then filtered to obtain a homogeneous solution (Dai, van Spreonsen *et al.*, 2013; Dai, Witkamp *et al.*, 2013).

Extraction process

A natural deep eutectic solvent-based ultrasonic-assisted extraction (NADES-UAE) method was applied to extract polyphenolic and caffeine content based on the literature (Wei *et al.*, 2015; Dai *et al.*, 2016). Briefly, the dried powder of coffee beans (1 gram) was mixed with NADES solution. The mixture solution was vortexed then extracted using NADES-UAE method which some combinations factor of extraction condition. After the extraction process, the extract solution and residue were separated using Buchner funnel. The extract solution was stored at room temperature and until ready to use.

Determination of total polyphenolic content

The total polyphenolic content determination was conducted by microplate reader 96 well method according to previous studies (Bobo-García *et al.*, 2014; Ahmad *et al.*, 2017a; Ahmad *et al.*, 2017b). Briefly, the extract or standard solution (20 µL) was added to 100 µL of 25% Folin-Ciocalteu reagent, homogenized and allowed for 4-6 minutes. Then sodium carbonate solution (75 µL) was added, homogenized and incubated for 2 hours at room temperature in the dark. The measurement of absorbance was performed using microplate reader 96 spectrophotometer UV-VIS (VersaMax™, USA) at a 750 nm wavelength. Gallic acid with concentrations from 12.5 to 200 µg/mL were used as a standard (Ahmad *et al.*, 2017a) and the regression formula was $Y = 0.0158X + 0.2064$ with $R = 0.9969$, where Y is concentration and X is absorbance. The equation was used to determine the TPC from each extract.

Determination of caffeine content

Determination of caffeine content was performed using high-performance liquid chromatography (HPLC) based on the study has been reported by (Ali *et al.*, 2012), with modification. The HPLC separation was performed using column C_{18} 4.6 mm × 150 mm, 5 µm (Shimpack), methanol: aqua demineralization (30:70) of the mobile phase, and detector UV-Vis at 270 nm. The sample injection of extract or standard solution was set at 20 µL with a flow rate of 1.3 mL/minutes. The retention time of caffeine was used as a standard based on peak area of chromatogram with some different concentration include 0.2, 0.4, 0.8, 1, and 1.2 µg/mL, respectively. The equation formula was obtained $Y = 63537X + 36507$ with $R = 0.99993$ and was applied to calculate the caffeine content in each extract, where Y is concentration and X is peak area.

RESULTS AND DISCUSSION

Extraction process

In this study, the development of extraction process based on the approach of green chemistry principles to obtain an efficiency of extraction conditions with the optimum yields of secondary target metabolite from the matrix sample. Extraction condition refers to the research that has been reported by (García *et al.*, 2016; Fernández *et al.*, 2017). Combination factors of

extraction condition were used in this study including NADES ratio, liquid-solid ratio, and extraction time. Extraction of coffee beans was performed using NADES with lactic acid-sucrose and citric acid-glucose compositions combined with ultrasonic-assisted extraction (NADES-UAE) and controlled temperature at 40°C to prevent degradation of compounds, mainly polyphenolic content (Wei *et al.*, 2015; Wei *et al.*, 2015; Fernández *et al.*, 2017).

In the present study, extraction of secondary metabolite (mainly polyphenolic and caffeine content) from green coffee beans was performed using NADES-UAE method. The selection of extraction method was based on the time efficiency, the amount of solvent used, the increase of surface area of the sample, the cost efficiency, and prevent the degradation of the various compounds. This method was chosen because of its rapid and comfortable for use, inexpensive, environmentally friendly, and available in the laboratory. Also, the process is suitable for the extraction of polyphenolic compounds that are vulnerable to oxidization when exposed to high light and temperature and may also increase the yield of secondary metabolite. Sonication is the production of sound waves that create a cavitation wavelength near the sample tissue, which disrupts the cell wall of the plant so that it releases the compounds contained in the cells (Xu *et al.*, 2015; Pham *et al.*, 2017). The efficiency of extraction using ultrasonic-assisted extraction method was influenced by time, power, and temperature (Bubalo *et al.*, 2016; Fernández *et al.*, 2017).

Some factors must be considered in the extraction process using the UAE method, i.e., extraction time, solvent combination ratio, power irradiation, extraction temperature, and sample-solvent ratio. In this study, the extraction process was conducted using 3 factors and 3 levels (including low, medium, and high) with 27 total combinations of extraction condition against total polyphenol and caffeine content. The parameter factors consisted of NADES ratio, the liquid-solid ratio, and extraction time, as can be seen in Table 1.

Table 1: Experimental design according to the combination of extraction condition factor using natural deep eutectic solvent based ultrasonic-assisted extraction (NADES-UAE).

No.	Parameters factor	Unit	Lactic acid - sucrose			Citric acid - glucose		
			Low	Medium	High	Low	Medium	High
1	NADES ratio	g/g	4	5	6	1	2	3
2	Liquid-Solid Ratio	mL/g	15	45	75	10	20	30
3	Extraction Time	Minutes	15	35	60	5	10	15

Table 2: Results of 10 highest total polyphenolic content of coffee beans extract obtained using lactic acid-sucrose as a solvent.

No.	Factor A: Lactic acid-sucrose ratio (g/g)	Factor B: Liquid-solid ratio (mL/g)	Factor C: Extraction time (Minutes)	Response: Total polyphenolic content (mg GAE/g)
1	2	10	15	87.01
2	1	20	15	84.43
3	3	20	15	68.73
4	2	20	10	64.54
5	2	30	5	64.50
6	1	20	5	63.57
7	2	30	15	62.41
8	3	10	10	56.48
9	3	20	5	56.23
10	2	10	5	53.69

Lactic acid-sucrose (with a ratio of 1:1, 2:1, and 3:1 g/g) and citric acid-glucose (4:1, 5:1, dan 6:1 g/g ratio) were selected as the following component of NADES. Then was added with aqua demineralization that aims to reduce viscosity and accelerate NADES preparation. Lactic acid and citric acid act as hydrogen bond acceptor (HBA), while sucrose and glucose act as hydrogen bond donor (HBD) (Lu *et al.*, 2016; Fernández *et al.*, 2017; Wang *et al.*, 2018).

Total polyphenolic content determination

Table 2 and Table 3, shows the use effect of NADES as a solvent (lactic acid-sucrose and citric acid-glucose) with NADES-UAE method based on a combination of extraction condition against total polyphenolic content. Based on the results in Table 2 was achieved the 10 highest of total polyphenolic content using some combination factor of extraction condition and the best value of 87.01 mg GAE/g sample with 2 g/g of lactic acid-sucrose ratio and 10 g/mL of the liquid-solid ratio for 15 minutes. While, in Table 3 was obtained the 10 highest of total polyphenolic content using some combination factor of extraction condition and the best value of 62.91 mg GAE/g sample using 5 g/g of citric acid-glucose ratio and 15 mL/g of the liquid-solid ratio for 15 minutes of extraction time.

The highest total polyphenolic content of 87.01 mg GAE/g sample were obtained using NADES with components of a combination of lactic acid and sucrose (2:1 g/g), the liquid-solid ratio of 10 mL, and extraction time of 5 minutes. The result has a higher total polyphenolic content than the study has been reported by (Hečimović *et al.*, 2011) with TPC of 33.12 mg GAE/g sample from coffee beans based on the degree of roasting. And on the other hand, the study has been reported by (Xu *et al.*, 2015) that the extraction using the optimum condition of the subcritical water

extraction (SWE) method has approximately the same level of 86.23 mg GAE/g sample. The use of lactic acid and sucrose as a component of NADES is higher than the use of citric acid and glucose with the highest total polyphenolic content of 62.91 mg GAE/g sample.

Table 3: Results of 10 highest total polyphenolic content of coffee beans extract obtained using citric acid-glucose as a solvent.

No.	Factor A: Citric acid-glucose ratio (g/g)	Factor B: Liquid-solid ratio (mL/g)	Factor C: Extraction time (Minutes)	Response: Total polyphenolic content (mg GAE/g)
1	5	15	15	62.91
2	5	15	60	61.86
3	6	15	35	49.09
4	4	15	35	64.54
5	4	45	15	30.27
6	4	45	60	29.34
7	6	45	60	28.26
8	6	45	35	27.75
9	6	45	15	24.10
10	5	75	15	22.46

Table 4: Results of the 10 highest caffeine content of coffee beans extract obtained using lactic acid-sucrose as a solvent.

No.	Factor A: Lactic acid-sucrose ratio (g/g)	Factor B: Liquid-solid ratio (mL/g)	Factor 3: Extraction time (Minutes)	Response: Caffeine content (mg/g)
1	2	30	5	1.30
2	2	30	15	0.73
3	3	20	15	0.69
4	2	20	10	0.66
5	1	30	10	0.62
6	3	20	5	0.61
7	1	20	15	0.58
8	3	30	10	0.52
9	2	10	5	0.48
10	3	10	10	0.48

Table 5: Results of the 10 highest caffeine content of coffee beans extract obtained using citric acid-glucose as a solvent.

No.	Factor A: Citric acid - glucose ratio (g/g)	Factor B: Liquid-solid ratio (mL/g)	Factor 3: Extraction time (Minutes)	Response: Caffeine content (mg/g)
1	4	15	35	4.45
2	6	15	35	4.21
3	5	15	60	3.86
4	4	45	15	3.22
5	5	45	35	2.91
6	5	15	15	2.82
7	6	45	15	2.70
8	6	45	60	2.55
9	5	75	60	2.04
10	6	75	35	1.53

Caffeine content determination

Table 4 and Table 5, demonstrated the use effect of NADES as a solvent (lactic acid-sucrose and citric acid-glucose) with NADES-UAE method based on a combination of extraction condition against caffeine content. The 10 highest of caffeine content in beverages extract was obtained in samples using lactic acid-sucrose as a solvent (as can be seen in Table 4) and citric acid-glucose (showed in Table 5). According to the combination factors of extraction condition was obtained the best caffeine content (4.45 mg/g sample) with combination factor of 4 g/g citric acid-glucose, 15 mL/g liquid-solid ratio, and 35 minutes extraction time (in Table 5) and 1.30 mg/g sample using 2 g/g lactic acid-sucrose ratio and 30 mL/g liquid-solid ratio for 5 minutes (in Table 4). Figure 1 demonstrated the chromatogram feature for the best value of extract based on difference NADES component used and

the extraction condition compared with caffeine standard (1.2 µg/mL).

The highest caffeine content (4.45 mg/g sample) was obtained using citric acid and glucose with a ratio of 5:1 g/g and the liquid-solid ratio of 15 mL/g for 35 minutes. This result was still relatively higher than extraction with roasting (0.06-2.25% mg/g) based on the study has been reported by (Hečimović *et al.*, 2011) and (Belguidoum *et al.*, 2014), while the result was higher than extraction using lactic acid and sucrose with the most top caffeine content of 1.30 mg/g sample (combination factors of extraction condition such as 2 g/g NADES ratio and 30 mL/g liquid-solid ratio for 5 minutes).

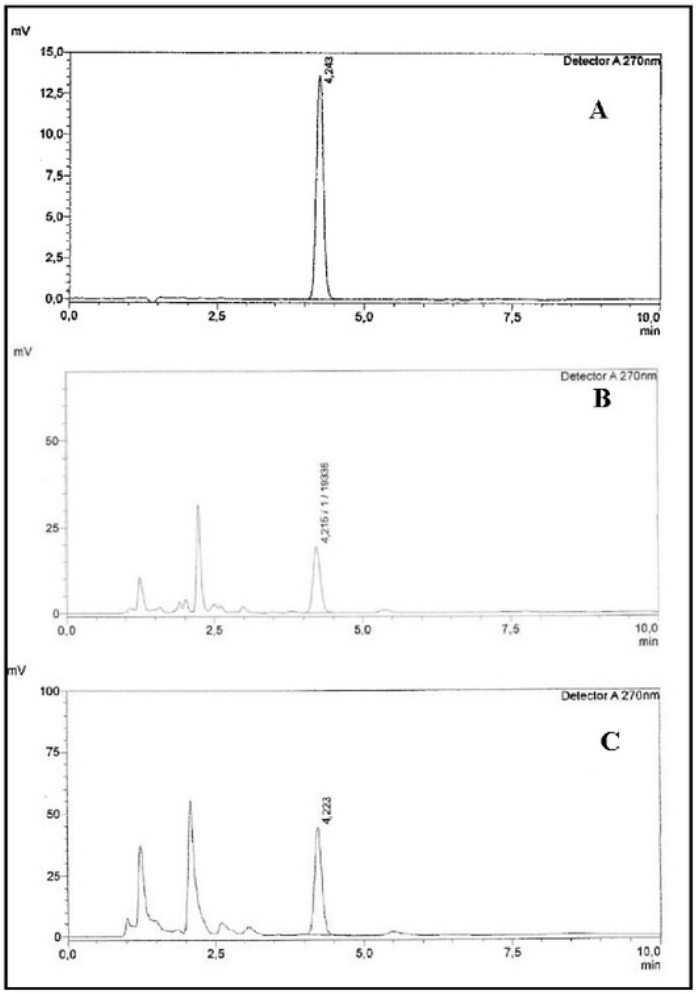

Fig. 1: Chromatogram feature of (**A**) caffeine standard (1.2 µg/mL), extracts from the solvent of lactic acid-sucrose (**B**) and citric acid-glucose (**C**).

Based on the obtained of total polyphenolic and caffeine content demonstrated that the use of different NADES components indicated the specificity of the extracted target compounds and was strongly influenced by some combination of extraction condition. These results were preliminary data for further research that focus on optimization of NADES-UAE method based on various extraction conditions using response surface methodology and aims to obtain an extract with maximum purity of the target compound from the coffee beans.

CONCLUSION

Based on the above results, the application of NADES-UAE method showed the differences specificity of the extracted compounds from coffee beans according to the differences NADES components. The highest total polyphenolic content of 87.01 mg GAE/g sample was obtained using NADES components of 2 g/g lactic acid-sucrose ratio and 10 mL/g liquid-solid for 5 minutes. While, the highest caffeine content of 4.45 mg/g was obtained using citric acid-glucose ratio of 5 g/g, liquid-solid of 15 mL/g, and extraction time of 35 minutes. These results were preliminary data for further research and obtained the green extraction method with rapid, easy, inexpensive, and environmentally friendly.

ACKNOWLEDGMENT

This work supported by the Directorate of Research and Community Engagement (DRPM) Universitas Indonesia via

Hibah PITTA 2018.

CONFLICT OF INTEREST

All authors have declared no conflict of interest.

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How to cite this article:
Ahmad I, Pertiwi AS, Kembaren YH, Rahman A, Mun'im A. Application of Natural Deep Eutectic Solvent-Based Ultrasonic Assisted Extraction of Total Polyphenolic and Caffeine Content from Coffe Beans (*Coffea Beans* L.) For Instant Food Products. J App Pharm Sci, 2018; 8(08): 138-143.

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