Effect of Amplitude on Ultrasonic Assisted Extraction of Caffeic Acid from AndrographisPaniculata

Vi Vien Chia, Sook Fun Pang, JoliusGimbun, Sureena Abdullah, Mashitah M. Yusoff

ABSTRACT: ***Andrographispaniculata belongs to Acanthaceae family is often used as a traditional herbal medicine for treatment of diabetes and hypertension owing to the presence of medically useful bioactive compounds. The bioactive compounds from plant material must be extracted before it can be formulated as functional food. This paper presents the effect of sonication amplitude on the yield of caffeic acid extraction from A. paniculata. At first, the effect of particle size, solvent type and solid to solvent ratio was studied using a one-factor-at-time method in an ultrasonic assisted extraction. The extracts were quantified and identified using ultra-performance liquid chromatography coupled with photodiode array detector. The highest yield of caffeic acid (5.53 mg/g DW) was found using the particle size of 125-250 µm, solvent made of 50% aqueous ethanol and the solvent concentration, solid solvent ratio of 0.05g/mL and at sonication amplitude of 50%. Findings from this work may provide a useful guide to enhance extraction of caffeic acid from A. paniculata.***

Keywords: AndrographisPaniculata, ultrasonic extraction, amplitude, caffeic acid

I. INTRODUCTION

Andrographispaniculata(King of Bitter) has attracted wide attention due to it is being vastly used by local people from Southeast Asia countries for traditional medication. *A. paniculata*has been labelled as a miraculous herbal product as it exhibits a broad spectrum of pharmaceutical activities (Hossain, Urbi, Sule, & Rahman, 2014). Specifically, Malaysian used *A. paniculata* to treat diabetes and hypertension (Jarukamjorn & Nemoto, 2008;Zhang & Tan, 2000). *A. paniculata* is also used for prevention and treatment of the common cold in Asia and Scandinavia (Thisoda *et al*., 2006).

Generally, *A. paniculata*contains a huge amount of bioactive compounds categorized under terpene group such as andrographolide, neoandrographolide, andrograpanin(Akbar, 2011). According to Praveen*et al*.(2014), this plant also contains phenolic compounds such as gallic acid, protocatechuic acid, β-resorcylic acid, chlorogenic acid.

Various extraction approaches have been applied to extract the bioactive compounds of *A. paniculata*. Among all, ultrasonic assisted extraction (UAE) allow a shorter extraction time and lesser solvent to extract the polyphenolic compounds compared to conventional method such as maceration (Jovanov *et al*., 2017). UAE is known to improve both the internal and external mass transfer which facilitates a greater extraction yield. The effect of sonication amplitude to the yield of caffeic acid extraction from *A. paniculata* via UAE is not yet well understood, and hence this is the aim of the current work. The polyphenols from the extract was then identified and analyzed via a sensitive, rapid and reliable ultra-performance liquid chromatography coupled with photodiode array detector (UPLC-PDA).

II. MATERIALS AND METHOD

The caffeic acid standard was obtained from Sigma-Aldrich (St.Louis, MO, USA). HPLC grade of acetonitrile, LCMS grade water and analysis grade ethanol were purchased from Fisher Scientific (Pittsburgh, PA). HPLC grade formic acid was purchased from Merck (Darmstadt, Germany). The*A. paniculata*plant was collected from Sungai Lembing, Pahang, Malaysia. The plant was washed thoroughly using deionized water before dried under open air and then oven-dried at the temperature of 40°C until no significant change in weight was observed. The dried raw material was finely ground and sieved into different types of particle size using an analytical sieve shaker. In addition, the dry *A. paniculata* was then checked for its moisture content using a moisture analyzer (AND MS-70, Japan).

The extracts were prepared using QsonicaSonicators Q700 equipped with a standard ultrasonic probe model CL-334(Newton, USA). Firstly, the effect of particle size of the powdered *A. paniculata* was studied using five size classes, i.e. <125µm, 125-250µm, 250-500µm, 500-800µm and >800µm. Then, the best solvent used to extract sample was determined using 0, 20, 50, 70 and 100% ethanol. Subsequently, the optimum solid to solvent ratio was studied at 0.01, 0.02, 0.05, 0.10 and 0.15g/mL. Finally, the extraction at different amplitude (50, 60, 70, 80 and 90 %)

Revised Manuscript Received on February 14, 2019.

ViVien Chia, Centre of Excellence for Advanced Research in Fluid Flow (CARIFF), Universiti Malaysia Pahang, 26300 Gambang, Pahang, Malaysia. Faculty of Chemical & Natural Resources Engineering, Universiti Malaysia Pahang, 26300 Gambang, Pahang, Malaysia.

Sook Fun Pang,Centre of Excellence for Advanced Research in Fluid Flow (CARIFF), Universiti Malaysia Pahang, 26300 Gambang, Pahang, Malaysia. Faculty of Industrial Sciences & Technology, Universiti Malaysia Pahang, 26300 Gambang, Pahang, Malaysia.

JoliusGimbun, Centre of Excellence for Advanced Research in Fluid Flow (CARIFF), Universiti Malaysia Pahang, 26300 Gambang, Pahang, Malaysia. Faculty of Chemical & Natural Resources Engineering, Universiti Malaysia Pahang, 26300 Gambang, Pahang, Malaysia.

Sureena Abdullah, Centre of Excellence for Advanced Research in Fluid Flow (CARIFF), Universiti Malaysia Pahang, 26300 Gambang, Pahang, Malaysia. Faculty of Chemical & Natural Resources Engineering, Universiti Malaysia Pahang, 26300 Gambang, Pahang, Malaysia.

Mashitah M. Yusoff, Faculty of Industrial Sciences & Technology, Universiti Malaysia Pahang, 26300 Gambang, Pahang, Malaysia

were performed using the predetermined best particle size, solvent type as well as the solid to solvent ratio for 10 minutes. The supernatant was then separated by centrifugation (Eppendorf 5810 R, Germany) at 10000 rpm for 15 minutes to obtain a clear solution. The plant extract was stored in a -80°C freezer until further use.

The identification and quantification of the polyphenol constituent was performed by Waters ACQUITY UPLC System equipped with the ACQUITY UPLC PDA Detector operated via Waters Empower 3 software. The mobile phases consisted of 0.1% formic acid in ultrapure water (Solvent A), 0.1% formic acid in acetonitrile (Solvent B), 20% of acetonitrile (Solvent C) and 100% of acetonitrile (Solvent D). Separation was achieved using Luna® Omega 1.6µm C18 column with a dimension of 100 x 2.1 mm. The runtime for a sample was 18 minutes with an injection volume of 5µL and flow rate at 0.15 mL/min at room temperature. A linear gradient of UPLC solvents was determined and the performed elution scheme were: 0-1 min, 78% A; 1-3.5 min, 77% A; 3.5-4.5 min, 75% A; 4.5-5.0 min, 73% A; 5.0-6.0 min, 70% A; 6.0-8.0 min, 69% A; 8.0-9.0 min, 68% A; 9.0-10.0 min, 65% A; 10.0-11.0 min, 60% A; 11.0-12.0 min, 55% A; 12.0-13.0 min, 50% A; 13.0-14.0 min, 5% A; 14.0-15.0 min, 5% A; 15.0-18.0 min, 78% A. The peak of caffeic acid in comparison with the standard was detected at 355 nm.

III. RESULTS AND DISCUSSION

Figure 1 shows the UPLC-PDA chromatogram of caffeic acid at the retention time of 2.155 minutes. The calibration curve was plotted as the function of the concentrations of standard caffeic acid versus their peak area. The six points calibration curve in the concentration range of 0.001mg/mL to 0.25mg/mL and shown good linearity with r^2 = 0.999. The concentration of caffeic acid in extract solutions were determined using the plotted calibration curve.

Effect of particle sizes: Figure 3shows the effect of various particle sizes of powdered*A. paniculata* on the yield of caffeic acid. It was found that the particle size ranged from 125-250µm gave the highest yield ofcaffeic acid (5.432 mg/g DW). Generally, a significant decline is seen as the particle

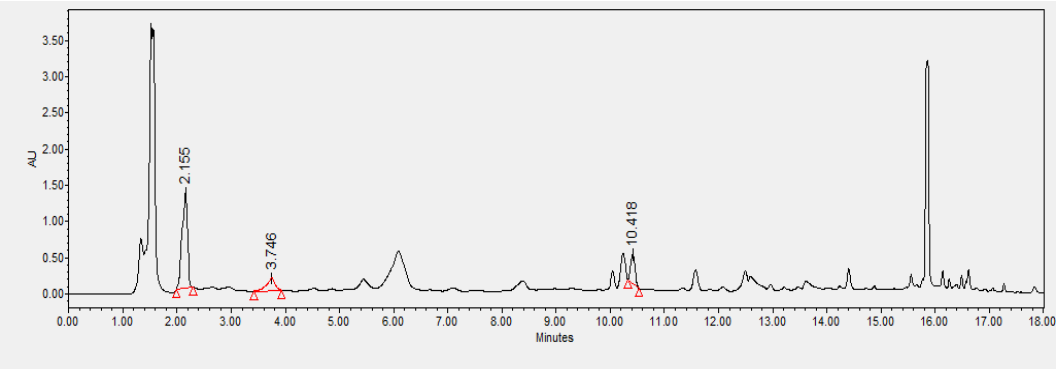

Fig. 1: UPLC-PDA chromatogram of caffeic acid at the retention time of 2.155 minutes

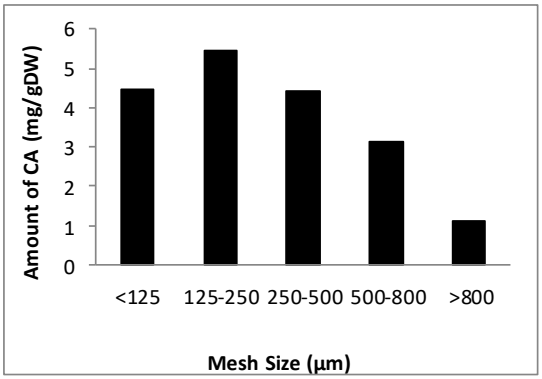

Fig. 2: Effect of particle size on yield of caffeic acid

size of *A. paniculata* is beyond 250µm and lowest yield was achieved at 800µm; 1.123 mg/gDW. This observation is due to the reduction of surface area available for the larger powder and hence less efficient mass transfer (extraction) process. The surface area per unit mass enhances with the decrease in particle size. Thus, smaller particle size affects the solvent-solute interactions by improving the contact of plant material with the solvent and offers a greater and active surface area per unit mass for mass transfer (Puspitasari, Martono, Pramono, & Widyarini, 2016).

However, the lowest particle size with very small fragments especially particle size of less than 125µm did not give the highest yield of the extract compounds. The finer particles tend to float on the solvent and causes the solvent have a hard time to pass through the sample during mixing and extraction process (Jovanovic et al., 2017). As a result, the extraction is less efficient. Meanwhile, large particle size also yield less desired compounds because of smaller surface area are available to allow the solvent to penetrate into the cell wall (Shi, 2015).

Effect of solvent concentration: Five different percentage of solvents were studied including 0 (water), 20, 50, 70 and 100% of ethanol. Ethanol was proposed in this study as many studies showed ethanol has the highest extraction efficiency towards the relatively high polarity of phenolic compounds (Thoo, Ng, Khoo, Wan Aida, & Ho, 2013). In an addition, polyphenol like caffeic acid is easily soluble in ethanol with its partially polar function of large hydrocarbon moiety and glycoside form of polyphenols (Jovanovic et al.,

2017). The interaction between plant material and solvent is enhanced by the presence of water in the organic solvent which creates a more polar medium and increases the effectiveness of swelling in plant material. However, free radicals may generate in high amount of water due to the ultrasound from UAE which resulted dissociation of water and cause an oxidative reaction to coexist with extraction (Rao & Rathod, 2015). Therefore, high amount of water shows a less optimum extraction yield of caffeic acid. The yield of caffeic acid by pure water (0.066 mg/g DW) is not as high as those of aqueous ethanol solvent (Figure 4). The yield of caffeic acid increased with the concentration of ethanol up to 50% and then began to decrease gradually at higher concentration. The best extraction solvent was achieved using 50% ethanol with the yield of 0.086 mg/g DW caffeic acid.

Effect of solid to solvent ratio: Figure 4 shows the effect of solid to solvent ratio on the yield of caffeic acid. It was observed that the highest yields were achieved at 0.01 g/mL (0.129 mg/g DW). Although there is no significant difference between 0.01 and 0.02 solid to solvent ratio but the amount of caffeic acid decline dramatically beyond 0.02g/mL solid solvent ratio. Thus, 0.01g/mL was selected as the optimum solid to solvent ratio. The highest yield of the compounds was observed at the lowest solid to solvent ratio and this can be due to the huge difference in concentration gradient between solid and solvent (Rao & Rathod, 2015). Thus, there is a higher mass transfer between the solid and solvent when there is lesser amount of solid available and in the meantime the volume of solvent is sufficient to immerse the entire sample during the extraction process. While, the lowest yield of caffeic acid was seen when larger amount of plant solid is available in the same amount of solvent and this condition tends to cause scattering of ultrasound waves (Rao & Rathod, 2015). As a result, lesser ultrasonic energy was transmitted into the plant material during extraction and eventually lesser bioactive compounds can be harvested (Jovanovic et al., 2017).

Effect of sonication amplitude: The best particle size (125-250µm), solvent concentration (50% ethanol) and solid solvent ratio (0.01 g/mL) from the initial screening were applied to study the effect of sonication amplitude. The energy releases by ultrasound are sufficient to disrupt the cell wall of the plant material and highest yield of the compounds can be achieved when the plant released its content into the solvent (Rao & Rathod, 2015). Generally, higher amplitude will generate a greater formation of the cavitation bubbles. However, the yield ofcaffeic acid decreases as the amplitude increases as shown in Figure 5. This can be explained as the studied compound is highly sensitive towards the surrounding heat whereby higher amplitude generates more heat during the extraction and eventually leads to oxidative degradation, thus resulted lower yield (Icyer et al., 2016). It was found that the 50% of amplitude provide a sufficient shear force to break the plant cell wall of *A. paniculata*and hence facilitating the release of caffeic acid.

CONCLUSION

The effect of particle size, solvent type, solid to solvent ratio and sonication amplitude on the caffeic acid extraction via UAE from *A. paniculata* was successfully elucidated. It was found that the highest caffeic acid yield (5.53 mg/g DW) was obtained using a particle size ranging from 125-250 µm,50% ethanol as solvent, solid to solvent ratio of 0.01 g/mL and sonication amplitude of 50%.

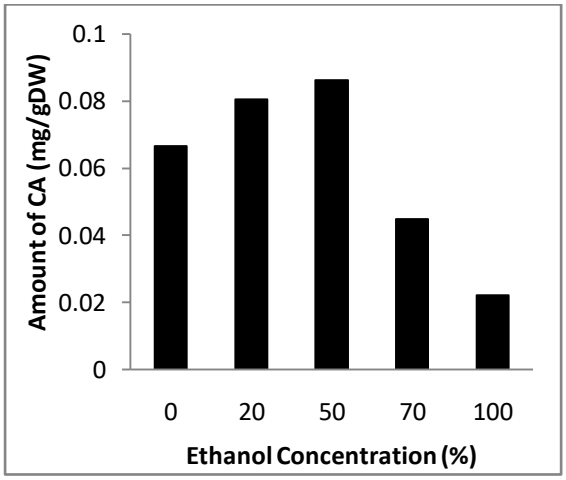

Fig. 3: Effect of solvent concentration on yield of caffeic acid

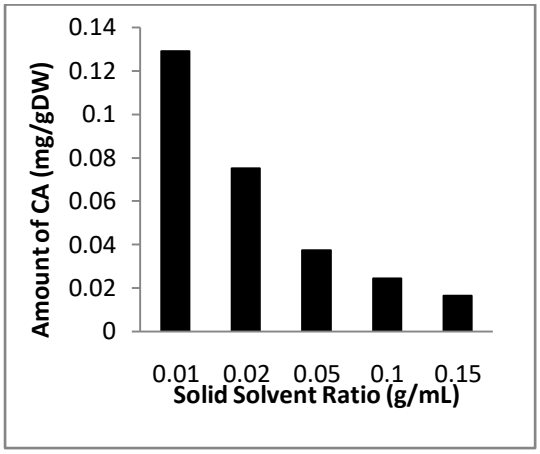

Fig. 4: Effect of solid solvent ratio on yield of caffeic acid

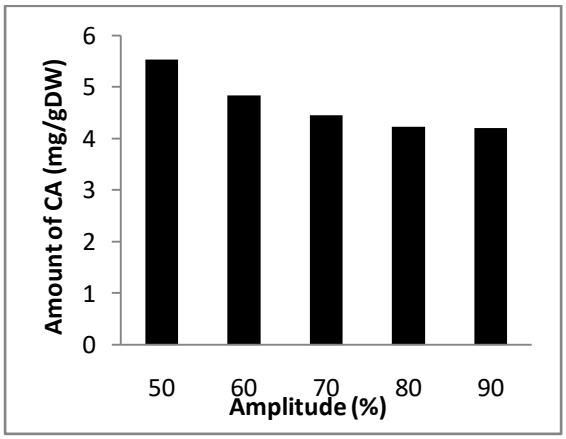

Fig. 5: Effect of amplitude on yield of caffeic acid

ACKNOWLEDGEMENTS

This research is supported by PGRS180324 and RDU1803121. Dr Pang Sook Fun is the recipient of UMP Post-Doctoral Fellowship in research.

REFERENCES

1. Akbar, S. (2011). *Andrographis paniculata*: A review of pharmacological activities and clinical effects. Alternative Medicine Review : A Journal of Clinical Therapeutic, 16(1), 66–77.
2. Hossain, M. S., Urbi, Z., Sule, A., & Rahman, K. M. H. (2014). Andrographis paniculata (Burm. f.) Wall. ex Nees: A review of ethnobotany, phytochemistry, and pharmacology. Scientific World Journal, 2014.
3. Icyer, N. C., Toker, O. S., Karasu, S., Tornuk, F., Bozkurt, F., Arici, M., & Sagdic, O. (2016). Combined design as a useful statistical approach to extract maximum amount of phenolic compounds from virgin olive oil waste. LWT - Food Science and Technology, 70, 24–32.
4. Jarukamjorn, K., & Nemoto, N. (2008). Pharmacological Aspects of *Andrographis paniculata* on Health and Its Major Diterpenoid Constituent Andrographolide. Journal of Health Science, 54(4), 370–381.
5. Jovanov, A., Petrov, P., Đorđev, V., Zdun C, G., Šavikin, K., & Bugarski, B. (2017). Polyphenols extraction from plant sources. Lekovite Sirovine, 37(December).
6. Jovanovic, A. A., Dordevic, V. B., Zdunic, G. M., Pljevljakusic, D. S., Savikin, K. P., Godevac, D. M., & Bugarski, B. M. (2017). Optimization of the extraction process of polyphenols from *Thymus serpyllum L*. herb using maceration, heat- and ultrasound-assisted techniques. Separation and Purification Technology, 179, 369–380.
7. Praveen, N., Poornananda, M. N., & Nayeem, A. (2014). Polyphenol Composition and Antioxidant Activity of *Andrographis Paniculata* L. Nees. Mapana J Sci, 13(4), 33–46.
8. Puspitasari, A., Martono, S., Pramono, S., & Widyarini, S. (2016). Optimization of extraction process and dechlorophyllation of ethanolic extract of *Andrographis paniculata* ness. International Journal of Pharmaceutical and Clinical Research, 8(5), 345–351.
9. Rao, P. R., & Rathod, V. K. (2015). Mapping study of an ultrasonic bath for the extraction of andrographolide from *Andrographis paniculata* using ultrasound. Industrial Crops and Products, 66, 312–318.
10. Shi, J. (2015). Functional food ingredients and nutraceuticals processing technologies.
11. Thisoda, P., Rangkadilok, N., Pholphana, N., Worasuttayangkurn, L., Ruchirawat, S., & Satayavivad, J. (2006). Inhibitory effect of *Andrographis paniculata* extract and its active diterpenoids on platelet aggregation. European Journal of Pharmacology, 553(1–3), 39–45.
12. Thoo, Y. Y., Ng, S. Y., Khoo, M. Z., Wan Aida, W. M., & Ho, C. W. (2013). A binary solvent extraction system for phenolic antioxidants and its application to the estimation of antioxidant capacity in *Andrographis paniculata* extracts. International Food Research Journal, 20(3), 1103–1111.
13. Zhang, X. F., & Tan, B. K. (2000). Antihyperglycaemic and anti-oxidant properties of *Andrographis paniculata* in normal and diabetic rats. Clinical and Experimental Pharmacology & Physiology, 27(5–6), 358–363.