

Extraction of Polyphenolic Content from *Peperomia pellucida* (L) Kunth Herb with 1-Ethyl-3-methylimidazolium Bromide as a Green Solvent

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Extraction of the polyphenolic content from *Peperomia pellucida* (L) Kunth herb (Piperaceae family) using 1-ethyl-3-methylimidazolium bromide as a solvent was attempted. The herbs were extracted using the ionic liquid-based microwave-assisted extraction method with some combination factors such as extraction time, microwave power, liquid-solid ratio, and ionic liquid concentration. The optimum yields of total polyphenolic content (13.750 µg GAE/g sample) were obtained by using a microwave power of 30 % Watt, extraction time of 10 minutes, the liquid-solid ratio of 14 ml/g, and ionic liquid concentration of 0.7 mol/l. Based on the results, compared to conventional organic solvent, the solvent of 1-ethyl-3-methylimidazolium bromide could provide higher extraction yields of polyphenolic content. Moreover, the extraction of a secondary metabolite from this herb becomes more rapid, easy, and efficient.

Key words: *Peperomia pellucida* (L). Kunth, 1-ethyl-3-methylimidazolium bromide, ionic liquid, microwave assisted extraction, total polyphenolic content

The use of ionic liquid solvent was done over the last decade. Various techniques, cost, and their complexity have been developed to extract the active components of the plant^[1]. Ideally, the use of extraction method

aimed to produce the secondary metabolites optimally, by considering the extraction method, which is a simple, rapid, safety, economical, eco-friendly and can be reproducible^[2-4]. The heat reflux extraction (HRE)

method is a conventional method and most appropriate to use an ionic liquid solvent compared with other. However, the use of the non-conventional methods has proven to be more effective and efficient to extract the active constituents of the plants^[5,6]. An ionic liquid solvent combined with the use of non-conventional extraction methods, such as ionic liquid-based microwave-assisted extraction (IL-MAE), negative-pressure cavitation-assisted extraction (IL-NPCE), ultra-high pressure assisted extraction (IL-UPE), supercritical fluid extraction (IL-SFE), and ultrasound-assisted extraction (IL-UAE)^[7,8]. Although all of the methods have special requirements, moreover in some cases has been performed a comparative analysis of these methods.

1-Ethyl-3-methylimidazolium bromide (EMIMBr) is one type of ionic liquid solvent. It used as a solvent for extracting compounds from the natural products. Study of ionic liquid as a solvent had reported such as the trans-resveratrol extraction from *Polygonum cuspidatum* using IL-MAE^[9]. Phenolic compound extraction from *Arctium lappa* L using IL-UAE and IL-MAE methods^[10], extraction of rhiosin and rhodionin from *Rhodiola rosea* using IL-UAE methods^[11].

Peperomia pellucida (L.) Kunth is a herb, belongs to a family of Piperaceae. The herbs are traditionally utilized to treat various diseases such as hypertension, diabetes mellitus, gout, headache and pain (abdominal pain)^[12,13]. Some studies had reported the plant has pharmacological properties as angiotensin converting enzyme (ACE) inhibitory^[14,15], gastroprotective^[16], antiinflammatory^[17], antisickling^[18], sunscreen^[19], antimicrobial and antioxidant^[20]. Polyphenol compounds have been isolated including; quercetin^[15], secolognan^[21], pelusidinA^[22] and chromene^[23]. However, to isolate the leading compounds (dereplication) or new compounds are difficult, it was due to the yield (particularly phenolic content) of this plant is poor. Therefore, the development of extraction methods conducted with the application of the green chemistry principles by using an ionic liquid solvent. Application of the IL-MAE for the secondary metabolite extraction from this plant has reported in the preliminary study previously^[24]. The use of EMIMBr as a solvent for the extraction of phenolic constituents has not reported. The use of ionic liquid solvent aims to obtain the extracts with maximum phenolic constituents.

Samples of *P. pellucida* herb were collected from North Mamuju, West Sulawesi, Indonesia on June 10-20, 2016. The voucher specimens identified at the Herbarium Bogoriense, Bogor, West Java, Indonesia. The sample was washed, dried and powdered using a grinder. The chemical materials were employed, such as EMIMBr as a solvent purchased from Shanghai Chen Jie Chemical, China. Sodium carbonate, Folin-Ciocalteu reagent, and gallic acid standard purchased from Sigma-Aldrich, UK. Aqua DM, methanol, n-hexane and ethyl acetate were purchased from PT. SmartLab Indonesia, Indonesia.

In the conventional extraction method, the dried powder of sample (3 g) was macerated with n-hexane 50 ml, allowed to stand for 24 h, and filtered and then evaporated to obtain a dry extract. Furthermore, the residue was macerated using ethyl acetate in the same procedure. In the non-conventional extraction method^[24], the dried powder of sample (3 g) was mixed with an ionic liquid solvent then extracted using an IL-MAE (Modena 900 Watt, with slight modification), which operated under some conditions. The residue and extract solution was separated by filtering using a cotton swab and cooled at room temperature. The obtained extract solution was left for 10 to 12 h to precipitate the desired extract.

Determination of the total polyphenolic content using a microplate reader 96 well method^[25,26]. A total of 20 μ l (1000 ppm) of the extract solution or the standard solution were added to 100 μ l reagent 25 % Folin-Ciocalteu solution, homogenized for one minute and then allowed to stand for 4 min. Then a 75 μ l sodium carbonate solution was added and homogenized for one minute. Absorbance measured at a 750 nm wavelength using a 96 well microplate reader after incubated for 2 h at room temperature in the dark. Gallic acid solutions (200, 100, 50, 25, and 12.5 μ g/l, respectively) were used as standards.

As far as we know, the selection of solvent to obtain extracts with maximum yield (total polyphenolic content) is a crucial step, especially for the optimization

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Accepted 25 September 2017

Revised 26 March 2017

Received 11 November 2016

Indian J Pharm Sci 2017;79(6):1013-1017

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of extraction methods that aim to acquire a target compound. The use of EMIMBr as a solvent is expected to attract to the optimum polyphenolic constituents by using the IL-MAE method. Some factor considered in this method, among others, the extraction time, the microwave power, the liquid-solid ratio, and the ionic liquid concentration shown in Table 1. Each extract with the yields of total polyphenolic content compared with a conventional method.

A standard calibration curve was performed using the microplate reader was shown in Table 2. Based on the results of linear regression, analysis has obtained automatically from the microplate reader (VersaMax™ ELISA Microplate Reader). The result was in accordance with the results had been reported in the previous studies^[25,27,28], and the Eqn. was $Y=0.023+7.812X$ with a correlation coefficient (R^2) of 0.999 (as shown in fig. 1). Where Y is the yields of total phenolic content, and X is the concentration of standard or sample. The equation was applied to determine the total polyphenolic content from herb samples using different types of solvents and extraction methods.

Based on the results of the absorbance measurements of the sample obtained from both the conventional and non-conventional methods, there are differences in the yields of total phenolic content. The optimum yields were obtained using non-conventional extraction methods in the range from 5.529 to 13.750 μg GAE/g sample such as the ionic liquid concentration of 0.7 mol/l; extraction time of 10 min; liquid-solid ratio of 14 ml/g and microwave power of 30 % Watt (Table 3). Whereas the yields were acquired using conventional extraction methods, both the n-hexane and ethyl acetate extracts were 3.408 and 7.823 μg GAE/g sample, respectively. Based on the above results, the use of EMIMBr solvent is higher compared to the organic solvent (fig. 2).

This study is an early stage in the development of extraction methods to obtain the target compound from medicinal plants rapidly, easily and efficiently.

TABLE 1: EXPERIMENTAL FACTORS OF AN IL-MAE USING EMIMBr AS A SOLVENT

Factor	Unit	Symbol	Range and level		
			Low	Medium	High
Extraction time	Min	A	10	15	20
Microwave power	% W	B	10	30	50
Ionic liquid concentration	mol/l	C	0.2	0.7	1.2
Liquid-solid ratio	ml/g	D	10	12	14

Furthermore, from the further study is in progress including the optimization of extraction method, isolation of biomarker compound, and screening of activity.

TABLE 2: RESULTS OF ABSORBANCE MEASUREMENT FROM GALLIC ACID STANDARD

Concentration ($\mu\text{g/ml}$)	Absorbance	Approximate absorbance	Standard deviation
	0.112		
12.5	0.118	0.116	0.004
	0.119		
	0.205		
25	0.212	0.209	0.004
	0.210		
	0.415		
50	0.405	0.405	0.009
	0.395		
	0.874		
100	0.832	0.843	0.027
	0.823		
	1.559		
200	1.531	1.57	0.045
	1.619		

The absorbance measurement at a wavelength of 750 nm using a 96 well microplate reader

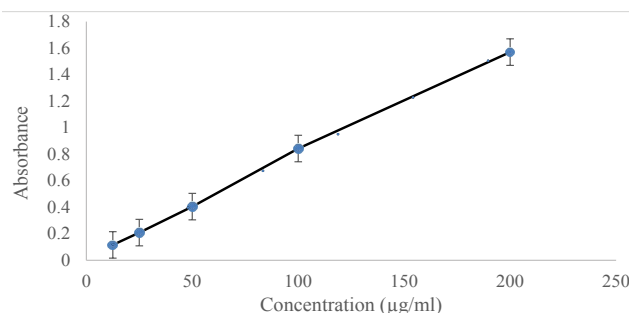


Fig. 1: Curve of linear regression analysis of gallic acid. The concentration series (—●—) and the linear concentration series (-----) of the gallic acid standard

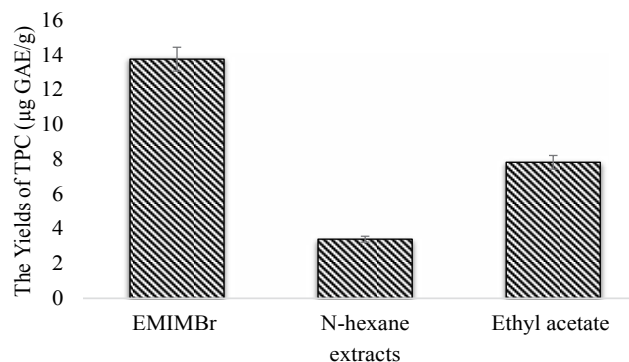


Fig. 2: The efficiency extraction of total polyphenolic content from *P. pellucida* herb. GAE ($\mu\text{g/g}$) is gallic acid equivalent

TABLE 3: RESULTS OF ABSORBANCE MEASUREMENT AND DETERMINATION OF TOTAL POLYPHENOL CONTENT OF *P. PELLUCIDA* EXTRACT OBTAINED USING IL-MAE

Run	Factor A	Factor B	Factor C	Factor D	ABS	Yield actual
1	15	30	0.7	12	1.048	10.931
2	15	10	0.2	12	0.663	6.824
3	10	30	0.7	14	1.312	13.750
4	20	30	0.7	14	0.922	9.590
5	10	30	0.7	10	1.094	11.428
6	15	50	0.7	14	1.061	11.076
7	15	30	0.2	10	0.541	5.529
8	10	30	0.2	12	0.672	6.923
9	15	50	0.2	12	0.857	8.897
10	20	30	1.2	12	1.060	11.062
11	15	30	0.7	14	1.029	10.728
12	20	50	0.7	12	0.983	10.244
13	20	30	0.2	12	1.280	13.412
14	15	50	0.7	10	0.972	9.593
15	15	10	1.2	12	0.955	11.684
16	15	50	1.2	12	0.922	10.536
17	10	50	0.7	12	1.118	14.131
18	20	30	0.7	10	1.017	11.755
19	15	10	0.7	10	1.348	10.276
20	10	10	0.7	14	1.125	8.651
21	10	10	0.7	12	0.986	10.604
22	20	10	0.7	12	0.883	11.531
23	15	30	1.2	10	0.834	8.989

Factor A is extraction time (minutes); factor B is microwave power (% Watt); factor C is an ionic liquid concentration (mol/l); factor D is the liquid-solid ratio (ml/g). ABS are approximate absorbance (three times). Yield actual is the total polyphenol content ($\mu\text{g/g}$, gallic acid equivalent; GAE)

Acknowledgements

This study supported by grants via “PITTA 2017” from Directorate of Research and Humanity Engagement (DRPM), Universitas Indonesia.

Conflict of interest:

The authors declare that they have no conflict of interest.

Financial support and sponsorship:

Nil.

REFERENCES

- Espino M, Fernández MA, Gomez FJV, Silva MF. Natural designer solvents for greening analytical chemistry. *Trends Anal Chem* 2016;76:126-36.
- Bogdanov MG, Kantlehner W. Simple prediction of some physical properties of ionic liquids: the residual volume approach. *Z Naturforsch B* 2009;64:215-22.
- Bogdanov MG, Petkova D, Hristeva S, Svinjarov I, Kantlehner W. New guanidinium-based room-temperature ionic liquids. Substituent and anion effect on density and solubility in water. *Z Naturforsch B* 2010;65:37-48.
- Jessop PG, Jessop D, Fu D, Phan L. Solvatochromic parameters for solvents of interest in green chemistry. *Green Chem* 2012;14:1245-59.
- Smith RM. Extractions with superheated water. *J Chromatogr A* 2002;975:31-46.
- Khoddami A, Wilkes M, Roberts T. Techniques for analysis of plant phenolic compounds. *Molecules* 2013;18:2328-75.
- Chemat F, Vian MA, Cravotto G. Green extraction of natural products: Concept and principles. *Int J Mol Sci* 2012;13:8615-27.
- Chemat F, Vian MA. Green chemistry and sustainable technology: Alternative solvents for natural products extraction. New York: Springer US; 2014.
- Du F, Xiao X, Li G. Application of ionic liquids in the microwave-assisted extraction of trans-resveratrol from *Rhizoma Polygoni Cuspidati*. *J Chromatogr A* 2007;1140:56-62.
- Lou Z, Wang H, Zhu S, Chen S, Zhang M, Wang Z. Ionic liquids based simultaneous ultrasonic and microwave assisted extraction of phenolic compounds from burdock leaves. *Anal Chim Acta* 2012;716:28-33.
- Zhu S, Ma C, Fu Q, Hu L, Lou Z, Wang H, *et al.* Application of ionic liquids in an online ultrasonic assisted extraction and solid-phase trapping of rhodiosin and rhodionin from *Rhodiola rosea* for UPLC. *Chromatographia* 2013;76:195-200.
- Hariana A. Medicinal Plants and Their Efficacy. 3rd ed. Jakarta: Penebar Swadaya; 2006 (in Bahasa).
- Heyne K. The Useful Indonesia Plants. 3rd ed. Jakarta: Departemen Kehutanan, Yayasan Sarana Wana Jaya; 2007.
- Saputri F, Mun'im A, Lukmanto D, Aisyah S, Rinandy J. Inhibition of angiotensin converting enzyme (ACE) activity

- by some Indonesia edible plants. *Int J Pharm Sci Res* 2015;6:1054-9.
15. Kurniawan A, Saputri F, Ahmad I, Mun'im A. Isolation of angiotensin converting enzyme (ACE) inhibitory activity quercetin from *Peperomia pellucida*. *Int J PharmTech Res* 2016;9:115-21.
 16. Roslida AH, Aini NZ. Evaluation of gastroprotective effect of the ethanolic extract of *Peperomia pellucida* (L) Kunth. *Pharmacologyonline* 2009;2:678-86.
 17. Arrigoni-Blank MF, Dmitrieva EG, Franzotti EM, Antonioli AR, Andrade MR, Marchioro M. Anti-inflammatory and analgesic activity of *Peperomia pellucida* (L.) HBK (Piperaceae). *J Ethnopharmacol* 2004;91:215-8.
 18. Abere TA, Okpalaonyagu SO. Pharmacognostic evaluation and antisickling activity of the leaves of *Peperomia pellucida* (L.) HBK (Piperaceae). *Afr J Pharm Pharmacol* 2015;9:367-74.
 19. Ahmad I. Penentuan nilai persentase eritema dan pigmentasi ekstrak herba Suruhan (*Peperomia pellucida* L.) secara in vitro. *J Sains dan Kesehatan* 2015;1:90-5.
 20. Oloyede GK, Onocha PA, Olaniran BB. Phytochemical, toxicity, antimicrobial and antioxidant screening of leaf extracts of *Peperomia pellucida* from Nigeria. *Adv Environ Biol* 2011;5:3700-9.
 21. Xu S, Li N, Ning MM, Zhou CH, Yang QR, Wang MW. Bioactive compounds from *Peperomia pellucida*. *J Nat Prod* 2006;69:247-50.
 22. Bayma JDC, Arruda MSP, Müller AH, Arruda AC, Canto WC. A dimeric ArC2 compound from *Peperomia pellucida*. *Phytochemistry* 2000;55:779-82.
 23. Susilawati Y, Nugraha R, Muhtadi A, Soetardjo S, Supratman U. (S)-2-Methyl-2-(4-methylpent-3-enyl)-6-(propan-2-ylidene)-3,4,6,7-tetrahydropyrano[4,3-g]chromen-9(2H)-one. *Molbank* 2015;2015:1-6.
 24. Ahmad I, Yanuar A, Mulia K, Mun'im A. Application of ionic liquid based microwave-assisted extraction of the secondary Metabolite from *Peperomia pellucida* (L) Kunth. *Pharmacogn J* 2017;9:227-34.
 25. Zhang Q, Zhang J, Shen J, Silva A, Dennis DA, Barrow CJ. A simple 96-well microplate method for estimation of total polyphenol content in seaweeds. *J Appl Phycol* 2006;18:445-50.
 26. Bobo-García G, Davidov-Pardo G, Arroqui C, Vírveda P, Marín-Arroyo M, Navarro M. Intra-laboratory validation of microplate methods for total phenolic content and antioxidant activity of polyphenolic extracts, and comparison with conventional spectrophotometric methods. *J Sci Food Agric* 2014;95:204-9.
 27. Medina-Remón A, Barrionuevo-González A, Zamora-Ros R, Andreas-Lacueva C, Estruch R, Martínez-González A, *et al.* Rapid Folin-Ciocalteu method using microtiter 96-well plate cartridges for solid phase extraction to assess urinary total phenolic compounds, as a biomarker of total polyphenols intake. *Anal Chim Acta* 2009;634:54-60.
 28. Ainsworth EA, Gillespie KM. Estimation of total phenolic content and other oxidation substrates in plant tissues using Folin – Ciocalteu reagent. *Nat Protoc* 2007;2:875-7.