

Reply to the reviewers' comments

Reviewer Number	Original comments of the reviewer	Reply by the author(s)	Changes done on page number and line number
1.	Remarks: Grammatical errors need to be corrected.	“To explore the use effect of ionic liquid as a green solvent in the polyphenolics extraction from <i>Peperomia pellucida</i> (L.) Kunth using 1-buthyl-3-methylimidazolium bromide ([BMIM]Br) and 1-buthyl-3-methylimidazolium chloride ([BMIM]Cl) with ionic liquid based microwave-assisted extraction (IL-MAE) method.” replaced with “The aims of the study was to explore the application effect of ionic liquid as a green solvent in the polyphenolics content extraction from <i>Peperomia pellucida</i> (L.) Kunth herbs using 1-butyl-3-methyl imidazolium bromide ([BMIM]Br) and 1-butyl-3-methyl imidazolium chloride ([BMIM]Cl).”	Page no. 1 and line no. 16-18
		“Based on the result was obtained the highest of total polyphenolic content including 18.287 µg GAE/g ([BMIM]Cl), 15.734 µg GAE/g ([BMIM]Br), 7.823 µg GAE/g (ethyl acetate), and 3.408 (n-hexane). Scanning electron microscopy demonstrated the extraction mechanism with significant physical changes in plant tissue residue after extraction treatment with different solvents.” Replaced with “The results showed that the effect of ionic liquid on the yield of total polyphenolics content, including 18.287 µg GAE/g (0.7 mol/l [BMIM]Cl concentration, 14 ml/l liquid-solid ratio, and 270 Watts microwave power for 10 minutes), and 15.734 µg GAE/g (0.7 mol/l [BMIM]Br concentration, 14 ml/l liquid-solid ratio, and 270 Watts microwave power for 15 minutes), whereas the SEM demonstrated the extraction mechanism with significant physical changes in matrix sample after treatment using different solvents.”	Page no. 1 and line no. 24-28
		“ <i>Peperomia pellucida</i> (L.) Kunth herbs (Piperaceae family) could be found in humid environments throughout the territory of Indonesia and traditionally used as an analgesic, anti-hyperuricemic, diabetes mellitus, and antihypertension. ^[1] Some study has reported pharmacological properties from this plant such as antihyperglycemic oxidative stress, dyslipidemia in diabetic rats, ^[2] anti-inflammatory, analgesic activity, ^[3] and antihypertension. ^[4] The herbs contain a secondary metabolite including essential oil, terpenes, alkaloid, phenols, and flavonoids. ^[5] Some compounds were isolated such as quercetin, ^[4] chromenes, ^[6] dillapiole, ^[7] xanthone patulosida A, ^[8] peperomins, ^[9] and pellucidin A. ^[10] ” replaced with “ <i>Peperomia pellucida</i> (L.) Kunth herbs is a Piperaceae family and can be found in wet environments throughout the territory of Indonesia. It is traditionally used as an analgesic, anti-hyperuricemic, anti-diabetes mellitus, and antihypertension. ^[1] Some studies have reported the pharmacological properties of this plant such as antihyperglycemic oxidative stress, dyslipidemia in diabetic rats, ^[2] anti-inflammatory, analgesic activity, ^[3] and antihypertension. ^[4] Also, reported the content of secondary metabolites including essential oil, terpenes, alkaloid, phenols, and flavonoids. ^[5]	Page no. 3 and line no. 3-9

	Some polyphenolic compounds such as quercetin, ^[4] chromenes, ^[6] dillapiole, ^[7] xanthone patulosida A, ^[8] peperomins, ^[9] and pellucidin A ^[10] were isolated.”	
	“.....this plant had no economic value and farmers still consider as a weed.” Replaced with “.....it has no economic value and weeds for farmers, especially in palm oil plantations.”	Page no. 3 and line no. 10-11
	“The ionic liquid is a green solvent that could be applied because it has the flexibility of ions combination to adjust the physicochemical properties of the target compounds and potential to replace the organic solvents that are flammable, volatile, and toxic. ^[11] Also, other considerations regarding the entire process, the economic and environmental impact” Replaced with “The ionic liquid is a green solvent that has the flexibility of ions combination to adjust the physicochemical properties of the target compounds and potential to replace the organic solvents that are flammable, volatile, and toxic. ^[11] In addition, the targeted compounds can be improved by considering safety, toxicological, economics, and environmental impact standpoint.”	Page no. 3 and line no. 14-17
	“The chemicals used in this study,” replaced with “The chemicals were used in this study”	Page no. 4 and line no. 18
	“The equipment used including.....” replaced with “The equipments were used including.....”	Page no. 4 and line no. 22
	“The dried powder of samples (3 g) was macerated with n-hexane 50 ml, allowed to stand for 24 h, filtered and evaporated to obtain a dry extract. Furthermore, the residue was macerated using ethyl acetate with the same procedure.” Replaced with “The dried powder of samples (3 gram) were macerated with 50 ml n-hexane, allowed to stand for 24 hours, filtered and evaporated to obtain a dry extract. Furthermore, the residue was remacerated using ethyl acetate with the same procedure.”	Page no. 5 and line no. 3-5
	“Extraction using ionic liquid based microwave-assisted extraction.” Replaced with “An ionic liquid based microwave-assisted extraction (IL-MAE) method was used to extracting polyphenolic content based on literature.”	Page no. 6 and line no. 7-8
	“As can be seen in Table 2 and Table 3, demonstrated the effect of extraction condition combination using [BMIM]Br and [BMIM]Cl with ionic liquid based microwave assisted extraction method. According to the results in Table 2 obtained the ten highest of yields with the combination of extraction condition factors and the best yields (15.734 µg GAE/g) using [BMIM]Br as a solvent. Whereas, the results in Table 3 obtained the ten highest of yields and the best yields (18.287 µg GAE/g) using [BMIM]Cl. In Figure 1 demonstrated that [BMIM]Cl could extract secondary metabolites with the highest of total polyphenolic content compared to [BMIM]Br solvent and conventional organic solvents (n-hexane and ethyl acetate).” Replaced with “Table 2 and Table 3, demonstrated the combination effect of extraction condition using [BMIM]Br and [BMIM]Cl with ionic liquid based microwave assisted extraction method. According to the results in Table 2 was obtained the 10 highest of yields with the combination of extraction condition factors and the best yields (15.734 µg GAE/g)	Page no. 6 and line no. 11-16

		using [BMIM]Br as a solvent. Whereas, the results in Table 3 was obtained the best yields (18.287 µg GAE/g) and the 10 highest of yields using [BMIM]Cl. Comparison of efficiency level between an ionic liquid as a green solvent and conventional organic solvent shown in Figure 1.”	
		“The extraction mechanism was performed using SEM (can be seen in Figure 2), shows significant physical changes in plant tissue after extraction treatment with different solvents. Based on the result, the cell wall surface changes in samples that have been extracted IL-MAE compared to the maceration method and the sample before the extraction process, whereas the changes of cell wall surface before and after the maceration extraction did not show significant differences.” Replaced with “In Figure 2, showed significant physical changes in matrix sample after treatment with different solvents. Based on the result, described the changes of cell wall surface in matrix samples that have been extracted using the IL-MAE compared to the maceration method.”	Page no. 6 and line no. 18-20
		“ <i>P. pellucida</i> is a plant rich in pharmacological properties. However, this herb had not been utilized as raw materials for drugs produced commercially. Some factors of the causes are among others constrained regarding cultivation, standardization, optimum yields, optimal extraction methods, and so on. One of the factors that play a major role in obtaining optimum yields is the development of extraction methods.” Replaced with “ <i>P. pellucida</i> herb is a plant rich in pharmacological properties. However, the plant has not utilized as raw materials for commercial herbal medicines because it constrained by the various factors such as cultivation, standardization, optimal results, optimal extraction methods, and so on. Development of extraction method is one of the factors that play a major role in obtaining the optimum yields.”	Page no. 6-7, line no. 23-25, 1
		“The capability enhancement of extracting certain chemical components could increase with the solvent hydrophobicity enhancement. ^[12] The hydrophobicity of both ionic liquids used in this study was [BMIM]Br<[BMIM]Cl,” replaced with “The solvent hydrophobicity enhancement has the ability to increase the extraction capability of the targeted secondary metabolite. ^[12] The hydrophobicity of both ionic liquids were used in this study, namely [BMIM]Br<[BMIM]Cl,”	Page no. 7 and line no. 14-16
		“The scanning electron microscopy (SEM) analysis.....” replaced with “The SEM analysis.....”	Page no. 7 and line no. 20

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Author Name Islamudin Ahmad, Arry Yanuar, Kamarza Mulia, and Abdul Mun'im		Title of the paper Metabolite Profiling Analysis of Conventional and Non- Conventional Extraction Methods on Secondary Metabolite from <i>Peperomia pellucida</i> (L.) Kunth using UPLC-Qtof-MS/MS System	Journal Name Journal of Young Pharmacist		
Author email islamudinahmad@farmasi.unmul.ac.id arry.yanuar@farmasi.ui.ac.id kmulia@che.ui.ac.id munimabdoel@gmail.com			PDF file name	JYP_279_17- QC-RD	MS Number JYP_279_17
Comment Number (As marked in Galley Proof)	Page Number, Line Number	As in Galley Proof	Modification to be done		Remarks
1.	Title	".....on A Secondary....."	".....on Secondary...."		"...A..." deleted
2.	Title	"...UPLC-Qtof-MS/MS..."	"...UPLC-QToF-MS/MS..."		replaced
3.	Author	Islamudin Ahmad ^{1,3,4*} , Kamarza Mulia ² , Arry Yanuar ³ , Abdul Mun'im ^{4*}	Islamudin Ahmad ^{1,3} , Kamarza Mulia ² , Arry Yanuar ³ , Abdul Mun'im ^{3*}		replaced
4.	Affilitation	" ^{1,3,4} Department....."	" ³ Department....."		replaced
5.	Abstract (line 6)	".....microwave-assisted extraction(IL- MAE)....."	".....microwave-assisted extraction (IL- MAE)..."		replaced
6.	Abstract (line 9- 10)	".... on profiling metabolite....."	".... on metabolite profiling....."		replaced
7.	Abstract (line 12)	"....([BMIM]BF ₄"	"....([BMIM]BF ₄)....."		Replaced
8.	Abstract (line 12)	"...ILMAE...."	"....IL-MAE...."		replaced
9.	Abstract (line 14)	Phone: ????	Phone: +62-852-1610-4550		added
10.	Abstract (line 15)	icapps2017.farmasiui@gmail.com	-		deleted
11.	Introduction (page 1, line 4)	"....peperomins,secolignan, ³"	"..peperomins, secolignan, ³"		Space added
12.	Introduction (page 1, line 4)	"This..."	"This..."		replaced

13.	Material and Methods (page 2, line 11)	"...ILMAE method)...."	"...(IL-MAE method)....."	replaced
14.	Matrial and method (page 2, line 18)	"2. Non-conventional ILMAE method"	"2. Non-conventional IL-MAE method"	replaced
15.	Material and Methods (page 2, line 43)	".....previous study. ^{12,23} including....."	".... previous study ^{12,23} including....."	replaced
16.	Material and Methods (page 2, line 44)	".....liquid\u2013solid....."	".....liquid-solid....."	replaced
17.	Material and Methods (page 2, line 45)	"....Box\u2013Behnken...."	"....Box Behnken...."	replaced
18.	Material and Methods (page 2, line 45)	".....of ILMAE method..."	"....of IL-MAE method...."	replaced
19.	Material and Methods (page 2, line 46)	".....of ILMAE method..."	"....of IL-MAE method...."	replaced
20.	Material and Methods (page 2, line 6)	"...0.1% formic acid(A) and....."	"....0.1% formic acid (A) and..."	replaced
21.	Material and Methods (page 2, line 12)	"....Effendorf tube."	"....Eppendorf tube."	replaced
22.	Results (page 2, line 5)	"...TIC..."	"....total ion chromatogram (TIC)....."	Replaced
23.	Results (page 2, line 7)	"....profiling metabolite....."	".....metabolite profiling....."	Replaced
24.	Results (page 2, line 9)	"....([BMIM]BF ₄)...."	"....([BMIM]BF ₄)...."	replaced
25.	Results (page 2, line 12)	".....properties.In Figure 2,"	"....properties. In Figure 2,"	replaced
26.	Results (page 2, line 18)	"....profiling metabolite....."	"....metabolite profiling....."	replaced
27.	Results, (page 3, line 1-3)	Figure 1: Peaks data of metabolite profiling analysis from extracts were obtained by ILMAE method (non-	Figure 2: Peaks data of metabolite profiling analysis from extracts were obtained by IL-MAE method (non-	Edited and bold

		conventional method) with [BMIM]BF ₄ as a solvent using UPLC-QToF-MS/MS system	conventional method) with [BMIM]BF₄ as a solvent using UPLC-QToF-MS/MS system	
28.	Table 2 (page 3)	Table 2: Results of LC-MS (m/z value) spectrum of extract was obtained using ILMAE method (non-conventional method)	Table 2: Results of UPLC-QToF-MS/MS (m/z value) spectrum of extract was obtained using IL-MAE method (non-conventional method)	Replaced
29.	Results (page 4, line 1)	Table 3: Results of LC-MS (m/z value) spectrum of extract was obtained using maceration method (conventional extraction method).	Table 3: Results of UPLC-QToF-MS/MS (m/z value) spectrum of extract was obtained using maceration method (conventional extraction method).	Replaced (Font arial 12 pt)
30.	Discussion (page 4, line 8)	“.....compound. ²³ including.....”	“.....compound ²³ including.....”	Replaced
31.	Discussion (page 4, line 9)	“....liquid\u2013solid.....”	“....liquid-solid.....”	replaced
32.	Discussion (page 4, line 20)	“.....Box\u2013Behnken...”	“....Box Behnken....”	replaced
33.	Discussion (page 4, line 1)	“....acetate.Each.....”	“....acetate. Each.....”	replaced
34.	Discussion (page 4, line 6)	“....database. ¹⁹ particularly.....”	“....database ¹⁹ particularly...”	Replaced
35.	References(poin 14)	“ <i>Bidens pilosa</i> ”	“ <i>Bidens pilosa</i> ”	In italic
36.		Q1. Kindly provide running title.	Metabolite profiling analysis on secondary metabolite from <i>Peperomia pellucida</i> (L) Kunth herb extracts	added
37.		Q2. Kindly provide Abbreviations used and Summary.	Abbreviation used <i>P. pellucida</i> : <i>Peperomia pellucida</i> (L.) Kunth; IL-MAE : ionic liquid microwave-assisted extraction; [BMIM]BF₄ : 1-butyl-3-methylimidazolium tetrafluoroborate; LC : liquid chromatography; HPLC : high performance liquid chromatography; UPLC-QToF-MS/MS : ultra-performance liquid chromatography – quadrupole time of flight mass spectrometry; Rt : retention time; TIC : total ion chromatogram; AUC : area under curve; m/z : mass per atomic charge.	

38.		Q2. Kindly provide Abbreviations used and Summary.	Summary - There were differences on profiling metabolite from both conventional and non-conventional extraction methods that was extraction method using organic solvent and ionic liquid solvent ([BMIM]BF₄) at the optimum condition. - The extract obtained using IL-MAE method had a peak depth with a well-separated Rt (retention time) value ranging from 0.5 to 7.5 minutes which means that the extracted compound was from polar to nonpolar properties. - The extract obtained using maceration method, the peak spread on a separate Rt ranges from 2.5 to 7.5 minutes. - Both extracts obtained have different area under curve (AUC) values with amount total of 22285 (IL-MAE) and 12679 (maceration), respectively, and showed the IL-MAE twice as large as that of the maceration. - conventional and non-conventional extraction method showed differences in metabolite profiling based on Rt value and mass spectrum m/z of each peak.	
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All the corrections have been incorporated using comments tool of PDF or scanned jpg image of pages can also be marked using red pen; Kindly send attachment to galleys@emanuscript.in

Dear Mr. Ahmad

We are pleased to inform that your manuscript "Metabolite profiling analysis of conventional and non-conventional extraction methods on a secondary metabolite from *Peperomia pellucida* (L) Kunth using UPLC-QToF-MS/MS system" is now acceptable after clearing the dues for publication of the manuscript.

The payment for author-side fee can be done online through credit card or by cheque. Please visit the journal's manuscript system and login into your account for the details.

Once the payment is received at our end, the manuscript would be processed further and you would receive an edited version of article in about 2-3 weeks from now to Journal of Young Pharmacist.

With warm personal regards,

Your sincerely,

Dr. S. Parasuraman, Ph.D.

Editor in-Chief Journal of Young Pharmacists

Metabolite Profiling Analysis of Conventional and Non-Conventional Extraction Methods on *Peperomia pellucida* (L.) Kunth Using UPLC-QToF-MS/MS System

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ABSTRACT

Objective: The purpose of this study was to observe the difference of extraction method (both conventional and non-conventional) based on metabolite profile using UPLC-QToF-MS/MS system from *Peperomia pellucida* (L.) Kunth. **Methods:** Dried samples were extracted using the conventional maceration method and the optimum of ionic liquid-based microwave-assisted extraction (IL-MAE) methods. Metabolite profiling was performed using UPLC-QToF-MS/MS system with some modifications adjusted to the instrument condition. The data was analyzed using Masslynx 4.1 software. **Results:** based on the results, there were differences on profiling metabolite from both conventional and non-conventional extraction methods that was extraction method using organic solvent and ionic liquid solvent (IL-MAE) the optimum condition. The extract obtained using IL-MAE method had a peak depth with a well-separated Rt (retention time) value ranging from 0.5 to 7.5 min which means that the extracted compound was from polar to nonpolar properties. The extract obtained using maceration method, the peak spread on a separate Rt ranges from 2.5 to 7.5 min. Also, both extracts obtained have different area under curve (AUC) values

with amount total of 22285 (IL-MAE) and 12679 (maceration), respectively, and showed the IL-MAE twice as large as that of the maceration. **Conclusion:** based on the results, conventional and non-conventional extraction method showed differences in metabolite profiling based on Rt value and mass spectrum m/z of each peak.

Key words: *Peperomia pellucida* (L.) Kunth, Hyphenated system, Ionic liquid-based microwave-assisted extraction, Maceration, Metabolite profiling.

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INTRODUCTION

Peperomia pellucida (L.) Kunth herbs (Piperaceae family) has been known to contain secondary metabolites such as terpenoids, alkaloids, saponins, dan polyphenols.¹ Some of them have been isolated (particularly polyphenolic groups) including pellucidin A,² peperomins,³ ignan,⁴ xanthone patuloside A,⁴ dillapiol, chromene,⁶ and quercetin.⁷ Also, the herbs have also been reported to have potential pharmacological properties.⁸

The bioactive compound from natural products mainly from plants source can be extracted by conventional (including maceration, reflux, soxhlet, percolation)⁹ and non-conventional extraction methods (such as microwave-assisted extraction, ultrasonic-assisted extraction, supercritical fluid extraction, and so on).¹⁰ However, the use of extraction methods is strongly influenced by the type of solvent used.

Ionic liquid based microwave-assisted extraction (IL-MAE) continued to be developed and applied in the extraction secondary metabolites from natural products. This method was developed based on the selection of solvents with a green chemistry principle approach in exploring the metabolite constituent of natural products. The ionic liquid is chosen as solvent because it has the flexibility and potential to replace conventional organic solvents that are volatile, flammable, and toxic.¹¹ Application of

ionic liquid as a solvent to extracting secondary metabolite and optimization of the IL-MAE method of polyphenolics content extraction from this herb has been performed in previous studies.¹²⁻¹³

Metabolite profiling is intended to distinguish between the known compound (dereplication) and new molecule directly present in the crude extract.¹⁴ Metabolite profiling in crude extracts is not an easy work as natural materials show a very diverse form of structure. In some compounds, the atomic sequence and its stereochemical orientation must be elucidated by de novo. Consequently, the ability of a single analytical technique in profiling metabolite content in the crude extract to date does not exist.¹⁵ Innovative development strategies from metabolite profiling in crude extracts where the researcher can be facilitated by the hyphenated system found during the last decade. The potential for chemical screening strategies has been significantly enhanced by the recent development of the hyphenated technique, which is capable of generating efficient metabolite separation, along with valuable structural information both online and at-line.¹⁶

One of the instruments with the hyphenated system used to analyze metabolite profiling with high accuracy and validity¹⁷⁻¹⁸ from crude extract is UPLC-QToF-MS/MS tandem system.¹⁹ The method has been

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successfully used for metabolite profiling analysis in some medicinal plants such as metabolite profiling of bioactive compounds from *Bidens pilosa*,¹⁴ metabolites profiling secondary metabolites of *Arabidopsis arabika*,²⁰ Analysis of steviol and its glycoside from *Stevia rebaudiana* leaves as a commercial sweetener,²¹ metabolite profiling of polyphenols from *Vaccinium berries*.²² While the metabolite profiling analysis of *P. pellucida* herb (using both conventional and non-conventional extraction) has not been reported.

Active compound isolation from *P. pellucida* herb requires specific techniques to obtain the compound optimally. Development of extraction methods (both conventional and non-conventional) and metabolite profiling with hyphenated systems to achieve the target compounds. This study aimed to know the difference of extraction method (both conventional maceration and non-conventional IL-MAE) based on metabolite profile analysis using UPLC-QToF-MS/MS system.

MATERIALS AND METHODS

Materials

Wild herb materials of *P. pellucida* were collected from the oil palm plantation at Baras sub-district, North Mamuju, West Sulawesi, Indonesia, and were identified at the Herbarium Bogoriense, Bogor, West Java, Indonesia. The fresh sample was washed, and dried at 50–60°C using drying oven, and the dried sample was powdered using a grinder. The powder obtained was stored at a cold temperature until analysis.

Material and General Equipments

The materials used in this study include n-hexane, ethyl acetate, aquadest (PT. Smart Lab Indonesia, Indonesia), 1-butyl-3-methylimidazolium tetrafluoroborate (Cheng Jie Chemical Co. LTD, China). Acetonitrile (LiChrosolv® for HPLC), Ethanol (Chromosolv® for HPLC), formic acid (Merck, Germany). The general equipment used in this study were Modena Microwave 900 Watt (Buono-MV3002), Rotary Evaporator (Buchi, Germany), UPLC-QToF-MS/MS System (Waters, USA), as well as equipment for maceration.

Extraction procedure

The extraction process was conducted using conventional (maceration method) and non-conventional (IL-MAE method) as follows:

1. Conventional Maceration Method

Dried sample (1 kg) was macerated using n-hexane solvent for 24 h, and extraction process was performed for 4–6 times. Next, the residual of samples was re-macerated using ethyl acetate with the same condition. The extract solution was evaporated using a rotary evaporator to obtain a dried extract.¹²

2. Non-conventional IL-MAE Method

For extraction process with the optimum ILMAE method was performed based on the previous study including microwave power, extraction time, ionic liquid concentration, and liquid:solid ratio had been involved. Response surface methodology and Box-Behnken design were used to obtain predictive model (multivariate quadratic regression equation Briefly, 30 grams of dried sample was extracted using the optimum condition of IL-MAE method with [BMIM]BF₄ as a solvent. The optimum condition of IL-MAE method was performed using ionic liquid concentration of 0.79 mol/l, the liquid-solid ratio of 11 ml/g sample, extraction time of 20 min, and microwave power of 30 %Watt.²³ For analysis, the extraction process was performed four times.

Metabolite profiling analysis

For determination of metabolite profiling was performed using UPLC- QToF-MS/MS System according to the literature,^{14,17,22,24} with

some modification adjusting to the instrument condition. Briefly, liquid chromatographic (LC) separation was performed using Xevo G2 ACQUITY with BEH C₁₈ column 1.7 µm, 2.1 x 50 mm (Waters Corp, Milford, MA, USA). Autosampler Tray and columns temperature was maintained at 40°C, the mobile phase consisting of water containing 0.1% formic acid and acetonitrile containing 0.1% formic acids (B) was used at a flow rate 0.3 ml/min. The elution gradient can be seen in Table 1.

Data acquisition was processed with Masslynx 4.1 software. Extract sample (10 mg) was weighed and dissolved in 100 ml methanol (LC-MS grade). One ml of the sample solution was taken into the Effendorf tube and was centrifuged at 13000 rpm for 10 min. The supernatant was separated into another Effendorf tube. The sample injection volume was set at 5 µl and the total running time was 10 min. The UPLC output system was connected to the mass spectrometer via the ESI interface and was operated in the positive ion detection mode. Nitrogen was used for desolvation of 500 L/h; the cone gas was set at 16 L/h. The capillary voltage was set at 3 kV, source temperature 110°C, and desolvation temperature 300°C.

RESULTS

For the results test of profiling metabolite using the same gradient conditions that were the mobile phase with the ratio between aquadest and acetonitrile (containing 0.1% formic acid) from 95:5 to 5:95 for 9 min as shown in Table 1.

As can be seen in Figure 1 and 2 demonstrated with peak smoothing and peak area integration (A) and TIC with peak smoothing (B) that showed difference of metabolite from both method that was extraction method using organic solvent and ionic liquid solvent ([BMIM]BF₄) at the optimum condition of the instrument. In Figure 1 a peak depth with a well-separated Rt (retention time) value ranging from 0.5 to 7.5 min was shown, which means that the extracted compound was from polar to nonpolar properties. Figure 2, the peak spread on a separate Rt ranges from 2.5 to 7.5 min. While in the min of 0.5 to 2.4 there was no peak. It indicates that the compound extracted by maceration method using ethyl acetate have nonpolar properties.

From both extracts based on different extraction methods (i.e., maceration and IL-MAE) the different result of metabolite profiling was obtained. Shown in Figure 1 which was the result of profiling metabolite of the extract obtained by IL-MAE, there was a peak especially in 0.4 to 3.0 min which was not present in the extract obtained by maceration (as can be seen in Figure 2). Whereas in Figure 2 there were peaks, especially at strong peak with Rt of 4.52 – 5.60 min while peak on metabolite profiling results from the extract obtained by IL-MAE show peak which was weak even not exist. While in the peak with Rt 6.20 there were on both extracts. Also, both extracts obtained has different AUC values in which extracts

Table 1: The elution gradient for metabolite profiling analysis using UPLC- QToF-MS/MS System (Waters).

No.	Time (Minute)	%A	%B
1	0	95	5
2	1	95	5
3	6	0	100
4	7	0	100
5	7.5	95	5
6	9	95	5

Where A is the mobile phase consisting of water containing 0.1% formic acid and B is the mobile phase consisting of acetonitrile containing 0.1% formic acid.

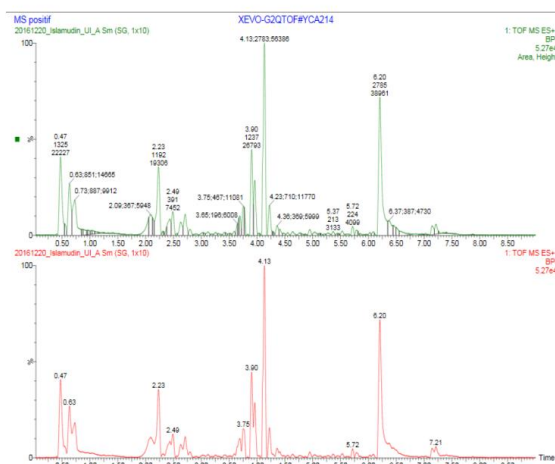


Figure 1: Peaks data of metabolite profiling analysis from extracts were obtained by IL method (non-conventional method) with [BMIM]BF₄ as a solvent using UPLC- QToF-MS/MS System. Where A is Total ion chromatogram (TIC) with peak smoothing and peak area integration; B is Total ion chromatogram with smoothing.

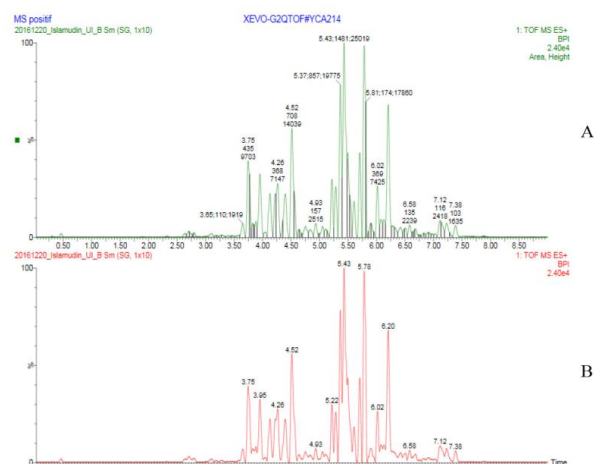


Figure 2: Peaks data of metabolite profiling analysis from extracts were obtained by maceration method (conventional method) with ethyl acetate as a solvent using UPLC- QToF-MS/MS System. Where A is Total ion chromatogram (TIC) with peak smoothing and peak area integration; B is Total ion chromatogram with smoothing.

Table 2: Results of LC-MS/MS (m/z value) spectrum of extract was obtained using IL method (non-conventional method).

No	Retention Time (Rt)	min	m/zvalue
1	0.4 – 2.3	0.468	139.1816; 140.1761; 141.1166; 323.3341; 507.4559; 691.6055; 875.7525; 1059.8871; 1244.0045; 1428.1177
		0.625	139.1782; 140.1761; 364.3413; 365.3409; 366.3379; 591.4858; 626.9603; 817.6363; 922.6903; 1043.7958; 1268.9130; 1496.0017
		0.730	139.1768; 140.1758; 365.3379; 410.1645; 591.4912; 626.9582; 817.6421; 922.6833; 1043.7909; 1268.9534; 1495.0270
		2.082	211.2456; 212.2475; 251.2299; 354.1744; 486.2294
		2.232	211.2516; 212.2471; 251.237; 355.1892; 565.2423; 647.2996; 818.2544; 1054.0790
2	2.3 – 2.9	2.436	223.2466; 245.2317; 252.2227; 340.1905; 565.2446; 603.3232
		2.490	171.1478; 245.2336; 246.2336; 251.2426; 433.2215; 481.2777; 595.3393
		2.669	195.1249; 196.1307; 283.2699; 447.2441; 593.3315; 685.3773
		2.715	195.1291; 196.1336; 402.2793; 477.2263; 522.3278; 687.3209
		2.805	179.1615; 219.1680; 308.3018; 403.2110; 566.4335; 724.3237; 769.3845; 911.3472; 1027.4069; 1174.3456; 1494.6885
3	2.9 – 3.5	2.919	201.2108; 219.2210; 235.2005; 323.3064; 407.2352; 430.2371; 531.3644; 650.2902; 847.4064; 899.3586
		3.091	151.1069; 209.1243; 211.1613; 276.1473; 385.2653; 407.2422; 515.3213; 647.4405; 733.3910
		3.109	177.1185; 207.1454; 314.2209; 331.2057; 407.2459; 548.3253; 549.3280; 671.3453; 783.3347; 883.4658
		3.348	207.1514; 225.1598; 305.2462; 421.2552; 529.3532; 549.3170; 685.3718; 685.3718; 773.4330; 957.6433; 1175.5614
		3.431	195.1424; 271.1469; 313.1953; 373.2277; 375.2440; 478.3654; 593.3487
4	3.5 – 4.5	3.685	212.2958; 304.3846; 313.1908; 318.3595; 529.3406; 625.3709; 754.4578; 841.4797; 975.5499
		3.756	207.1587; 235.1687; 236.1685; 403.2746; 404.2841; 529.3494; 753.4700; 827.4679; 952.3814; 1050.5581; 1189.4120; 1438.4369
		3.900	225.2053; 384.4238; 285.4135; 332.4052; 525.2990; 561.3804; 739.4343; 817.4390; 949.5057; 1075.5227
		3.953	300.1483; 315.1792; 316.1764; 332.3993; 466.3031; 629.3246; 739.4461; 785.4274; 963.5539; 999.5621; 1111.6162
		4.129	195.1401; 312.4749; 313.4536; 360.4524; 581.3390; 653.3799; 739.4486; 869.4777; 986.4565; 1124.5309
5		4.218	195.1435; 322.4378; 329.1913; 330.1898; 331.2008; 441.4445; 619.3845; 739.4498; 949.5218; 1174.6296; 1286.6456
		4.959	149.0787; 277.2780; 301.2099; 522.4457; 623.3851; 766.6262; 898.7619; 987.7861; 1174.6445; 1313.7004; 1495.8024
		5.714	139.1722; 251.1402; 308.3557; 309.3383; 419.3854; 565.3870; 581.3549; 637.4206; 638.4244; 639.4299; 858.8097; 896.7033; 1053.4819; 1272.7568; 1460.0713
		6.218	149.0884; 150.0798; 391.3789; 413.3726; 429.3433; 457.3602; 638.7296; 803.6777; 872.8376; 1019.749
		7.155	139.1703; 149.0715; 370.3681; 413.3850; 607.5057; 663.5709; 843.6861; 887.7139; 888.7165; 889.7233; 890.7280; 985.9030; 1326.0479
		7.206	139.1727; 370.3823; 413.3847; 677.5908; 843.6845; 871.7186; 872.7230; 873.7269; 874.7251; 1090.8916; 1155.9614; 1342.0386; 1470.1021

Table 3: Results of I MS (m/z value)spectrum of extract was obtained using maceration method (conventional extraction method).

No	Retention Time (Rt)	Min	m/zvalue
1	3.34 – 4.3	3.635	181.1697; 219.1656; 304.3615; 387.3539; 753.4727; 814.4013; 924.4348; 1206.4136; 1285.6440
		3.756	219.1701; 235.11686; 236.1662; 329.2194; 403.2748; 427.2461; 632.3526; 827.4625; 81.4370; 976.5239
		3.950	219.1695; 315.1745; 316.1740; 427.2531; 553.3780; 682.2105; 773.4325
		4.140	219.1689; 312.4491; 313.4427; 315.1689; 427.2712; 643.3893; 720.4321; 947.6596; 983.5687; 1143.5848; 1324.7644; 1436.8271
		4.272	219.1684; 221.1547; 329.1894; 330.1904; 427.2780; 627.3904; 711.3359; 803.5481; 967.4610
2	4.3 – 5.3	4.400	219.1678; 221.1811; 299.1703; 304.2689; 411.2731; 627.3802; 699.4662; 737.5193; 880.5945
		4.512	221.1875; 222.1843; 411.2766; 553.4916; 627.3677; 795.5170; 833.5162; 981.5439; 1091.9723
		4.941	203.2382; 219.1720; 221.1771; 387.2785; 419.3132; 639.3691; 641.3979; 810.6937; 939.6212; 1031.7057
		5.213	219.1744; 275.2413; 387.2763; 607.3751; 610.3864; 611.3885; 669.4666; 879.4844; 1022.9031; 1217.7123; 1269.7792; 1494.8412
		5.277	219.1739; 386.3646; 607.3882; 609.4003; 635.3800; 626.3835; 627.3950; 782.6851; 829.6377; 1029.4808; 1233.7205; 1357.7954; 1434.646
3	5.3 – 5.85	5.363	219.1739; 387.2882; 509.3809; 535.3666; 610.3857; 611.3904; 782.6791; 879.5152; 1086.8999; 1218.17064; 1318.7473
		5.438	219.1713; 412.3825; 535.3679; 593.3825; 594.3857; 609.3829; 625.4382; 782.6993; 1127.7306; 1185.7092; 1354.2301
		5.560	219.1709; 221.1738; 341.2921; 414.4041; 415.4006; 535.3690; 593.3873; 594.3924; 743.4485; 829.6261; 919.6580; 1197.0258
		5.714	203.2279; 219.1732; 308.3825; 309.3676; 535.3777; 593.3900; 637.4399; 829.6188; 919.6422; 1256.0709; 1313.7601
		5.785	219.1567; 334.4149; 335.3990; 356.3844; 607.4096; 689.6401; 813.6051; 903.6359
4	5.85 – 7.5	5.907	203.2272; 219.1738; 220.1809; 348.4148; 349.4074; 387.2815; 611.5715; 612.5655; 829.6135; 905.6819; 1097.9205; 1188.9225; 1376.1067
		6.014	219.1734; 221.1704; 336.5163; 362.4325; 427.3952; 551.4983; 815.6162; 887.6426; 937.7065; 1042.7949; 1167.8545
		6.204	149.0832; 219.1691; 387.2775; 413.3725; 429.3481; 613.5667; 797.6502; 865.6614; 961.7382; 1065.7802
		6.491	219.1746; 335.3382; 387.2788; 429.4297; 613.5734; 797.6614; 905.7272; 921.7256; 922.7267; 923.7308; 1033.8214; 1159.9659
		7.141	219.1692; 370.3982; 541.3345; 609.3832; 871.7190; 903.7157; 904.7144; 905.7189; 906.7194; 1081.8163; 1275.9602; 1497.1693
		7.238	219.1591; 370.3939; 593.3926; 705.6019; 813.7011; 871.7213; 872.7220; 873.7233; 874.7306; 1093.7841; 1166.9430; 1296.0737; 1433.9531
		7.395	219.1620; 370.3899; 565.3514; 705.6183; 813.7059; 814.7114; 815.7146; 888.7054; 1093.8440; 1166.9338; 1412.1720

were extracted by IL-MAE and maceration methods with amount total of 22285 and 12679, respectively, where the extracts of IL- MAE was twice as large as that of the extracts of maceration method.

DISCUSSION

In this study, non-conventional extraction was performed using ILMAE method based on a combination of optimum extraction condition parameters, as well as 18.6 min extraction times, 30 %Watt microwave power, 11 ml/g liquid-solid ratio, and 0.79 ml/L [BMIM]BF₄ in water. [BMIM]BF₄ was chosen because based on the comparison of effectiveness in extracting the polyphenol class from this herb in the previous study, and the liquid ionic solvent was the best solvent to extract the target compound.²⁴ling microwave power, extraction time, ionic liquid concentration, and liquid\solid ratio had been involved. Response surface methodology and Box\uehnhken design were used to obtain predictive model (multivariate quadratic regression equation While the conventional extraction using the maceration method

with n-hexane then followed by ethyl acetate extract obtained was dried and stored in a cool place and prepared for analysis.

Strategies for the analysis and identification of natural plant compounds with profiling metabolite approach using UPLC-QToF-MS/MS tandem system based on peaks at a particular retention time (Rt) as data and compared with the database.¹⁹ularly natural product research. Obtaining precise information on the chemical composition of complex natural extracts (metabolomes In metabolite profiling analysis, a very high resolution of chromatography was required to detect the metabolites of the associated matrix complex.²⁶ The reduction of particle size in chromatography allows higher separation efficiency and shorter analysis times.¹⁶

In this study, metabolite profiling analysis was performed only limited to the results of the investigation using UPLC-QToF-MS/MS tandem system and obtained peak distribution from each extract at the same eluent condition based on total ion chromatogram (TIC) with peak smoothing and peak area integration. The m/z spectrum value in each peak indicates compound predictions according to the experimental

molecular weight approach. This study does not use standards as controls, however, focusing only on the differences in the components present in each extract based on the Rt and m/z spectrum value.

Based on the results of metabolite profiling analysis, it was shown that the results of spectrum m/z gave different peaks on each extract. However, in this study, the m/z spectrum cannot be determined precisely given the absence of a comparison standard to be used as a benchmark on each peak produced. In addition, these profiles correlate with total polyphenolics content from extracts of each method.^{13,23} Therefore, only the optimum conditions obtained from the instrument used to separate the class of compounds contained in the extract based on the technique used, and the preliminary data related to the use of liquid ionic solvents as an alternative solution with a green chemical principles approach.

CONCLUSION

Based on the above results, metabolite profiling was determined and analyzed using UPLC- QToF-MS/MS System and both conventional and non-conventional extraction method showed differences in metabolite profiling based on Rt value and mass spectrum m/z of each peak.

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CONFLICT OF INTEREST

The author declares that he has no conflict of interest.

ABBREVIATIONS USED

SUMMARY

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