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Original Article

Extraction of Quercetin from *Nothopanax scutellarium* Leaves via Ionic Liquid-based Microwave-assisted Extraction

Ika Aulia Rahmi[1], Mahdi Jufri[2], Abdul Mun'im[3,*]

ABSTRACT

Introduction: *Nothopanax scutellarium* leaves have been used in Indonesian traditional medicine to treat several diseases. Previous studies used conventional extraction methods with large volumes of organic solvents, long extraction times, and low levels of quercetin content. This study was aimed to identify the optimal solvent among different ionic liquids that has the highest quercetin content. **Methods:** Ionic liquids including 1-butyl-3-methylimidazolium bromide, 1-butyl-3-methylimidazolium tetrafluoroborate, 1-butyl-3-methylimidazolium chloride, 1-butyl-3-methylimidazolium hydrogen sulfate, and 1-hexyl-3-methylimidazolium bromide, for extracting quercetin from *N. scutellarium* leaves using microwave-assisted extraction under the following conditions: ratio, 1:10; operation time, 10 min; and power, 10 W. Then, quercetin was fractionated using ethyl acetate and separated using 0.01 mol/L sodium bocarbonate, dipotassium phosphate or sodium cloride. The total flavonoid content was determined using a UV-Vis spectrophotometer, and quercetin content was determined using HPLC. **Results:** Extraction with 1-butyl-3-methylimidazolium chloride using NaCl as the separation salt was associated with the highest total flavonoid (360.57 mg/g) content among the ILs, whereas 1-butyl-3-methylimidazolium tetrafluoroborate combined with sodium chloride generated the highest quercetin content (26.13 mg/g). **Conclusion:** 1-butyl-3-methylimidazolium tetrafluoroborate is the optimal solvent for extracting quercetin from *N. scutellarium* leaves.
Key words: Flavonoid, Ionic liquid, Green extraction, Green technology, Mangkokan Leaf.

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INTRODUCTION

Mangkokan (*Nothopanax scutellarium* Merr.) leaves have been used traditionally to treat various conditions such as alopecia, wounds, anuria, and body odor.[1] Based on prior studies, mangkokan leaves can promote hair growth.[2-4] Although the mechanism of this effect is unclear, the activity of mangkokan leaves was believed to be attributable to its flavonoid content.

Quercetin is a major flavonoid in most plant extracts.[5] Quercetin is known for anti-oxidant, anti-inflammatory, anti-obesity, anti-hypertensive, vasodilatory, anti-hypercholesterolemic, anti-atherosclerotic, anti-bacterial, and anti-biofilm effects.[6-9] However, the conventional method for extracting this flavonoid uses large volumes of organic solvent. Furthermore, this process is limited by long extraction times and low active compound levels. In ethanol extracts of mangkokan Leaf an average at 1.53%, in ethyl fractions acetate an average at 4.79%, and ethyl acetate extract at 1.64%, ethyl acetate fraction at 5.83%.[2-3]

Several studies used ionic liquid (IL)-based microwave-assisted extraction (MAE), which has great advantages for extracting plant with high extraction levels and short extraction times.[10-11] The main advantages of MAE compared with conventional extraction techniques include the use of smaller amounts of solvents, lower costs and improved extraction quality because of the reduced degradation of thermolabile constituents.[12-16]

The use of 1-butyl-3-methylimidazolium bromide ([BMIM]Br), 1-butyl-3-methylimidazolium tetrafluoroborate ([BMIM]BF_4), 1-butyl-3-methylimidazolium chloride ([BMIM]Cl), 1-butyl-3-methylimidazolium hydrogen sulfate ([BMIM]HSO_4), and 1-hexyl-3-methylimidazolium bromide ([HMIM]Br) as environment friendly solvents for extracting flavonoids from mangkokan leaves has never been reported. ILs are expected to represent safe alternatives to volatile organic solvents.

MATERIALS AND METHODS

Herbal materials

Mangkokan leaves were obtained from BALITRO, Bogor, West Java, Indonesia and analyzed by the Indonesian Institute of Sciences. The leaves were washed and dried in an oven, and the dried sample was crushed using a grinder.

Chemical materials and equipment

[BMIM]Br, [BMIM]BF_4, [BMIM]Cl, [BMIM]HSO_4), and [HMIM]Br were obtained from Shanghai Cheng Jie Chemical Co. LTD (China). Other chemicals used in this study included $NaHCO_3$ (Merck, Germany), K_2HPO_4 (Merck, Germany), NaCl (Merck, Germany), ethyl acetate (Bratachem, Indonesia), methanol (Merck, Germany), glacial acetic acid (Merck, Germany), Aqua Pro Injection (PT. Ikapharmindo Putramas, Indonesia), quercetin standard (Sigma-Aldrich, Siangapore), and distilled water (CV. Satya Darmawan, Indonesia). The equipment used in the

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study included a UV-Vis spectrophotometer (Shimadzu, Japan), 900 W microwave (Buono MV-3002), VM-10 vortex mixer (WISD, Ireland), centrifuge (Laboratory Supply Company Ollmann & Co, Germany), HPLC (Shimadzu, Japan), and TLC UV Scanner (Camag, Switzerland).

Extraction methods

One gram of the dried mangkokan leaf sample was placed in a boiling flask and mixed with ILs, followed by MAE. The operating conditions included a running time of 10 min, IL concentration of 1.5 mol, 10 mL/g ratio, and power of 10 W. The extracts were filtered using filter paper.

Fractionation and separation with salt

The filtrates were fractioned using ethyl acetate. The extract was separated using 0.01 mol/L $NaHCO_3$, K_2HPO_4, or NaCl (1:1 ratio). Each solution was vortexed for 20 s and then centrifuged for 15 min at 3000 rpm. The organic phase containing quercetin was the top layer, which was dried in a water bath and hydrolyzed using 4 N HCl. The dried extract was weighed, diluted with deionized water to 5 mL, and used for further analysis.

Flavonoid identification

Flavonoids were identified using TLC. Extracts were spotted on TLC plates using 5-µL microcapillary tubes. Quercetin standard was used for comparison. Elution was conducted in a closed chamber that was already saturated with n-hexane:ethyl acetate:acetic acid (7:2.5:0.5) as the mobile phase. The plate was dried, sprayed using $AlCl_3$, and visualized under UV light for fluorescence. The R*f* value was calculated.

$$Rf = \frac{\textit{the distance traveled by substance}}{\textit{the distance traveled by solvent}}$$

Total flavonoid determination

The total flavonoid contents of extracts were determined using quercetin as a standard. Various amounts of quercetin (up to 10 mg) were dissolved in 10 mL of methanol to prepare concentrations of 10–100 ppm to determine the linearity of quercetin standard. After preparing the extract, as much as 1 mL was removed and mixed with 3 mL of 96% methanol, 0.1 mL of $AlCl_3$, 0.1 mL of 1 M sodium acetate, and 2.8 mL of double-distilled water. The solution was incubated at room temperature for 30 min, and the absorbance was measured using a UV-Vis spectrophotometer at 410 nm. The total flavonoid content was calculated from the calibration curve, and the results are presented as mg/g.

Quercetin determination using HPLC

The quercetin contents of the extracts were analyzed using HPLC. HPLC was performed in the isocratic mode using a C18 bonded-silica gel column (5 µm, 150 × 4.6 mm, GL Sciences, Japan) and an LC-20AT HPLC system equipped with a UV-Vis detector (SPD-20A). The mobile phase was methanol:Aqua Pro Injection:glacial acetic acid (65:34:1). The UV detector wavelength was 370 nm. The flow rate was 1 mL/min, the run time was 10 min, and the injection volume was 20 µL. The sample was filtered using a 0.45-micron syringe filter before analysis.

RESULTS

Flavonoid identification

The results of TLC analysis are presented in Figure 2. R*f* for each extract was compared with that of quercetin standard (0.38).

Total flavonoid determination

The standard solution was analyzed for linearity. A linear response was identified (r^2 = 0.9955), and the equation was y = 0.0078x − 0.0052. Extracts obtained using different ILs and three different salts were analyzed via UV-Vis spectrophotometry. The [BMIM] Cl extract using NaCl as the separation salt had the highest flavonoid content (360.57 mg/g), as shown in Table 2.

Quercetin determination using HPLC

A linear response was observed (y = 65,196x − 270952, r^2 = 0.9998). The [BMIM] BF_4 extract separated using NaCl exhibited the highest quercetin content among the solvents (26.13 mg/g), as shown in Table 3. The chromatogram is presented in Figure 3.

Figure 1: Mangkokan leaves (*Nothopanax scutellarium* Merr.).

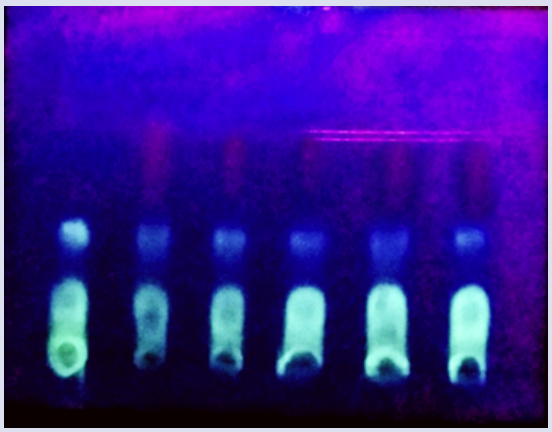

Figure 2: Flavonoid identification. The results are presented for (a) quercetin and the mangkokan leaf extracts using (b) 1-hexyl-3-methylimidazolium bromide, (c) 1-butyl-3-methylimidazolium hydrogen sulfate, (d) 1-butyl-3-methylimidazolium bromide, (e) 1-butyl-3-methylimidazolium chloride, and (f) 1-butyl-3-methylimidazolium tetrafluoroborate as ionic liquids.

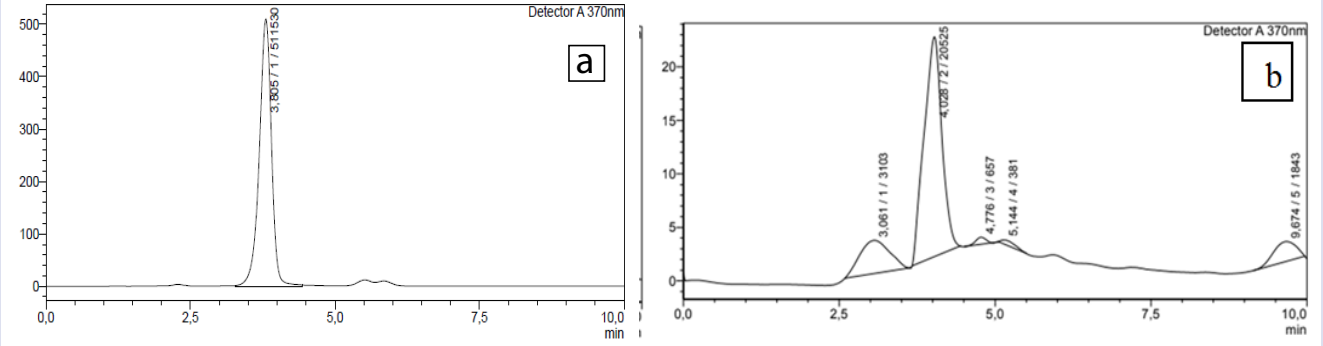

Figure 3: Chromatograms of (a) quercetin and (b) the 1-butyl-3-methylimidazolium tetrafluoroborate extract of mangkokan leaves.

Table 1: *Rf* values of mangkokan leaf extracts.

Mangkokan leaf extract	Rf
[BMIM]Br	0,36
[BMIM]BF_4	0,38
[BMIM]Cl	0,36
[BMIM]HSO_4	0,36
[HMIM]Br	0,38

Note: [BMIM] Br is 1-butyl-3-methylimidazolium bromide, [BMIM]BF_4 is 1-butyl-3-methylimidazolium tetrafluoroborate, [BMIM]Cl is 1-butyl-3-methylimidazolium chloride, [BMIM]HSO_4 is 1-butyl-3-methylimidazolium hydrogen sulfate, [HMIM]Br is 1-hexyl-3-methylimidazolium bromide.

Table 2: Effect of ionic liquids and salt addition on the total flavonoid content.

Extract	Total flavonoid content (mg/g)
[BMIM]Br + $NaHCO_3$	19.79 ± 0.33
[BMIM]Br + NaCl	23.09 ± 0.21
[BMIM]Br + K_2HPO_4	28.94 ± 0.32
[BMIM]Cl + $NaHCO_3$	325.36 ± 8.48
[BMIM]Cl + NaCl	354.2 ± 6.38
[BMIM]Cl + K_2HPO_4	204.09 ± 2.14
[BMIM]BF_4 + $NaHCO_3$	148.79 ± 1,85
[BMIM]BF_4 + NaCl	337.12 ± 8.06
[BMIM]BF_4 + K_2HPO_4	149.09 ± 9.62
[BMIM]HSO_4 + $NaHCO_3$	88,.8 ± 2.74
[BMIM]HSO_4 + NaCl	74.67 ±1.64
[BMIM]HSO_4 + K_2HPO_4	113.44 ± 0.61
[HMIM]Br + $NaHCO_3$	42.33 ± 0.14
[HMIM]Br + NaCl	57.19 ± 1.57
[HMIM]Br + K_2HPO_4	66.62 ± 1.22

Table 3: Effect of ionic liquids and salts addition on quercetin content.

Extract	Quercetin content (mg/g) ± SD
[BMIM]Br + $NaHCO_3$	4.03 ± 0.33
[BMIM]Br + NaCl	3.67 ± 0.21
[BMIM]Br + K_2HPO_4	8.53 ± 0.32
[BMIM]Cl + $NaHCO_3$	12.9 ± 8.48
[BMIM]Cl + NaCl	22.6 ± 6.38
[BMIM]Cl + K_2HPO_4	6,41 ± 2,14
[BMIM]BF_4 + $NaHCO_3$	11.96 ± 1.85
[BMIM]BF_4 + NaCl	26.13 ± 8.06
[BMIM]BF_4 + K_2HPO_4	24.62 ± 9.62
[BMIM]HSO_4 + $NaHCO_3$	2.33 ± 2.74
[BMIM] HSO_4 + NaCl	3.08 ± 1.64
[BMIM]HSO_4 + K_2HPO_4	4.92 ± 0.61
[HMIM]Br + $NaHCO_3$	2.99 ± 0.14
[HMIM]Br + NaCl	4.34 ± 1.57
[HMIM]Br + K_2HPO_4	1.86 ± 1.22

DISCUSSION

Mangkokan leaves were extracted via ILMAE, and the extracts were fractionated using ethyl acetate. ILs have non-volatile characteristics. Organic solvents such as ethyl acetate can be used to separate non-volatile compounds.[17] Then, the extracts were separated using different salts. This process precipitates residues in ILs, resulting in significant increases in extraction yields.[18]

The determination of the total flavonoid content was conducted by a colorimetric method using a UV-Vis spectrophotometer. In this study, the highest flavonoid content in mangkokan leaf extract was achieved using [BMIM]Cl, whereas [BMIM]BF_4 provided the highest quercetin content. This could be attributable to the presence of other flavonoid contents or differences in the principle of analysis, but further research is needed to clarify this issue. UV-Vis spectrophotometry is one of the most widely used methods for the determination of total flavonoid content because of its low implementation costs and availability in the laboratory.[19-20] However, HPLC is a more sensitive and selective analytical procedure for measuring isolated compounds, and it is widely used for all classes of flavonoids.[20]

The use of [BMIM]BF_4 for the success extraction of natural products was previously reported.[21-22] This finding is attributable to the fact that ILs can be used as both polar and non-polar solvents.[23] ILs can also be used in inorganic, organic, and polymer-solvent systems.[24] In addition, ILs are more environmentally benign solvents than the volatile organic solvents commonly used for extraction.[25] From this study, anion and cation is strongly influence an extraction result. Different anion and cation were studied and differences in their extraction yield were readily apparent, as shown in Table 3. The results showed that the ionic liquids based on Cl^- were the more efficient for total flavonoid, with BF_4– being the most efficient for quercetin. The different of cations including Bmim+ and Hmim+ were evaluated and the results are shown in Table 3. The extraction yield decreased when the alkyl chain length of the cation was increased from butyl to hexyl. The results suggested that the increasing alkyl chain length influences the extraction yield. Proton acidity and hydrophobicity for cations increased from buthyl to hexyl at the 1-position of the 1-alkyl-3-methylimidalizium ring. Both the proper hydrogen bonding and hydrophobic interactions of Bmim+ resulted in stronger solvation interactions with flavonoids followed by higher extraction yields than $[Hmim]^+$.

The different of salt using in partition affects extraction yields that different for each ionic liquids. Sodium cloride obtains the highest extraction quercetin compared with other salt in mangkokan leaves extraction using [BMIM]BF_4 solvent. Each ionic liquids has different optimum partition salt. It is because the mechanism of the salting-out effect is very complex due to the interplay of different types of

interaction between the solutes and the solvent. Hydration theories and the related water-structuring effects (kosmotropy) and semiquantitative way the intensity of the salting-out effects.

There were several factors that affect ionic liquid in this extraction process that has not been conducted in this study; extraction time and microwave power, temperature, and plant characteristics that must be consider for further study. The quantity of the extracted compound can be increased by increasing the extraction time, but it increase the risks of thermolable component.[26] The extraction time and microwave power was very sensitive parameters and both was needed to be handled simultaneously.[27] A combination of low or medium power with longer exposure is generally chosen to optimize MAE procedures, but study showed that various strengths from 500 W to 1000 W have no significant effect on flavonoid yields.[26] Temperatures in the range 60-90 °C have been used most often in MAEs carried out at atmospheric pressure.[27] The choice of extraction temperature depends on the stability and extraction results of the active compound target. Sample characteristics affect MAE performance. The small particle size will cause difficulties in separating the extract from the residue and additional cleaning steps may have to be used. In addition, fine samples treated with solvents for 90 minutes before extraction can improve MAE heating efficiency, increase diffusion and increase the mass transfer of active compounds to the solvent. However, in a number of reported cases, a long pre-treatment time did not improve the extraction results because the active ingredients might have left the sample matrix before extraction.[28]

CONCLUSIONS

The highest quercetin content was observed for mangkokan leaves extracted using [BMIM]BF_4 combined with NaCl as the separation salt (26.13 mg/g), confirming its utility for extracting quercetin from this medicinal plant.

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CONFLICTS OF INTEREST

None.

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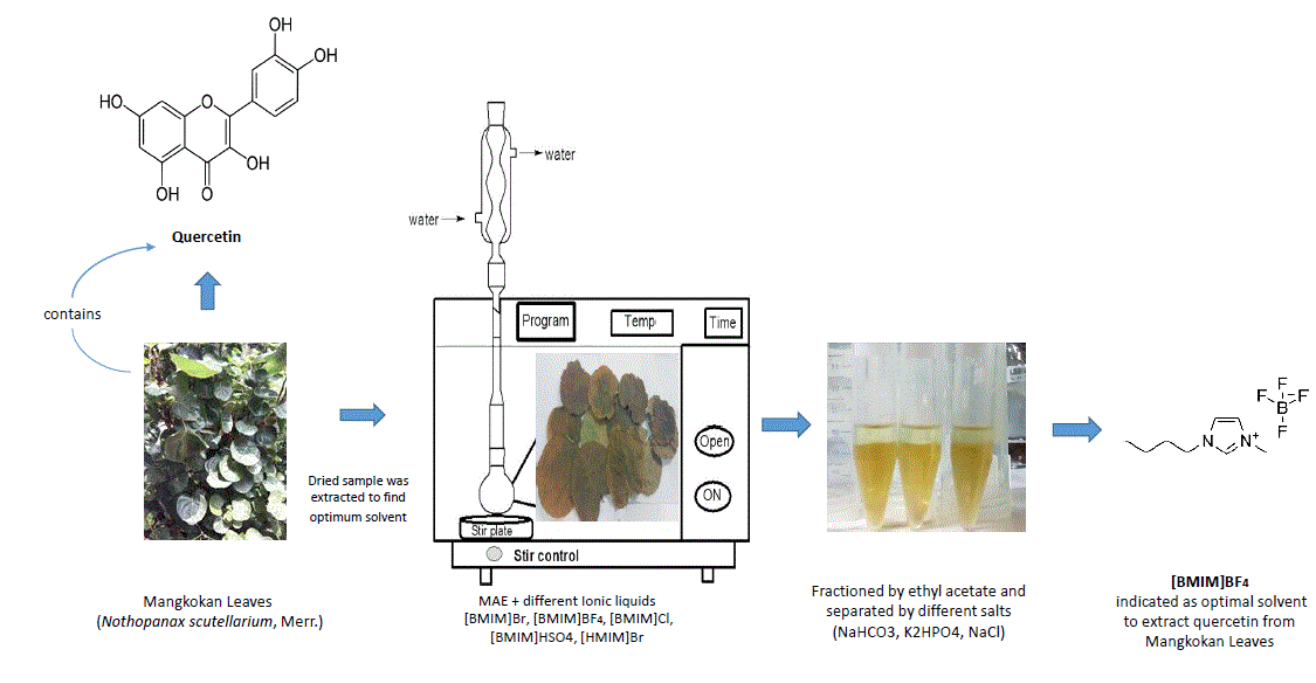
GRAPHICAL ABSTRACT



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