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Phyllantin Extraction Using Imidazolium Ionic Liquids with Chloride and Bromide Anion by Microwaved Assisted Extraction Method

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Abstract

Phyllanthin is known to have therapeutic properties such as immunomodulator, nephroprotective, and anticancer. Phyllanthin is a lignan compound that is commonly found in plants of the Phyllanthus genus, one of which is green meniran (*Phyllanthus niruri*). Solvent Ionic Liquid (IL) is one of the alternative solvents that are widely used for the extraction of compounds from a plant. The purpose of this study were to compare the extraction yield of philanthine compounds from meniran herbs using IL solvent by Microwave Assisted Extraction (MAE) with methanol by maceration method, and to determine the IL solvent that could produce the highest phyllanthin content. Optimization were carried out using the independent variables IL solvent concentration (0.25 M, 0.75 M, and 1.25 M) and sample-solvent mixture (1:10, 1:12, and 1:14). The variable modeling is designed using Response Surface Methodology that produced 9 runs of combination of solvent concentration and sample-solvent mixture ratio. The phyllanthin assay was using High Performance Liquid Chromatography (HPLC) with ethanol-water mixture (66:34) as mobile phase and wavelength at 229 nm for detection. The optimum extraction conditions showed the best results for imidazolium chloride IL at 1st run with a concentration of 0.75 M, the sample-solvent mixture of 1:14 and phyllanthin content of 0.1783 mg/g while imidazolium bromide IL at 7th run with a concentration of 0.75 M and the sample-solvent mixture of 1:12 and phyllanthin content of 0.23 mg/g. However, the methanol maceration method could only extract phyllanthin as much as 0.1319 mg/g. Based on these results, the use of IL-MAE was more effective than the methanol maceration method due to higher phyllantin extract.

Keywords: phyllanthin, ionic liquid, microwaved assisted extraction, ionic liquid imidazolium, chloride anion, bromide anion

1. Introduction

Indonesia is one of the countries that has a high level of biodiversity where 19,871 plants have been used as medicine for various diseases [1]. *Phyllanthus niruri*, or commonly known as green meniran, is one of the medicinal plants known to have various functions in medicine [2].

Phyllanthus niruri is widely distributed in tropical and subtropical areas of Central and South America and Asia (including India and Indonesia) [3]. *Phyllanthus niruri* has been used as a medicine for various diseases, such as dysentery, influenza, vaginitis, tumors, diabetes, diuretics, jaundice, kidney stones, and dyspepsia. This plant is also useful for treating hepatotoxicity, hepatitis B, hyper-glycemia, and diseases caused by viruses and bacteria [4].

Some secondary metabolites have been investigated in green meniran plants, like alkaloids, benzenoid, coumarin, flavonoid, lignan, lipid, phytallate, sterol, tannin, and triterpene [3]. One of the secondary metabolites of the lignan group that has been shown to have pharmacological effects is phyllanthin. Phyllanthin has been proved to has several pharmacological effects, such as reducing the growth of solid tumor masses, hepatoprotective, curing diabetes and various liver disorders caused by chemicals and viruses including hepatitis, antifibrotic and anti-inflammatory [5,6].

Phyllanthin compounds from *Phyllanthus niruri* are commonly extracted by conventional methods namely maceration with organic solvents. The maceration method has several drawbacks such as the use of a lot of solvents and low efficiency [7]. Therefore, it is necessary to use other method that is more efficient and effective to improve phyllanthin extraction. Microwave Assisted Extraction (MAE) has been noticed by several studies that has a high level of efficiency when used in the extraction process [8,9]. This method is modern automatic extraction method that has several excellences among others decrease solvent usage and extraction time, improving sample output and able to extract multiple samples. MAE involve the use of traditional solvent extraction and microwave energy to support the extraction process [10].

Organic solvents are generally used in the extraction of compounds, however, most of the organic solvents are volatile at room temperature causing pollution to the environment, as well as increasing the potential for accidents in the extraction process. Therefore, it is needed to explore alternative solvents that is more environmentally friendly. One of the alternative solvents that can be used is ionic liquid (IL), which shows very good properties, such as negligible vapor pressure, wide liquid temperature range, high thermal and chemical stability, adjustable viscosity, easy to dissolve in water and organic solvents, as well as good solubility and extraction ability for various organic compounds [11]. IL solvent can be recycled so that it can be used for several times without reducing its purity [12]. In addition, IL can also efficiently absorb and transfer microwave energy [13].

To the best of our knowledge, most recent researches that studied phyllantin extraction employed conventional method or non-conventional method with organic solvent [4,5,14–17]. Therefore, this study aimed to develop optimum condition of phyllantin extraction by non-conventional method namely MAE using IL solvent and compare the yield with conventional (maceration) method with organic solvent. The experimental design variables used in this study were the ratio of the mixture sample-solvent and the concentration of the ionic solvent. The yield of the extract was determined using HPLC (High Performance Liquid Chromatography) with a UV-Vis detector.

2. Materials and Method

2.1 Chemicals

Most of chemicals used in the study were procured from Chengji, China among other 1-decyl-3-methyl-imidazolium chloride, 1-dodecyl-3-methylimidazolium chloride, 1-tetradecyl-3-methylimidazolium chloride, 1-decyl-3-methylimidazolium bromide, 1-ethyl-3-methyl-imidazolium bromide and 1-hexadecyl-3-methylimidazolium bromide. The solvents used in the HPLC analyses were of HPLC grade purchased from Merck as well as phyllanthin standard and the salts (K_2HPO_4, K_2CO_3, Na_2HPO_4). Deionised and distilled water were obtained from Milli-Q system (Millipore, USA).

2.2 Plant material

The sample was obtained from aerial parts of *Phyllanthus niruri*. A total of 800 mg of the whole plant were dried in an oven for 3 days and then crushed into powder using a grinder. The powder sample was stored in a tightly closed container at room temperature prior to analysis.

2.3 Microscopic analysis

The coarse powder was used to study specific fragments and other microscopic characteristics. The slide was prepared by placing a few milligrams of the plant powder and mounted with two drops of acidified chloral hydrate solution and enclosed with the cover slip.

2.4 Ionic liquid preparation

Each ionic solution was dissolved with demineralized water, and made into 3 concentrations (0.25 M, 0.75 M, and 1.25 M). The sample-solvent mixtures were prepared at the following ratio: 1:10, 1:12, and 1:14.

2.5 Ionic liquid-based microwave-assisted extraction

Several screenings were conducted prior to MAE analysis among others irradiation time, salt solvents and ionic liquid using procedure described by previous researcher [18]. One gram of plant powder was dissolved with the selected ionic liquid solvents. The IL concentrations used were 0.25 M, 0.75 M, and 1.25 M with a solvent: sample ratio of 1:10, 1:12, and 1:14. The extractions were conducted 9 runs based on parameter designed by Response Surface Methodology (RSM) with the type of Full Factorial Design (FFD) as shown in Table 1. The output power was 120 W. The extract was filtered through a 0.45 µm membrane. The filtered solution was then divided into three equal solutions. Each of these solutions will be extracted with hexane solvent by a salting-out technique using K_2HPO_4 salt in a separatory funnel. The organic solution was drained and evaporated using a rotary vacuum evaporator (Buchi Switzerland). The extract was redissolved with the mobile phase and then injected into HPLC in duplicate.

Table 1. Parameter variation design.

Run	Factor 1 A: Concentration (mol/L)	Factor 2 B: Rasio (g/mL)
1	0.75	1:14
2	1.25	1:10
3	0.75	1:10
4	0.75	1:12
5	0.25	1:10
6	1.25	1:14
7	1.25	1:12
8	0.25	1:14
9	0.25	1:12

2.6 Methanol based maceration extraction

A total of 50 grams of meniran powder was extracted with 500 mL methanol in a brown bottle. The sample was left overnight and occasionally stirred. After 24 hours, the plant extract was then collected and filtered. The residue was then re-maceration with the addition of 500 mL of solvent. This procedure was then repeated. The filtrate was separated from the solvent using a rotary vacuum evaporator. The viscous extract obtained was calculated for its yield and injected into HPLC.

2.7 HPLC analysis

The HPLC system was using HPLC Shimadzu LC-20AT. The HPLC was equipped with UV-Vis detector (Shimadzu SPD-20A). Chromatographic separation was performed on Inertsil ODS HPLC Column, 5 µm, 250 x 4.6 mm. The mixture of solvent ethanol:water (66:34 v/v) was used as the mobile phase with 1.0 mL/min flow rate, 20 µL injection volume. The absorbance was measured at a wavelength of 229 nm for the detection of phyllathin [19]. Phyllanthin standard was prepared in mobile phase solution at concentration of 200 ppm. The solution was stored at 4°C and protected from light prior to analysis. This HPLC method for the quantitative estimation of phyllanthin was validated by using method validation parameters like precision, accuracy, linearity, LOD, LOQ and system suitability test prior to analysis.

3. Results and Discussion

3.1 Microscopic analysis

The microscopical studies of the coarse powder showed specific fragments among others seed coat, calcium oxalate druse crystals, upper epidermis with prism crystal of calcium oxalate, lower epidermis with stomata (Fig. 1).

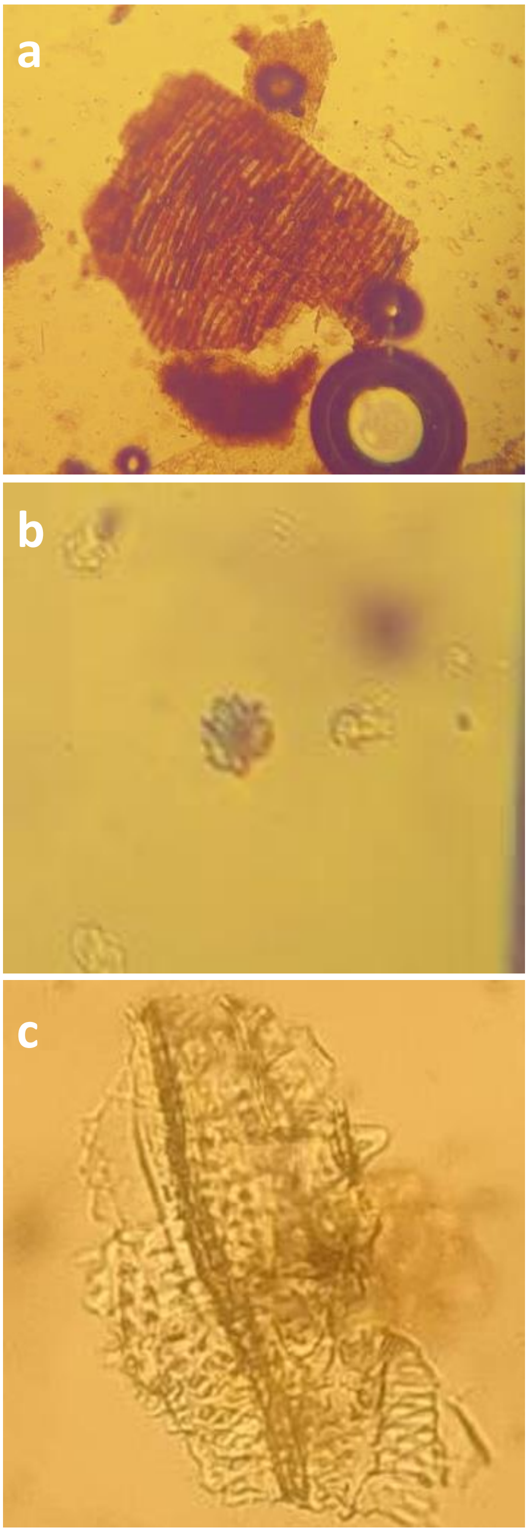

Figure 1. (a) Tangentially fragment of fruit wall, (b) rosette shape of calcium oxalate crystal, (c) vascular bundle, (d) and (e) epidermis with stomata (continued on the next page).

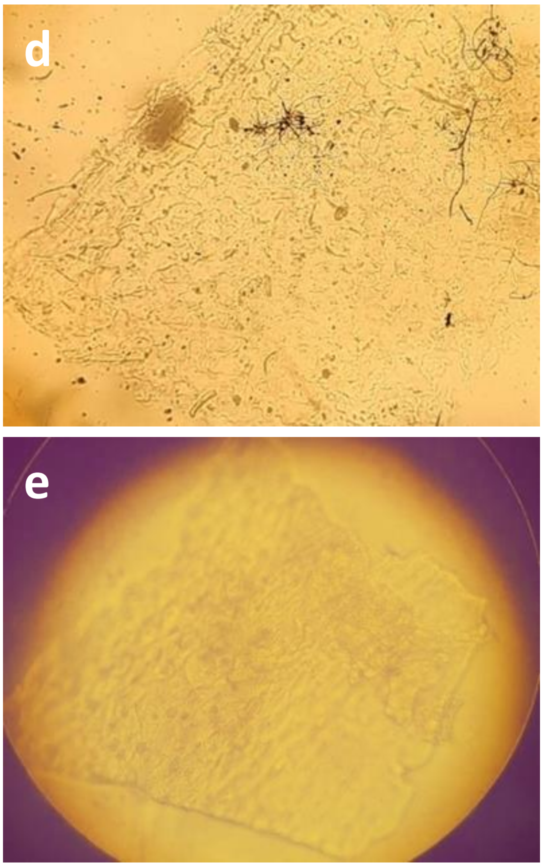

Figure 1. (a) Tangentially fragment of fruit wall, (b) rosette shape of calcium oxalate crystal, (c) vascular bundle, (d) and (e) epidermis with stomata (continued from previous page).

3.2 Methanol based maceration extraction

The crude extract obtained from the maceration technique is shown in the table below (Table 2). The concentration of the extract was found to be 0.1319 mg/g even though the extraction has been replicated 3 times for 3 days. This result is much lower than other research that used other conventional method with methanol solvent [16]. The methanol was chosen as the solvent due to similarity of polarity with phyllanthin [20].

Table 2. Average quantitative data from maceration results with methanol.

Weight (g)	Yield (%)	Area	Concentration (ppm)[a]	Concentration to powder (mg/g)	% Concentration to powder	Concentration to extract (mg/g)
5.05	10.1	2525842	65.32	0.1319	0.013	1.306

[a] standard deviation = 3.75

3.3 Ionic liquid-based microwave-assisted extraction

The extractions were conducted 9 runs to obtain optimum yield of phyllanthin. The parameter variations were designed by the RSM. This methodology is a sum of mathematical and statistical techniques for building empirical models, in which a response is influenced by several variables and the goal is to optimize the response. RSM is useful in statistical experimental designs (particularly two-level factorial or fractional factorial designs), regression modeling techniques, and optimization methods [21]. The results are shown in Table 3. It can be seen from the tables that optimum yields of phyllanthin are 0.1783 mg/g and 0.23 mg/g for imidazolium chloride IL and imidazolium bromide IL, respectively. These values shows that bromide anion-based IL could extract phylanthin better than chloride anion-based IL. Moreover, these yields were achieved at the same solvent concentration of 0.75 M for both IL. Nevertheless, ratios of the sample-solvent were different for both IL i.e., 1:14 for imidazolium chloride IL and 1:12 for imidazolium bromide IL. Imidazolium chloride IL needed higher solvent ratio than imidazolium bromide IL. This indicate that imidazolium bromide IL is more efficient than imidazolium chloride IL. This result is in line with previous research that used imidazolium bromide IL by MAE technique that obtained high yield [18].

Moreover, a lower liquid-solid ratio will induce higher extraction yield. This is because an increase of solvent volume will reduce the extraction efficiency due to ineffective heating by irradiation that caused by shorter irradiation time [22].

As aforementioned, the optimum yields were obtained at high solvent concentration. Lower solvent concentration may cause incomplete extraction resulted in low yield. Commonly, higher IL concentration may increase the yield. However, there is limit of this increase where higher IL concentration will decrease the yield due to increase of viscosity that cause a decrease of mass transfer of the solute of the expected compound [23].

3.4 Comparison of extraction yield of maceration method with MAE method

As aforementioned, the phyllanthin obtained from maceration method was 0.1319 mg/g. However, the phyllanthin yields were found higher from MAE method which were 0.1783 mg/g and 0.23 mg/g for imidazolium chloride IL and imidazolium bromide IL respectively. It can be concluded that the extraction using the IL-MAE method is more efficient in obtaining phyllanthin compounds from meniran herbs compared to the maceration method. This is probably due to the use of microwaves that can damage or break plant cell walls so that the contained compounds can be released and carried away by the solvent better than maceration method [16]. Furthermore, MAE method is more efficient due to shorter extraction time and the use of less solvent.

Table 3. Phyllanthin concentrations obtained from MAE extraction with imidazolium chloride IL (A) and imidazolium bromide IL (B).

Run	Solvent concentration (mol/L)	Ratio sample : solvent	Phyllanthin concentration to coarse powder by imidazolium chloride IL (A) (mg/g)	Phyllanthin concentration to coarse powder by imidazolium bromide IL (B) (mg/g)
1	0.75	1:14	0.1783	0.21
2	1.25	1:10	0.0314	0.07
3	0.75	1:10	0.1212	0.20
4	0.75	1:12	0.1543	0.23
5	0.25	1:10	0.0357	0.11
6	1.25	1:14	0.0592	0.06
7	1.25	1:12	0.0492	0.10
8	0.25	1:14	0.0872	0.10
9	0.25	1:12	0.0423	0.12

4. Conclusion

Ionic Liquid solvent with MAE method (IL-MAE) can effectively extract phyllanthin compounds from meniran herbs compared to conventional method namely maceration method. The IL-MAE method can extract higher phyllanthin compound than maceration method with shorter extraction time and less solvent. Moreover, the present results also demonstrate that the Imidazolium Bromide IL was more effective to extract phyllanthin compound than Imidazolium Chloride IL. In conclusion, IL-MAE with Imidazolium Bromide IL will be a promising extraction technique that is environmentally friendly, effective and efficient.

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