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Study of microwave-assisted extraction of polyphenol from *Phyllanthus urinaria*

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ABSTRACT

Background: Phyllanthus urinaria, found extensively in tropical Asian countries, possesses numerous biological activities attributed to its polyphenol compounds.

Objective: This study aims to optimize polyphenol extraction from Phyllanthus urinaria using microwave assistance.

Materials and Methods: First, the effects of parameters including ethanol concentration (used as the solvent) (40–80% v/v), material-to-solvent ratio (1:10–1:50 w/v), extraction time (30–150 minutes), and extraction temperature (30–70 °C) on conventional extraction were investigated. Subsequently, the effects of microwave pretreatment prior to extraction during the microwave-assisted extraction (MAE) process, including microwave power (100–700 W) and irradiation time (1–9 minutes), were evaluated. Optimal extraction conditions were determined based on total flavonoid content (TFC), total phenolic content (TPC), and antioxidant activity (DPPH scavenging assay) of the extract. The second-order kinetic model was also used to compare the efficiency of extraction methods.

Results: Results indicated that a microwave power of 250 W and irradiation time of 3 minutes were optimal for material pretreatment prior to extraction. Subsequent extraction parameters included ethanol concentration of 60% (v/v), solventto-material ratio of 1:40 (w/v), extraction temperature of 50° C, and extraction time of 60 minutes. Under these conditions, the extract exhibited maximum levels of TPC (277.99 \pm 5.47 mgGAE/gDW), TFC (38.90 \pm 0.58 gQE/gDW), and TEAC $(280.08 \pm 0.75 \mu molTE/gDW)$, which were 22.5%, 36.1%, and 29.4% higher, respectively, compared to the control without microwave treatment.

Conclusion: Furthermore, the second-order kinetic model demonstrated higher initial extraction rate (h), extraction rate constant (k), and extraction capacity (C_e) for MAE compared to conventional extraction.

Keywords: Antioxidant capacity; kinetic model; microwave-assisted extractio; phyllanthus urinaria; polyphenol

BACKGROUND

Phyllanthus urinaria, a member of the *Phyllanthus* genus (Euphorbiaceae), is extensively cultivated in Vietnam and tropical regions. This herbaceous plant is currently used as an ingredient in traditional Asian medicines. 1,2,3 Currently, 93 compounds have been found and structurally identified from *Phyllanthus urinaria* extracts, including 16 tannins, 21 phenolics, 12 flavonoids, 22 lignans, 13 terpenoids, and various secondary metabolites.^{1,4} These compounds, especially polyphenols, have been shown to have the ability to limit the formation and inhibit free radicals, thereby reducing their negative impacts on the body and lowering the incidence of chronic diseases such as diabetes, cancer, hypertension, and cardiovascular diseases.^{5,6} Polyphenols have many applications in pharmaceutical and food technology. The application of polyphenols in food fits the current trend of using natural compounds to replace conventional preservatives to ensure the health of consumers. Polyphenol extract is also used in functional foods thanks to its high antioxidant capacity.

Extraction is one of the well-known techniques to obtain the bioactive compounds; however, bioactive compounds are very sensitive and can be easily decomposed under adverse conditions. Various assistance techniques such as ultrasound, enzyme, and microwave have been used to increase extraction capacity and efficiency. Among these techniques, microwave-assisted extraction (MAE) has recently been widely applied for obtaining bioactive compounds, which significantly improves the extraction yield, and is regarded as green technology.⁷ Microwaves are electromagnetic non-ionizing radiation with frequencies from 300 MHz to 300 GHz, which cause heating based on dipolar rotation and ionic conduction. Dielectric heating from MAE is appropriate for heat-labile bioactive compounds. In addition, MAE has become an effective method for the selective extraction of target compounds in materials due to the difference in microwave absorption among various chemical substances.⁸ Compared to conventional methods, the upsides of MAE methods are shortening extraction time, minimizing organic solvent consumption, and increasing extractabilit.⁹ Water, methanol, and ethanol are the typical polar solvents used in MAE for extracting bioactive compounds from *Phyllanthus*. The obtained extracts are known for their richness in phenolic compounds, flavonoids, and tannins, which may demonstrate varying degrees of antioxidant activity attributed to their hydroxyl groups. Consequently, the

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majority of *Phyllanthus*' bioactivities may be associated with these hydroxyl-rich polyphenol compounds. 10

There have been many studies on the chemical composition, biological and pharmacological activities of *Phyllanthus*.^{1,4,5} Microwave-assisted extraction (MAE) is a highly effective method for extracting polyphenols from plant materials.⁸ Suktham et al. $(2021)^7$ demonstrated the effectiveness of MAE in obtaining high amounts of anthraquinones and polyphenols from *Morinda citrifolia* roots, while Li et al. (2019)¹¹ reported increased polyphenol yields from *Phyllanthus emblica* fruits using MAE. Verma et al.⁹ optimized the extraction of polyphenols from *Phyllanthus amarus* using MAE and found that microwaves significantly reduced extraction time while increasing the TPC yield. However, these studies did not investigate the kinetic parameters of the extraction process, which are crucial for clearly demonstrating the method's effectiveness. For *Phyllanthus urinaria*, ultrasonic-assisted extraction was used to extract polyphenols,¹² but MAE has not yet been applied. Solid-liquid extraction is a multi-component process where mass transfer occurs at varying rates. Kinetic modeling provides valuable insights into the extraction mechanism, aids industrial scaling, and optimizes process design by reducing energy, time, and solvent usage. Lazar et al. (2016) successfully applied the second-order kinetic model to describe polyphenol extraction from spruce bark using ultrasound.¹³ Similarly, Qu et al. (2010) used a kinetic model to compare the extraction processes of polyphenols from pomegranate marc under different parameters, such as temperature, particle size, and solid/solvent ratio.¹⁴ It is apparent that, despite its importance in comparing and optimizing process, extraction kinetics of polyphenol from *Phyllanthus* remain underexplored.

In that context, this study aims to utilize the MAE technique for extracting polyphenols from *Phyllanthus urinaria* and to identify suitable extraction conditions for obtaining polyphenol-rich extracts with high antioxidant activity. Additionally, the kinetic parameters of the extraction processes were determined to compare the efficiency of conventional extraction and MAE methods.

MATERIALS AND METHODS

Chemicals and reagents

Trolox, quercetin, gallic acid, 2,2-diphenyl-1-picrylhydrazyl (DPPH), and Folin-Ciocalteu reagents were supplied by Sigma-Aldrich (USA). All the other chemicals and organic solvents were of analytical reagent grade.

Sample preparation

Phyllanthus urinaria was purchased from the oriental medicines market in District 5, Ho Chi Minh City, Vietnam. The herbs were transported to the laboratory at Saigon Technology University and dehydrated at 60°C in a hot air convection dryer until the moisture content was about 10-12%. The dried herbs were ground into powder, sieved through an 80-mesh screen, and stored in vacuum PA packaging at -10°C for further experiments.

Microwave-assisted extraction procedure

The extraction processes were carried out following the procedure of Verma et al. (2016) with slight modification. ⁹ First, conventional extraction was conducted by mixing the sample with ethanol solution (used as the solvent) in a 200 mL three-necked round-bottom flask, followed by heating in a water bath (Memmert, Germany) and filtering through 20–25 µm paper to obtain the crude polyphenol extract. The investigated extraction conditions included ethanol concentration (40–80% v/v), material-to-solvent ratio (1:10–1:50 w/v), extraction time (30–150 minutes), and temperature (30–70°C).

For microwave-assisted extraction (MAE), the sample was mixed with an ethanol solution at the optimal concentration and solid-to-solvent ratio, determined in a previous conventional extraction experiment. The mixture was then irradiated in a microwave oven equipped with reflux condensation (UWave-2000, SINEO Microwave Chemistry Technology Co., Ltd., operating at 2.45 GHz and 0–1000 W) under irradiation power levels of 100–700 W and times of 1–9 minutes. After microwave pretreatment, extraction was performed at the same optimal temperature and duration as in the conventional process. The experiments were designed as one factor at a time (OFAT), and the TPC, TFC, and Trolox equivalent antioxidant capacity (TEAC) of the extract were evaluated.

Determination of total phenolic content

The TPC was determined as described by Liu et al.¹² 125 μ l of the extract and 125 μ l of Folin-Ciocalteu reagent were added into the test tube. Next, 1.5 ml of distilled water was added, and the mixture was incubated for 6 minutes. Then 1.25 ml of sodium carbonate solution (Na_2CO_3) 7% was added, and the tubes were shaken.

After 90 minutes of reaction without light exposure, the absorbance was determined at 760 nm in a UV-Vis spectrophotometer (10S UV-Vis, Thermo Genesys, USA). The TPC is expressed as milligrams of standard gallic acid equivalent per gram of dried sample (mg GAE/gDW).

Determination of total flavonoid content

The TFC was determined according to the method described in the study of Carmagnani et al.³ 1 ml of the extract and 0.3 ml of 5% sodium nitrite were added in a test tube and mixed well. After 5 minutes, 0.5 ml of 2% aluminum chloride solution was added. After 6 minutes, 0.5 ml of sodium hydroxyl 1M was added; the mixture was shaken for 10 minutes at room temperature, followed by measurement of the absorbance at 415 nm using a UV-Vis spectrophotometer. TFC is expressed as milligrams of standard quercetin equivalent per gram of dried sample (mg QE/gDW).

Determination of Trolox equivalent antioxidant capacity

The extract's TEAC was analyzed using the free radical scavenging method.¹² The reaction medium comprised 150µl of the extract and 2.85ml of DPPH radical solution 0.5 mM in ethanol. The reaction was done after 30 minutes at room temperature without light exposure. Then, the absorbance was measured at 517nm by a UV-Vis spectrophotometer. The extract's antioxidant capacity is expressed in μmol of Trolox equivalent per gram of dried sample (μmolTE/gDW).

Determination of kinetic parameters of the extraction process

The kinetic parameters of the extraction process were determined according to the study of Hong Van Le *et al*.¹⁵ The general second-order model can be written as:

$$
\frac{\mathrm{dCt}}{\mathrm{dt}} = k (C_{\mathrm{e}} - C_{\mathrm{t}})^2 (1)
$$

In which *k* is the second-order extraction rate constant (g DW/mg , min), Ce and Ct (mg/g DW) are the concentrations of the extracted compound at equilibrium and at a given time t, respectively.

The second-order extraction's integrated rate law, considering the boundary conditions from $Ct = 0$ to Ct and $t = 0$ to t, can be expressed as equation (2) or a linearized form given by equation (3). The initial extraction rate (h, mg/gDW.min) equals Ct/t as t approaches 0 and can be determined based on equation (4).

$$
C_t = \frac{Ce^2kt}{1 + Cekt} \tag{2}
$$
 $\frac{1}{Ct} = \frac{1}{kCe^2t} + \frac{1}{Ce} \tag{3}$ $h = kC_e^2 \tag{4}$

Based on equations (3) and (4), C_t can be expressed by equation (5):

$$
\frac{t}{Ct} = \frac{1}{Ce} \cdot t + \frac{1}{h} \quad (5)
$$

Therefore, the initial extraction rate (h), second-order extraction rate constant (k), and extraction capacity (Ce), can be experimentally determined based on the linear equation between t/Ct and t.

Statistical analysis

All experiments were carried out in triplicate. The results in the study were presented as the mean value of three repetitions and standard deviation. One-way ANOVA and Tukey test with p<0.05 were used to determine the significant differences between the means using JMP 13 software.

RESULTS AND DISCUSSIONS

Effects of extraction parameters on the conventional extraction process

Effect of material-to-solvent ratio

The effect of material-to-solvent ratio on total polyphenol compounds and antioxidant capacity of *Phyllanthus urinaria* extract is illustrated in Figure 1.

The results show that the extract's TPC, TFC and TEAC increased as the ratio of material to solvent increased from 1:10 to 1:40. At the ratio of material to solvent from 1:40 to 1:50, the TPC, TFC and TEAC reached the plateau values of 171.44 \pm 2.33 mgGAE/gDW, 21.03 \pm 0.68 mgQE/gDW and 208.05 \pm 2.50 μmolTE/gDW, respectively. These findings align with the mass transfer principle in extraction, where the concentration gradient between liquid and solid phases drives mass transfer. Increasing the material-to-solvent ratio enhances the concentration gradient, thereby improving the diffusibility of compounds into the solvent. However, once equilibrium is reached, the content of extracted compounds does not increase further due to the minimal concentration gradient between phases¹⁶. Additionally, some researchers have suggested that higher solvent amounts lead to greater dissolved oxygen levels in the solvent, which can reduce both polyphenol content and antioxidant activity. Thus, excessively high solvent ratios may not be advantageous for polyphenol extraction. The observed result in this study is consistent with previous studies of polyphenol and other antioxidant compounds extraction from *Centella asiatica,¹⁷ Jasminum subtriplinerve*, ¹⁸ and soybean sprouts.¹⁹

Figure 1. Effect of material-to-solvent ratios on TPC, TFC and TEAC *Different letters on top of bar charts represent the difference between means with p<0.05 Error bars represent the values of ±SD of the means.*

Effect of ethanol concentration

Antioxidants such as polyphenols show different solubility in polar or non-polar solvents. Depending on the structure, the quantity, and the arrangement of the antioxidant groups (-OH radicals) in the raw material, the type of solvent used to extract polyphenols are different.²⁰ Due to its high efficiency and low toxicity, the binary solvent ethanol-water is often used for polyphenol extraction. Bioactive phytochemicals such as phenolics and flavonoids can be dissolved in less polar solvents; the solubility of these compounds is based on their polar properties. Therefore, using ethanol-water at different concentrations to determine the highest extraction yield of polyphenol is crucial.^{19,20}

As seen in Figure 2, the increase in ethanol concentration improved the extraction yield, and the extract's TPC, TFC and TEAC reached the maximum value of 187.32 ± 3.20 mgGAE/gDW, 24.12 ± 0.88 $mgQE/gDW$ and $211.06 \pm 0.54 \mu molTE/gDW$ respectively, at 60% ethanol concentration. The extraction yield of polyphenols and the antioxidant capacity of *Phyllanthus urinaria* extract at 60, 70, and 80% ethanol concentration were insignificant $(p>0.05)$, meaning that exceeded ethanol concentration did not enhance the extractability. These results coincide with the findings of previous studies; the extraction yield of bioactive compounds from soybean sprout, grape, and grape stem^{19,21,22} could be enhanced by using the appropriate ethanol concentration.

Figure 2. Effect of ethanol concentration on TPC, TFC and TEAC *Different letters on top of bar charts represent the difference between means with p<0.05 Error bars represent the values of ±SD of the means.*

Effect of extraction temperature

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The impact of extraction temperature is shown in Figure 3. At 50°C, the extract had the greatest TPC, TFC, and TEAC values, which were $189.46 \pm 3.20 \text{ mg}$ GAE/gDW, $23.48 \pm 1.20 \text{ mg}$ OE/gDW, and $210.50 \pm 1.20 \text{ mg}$ 0.54μ molTE/gDW, respectively. The temperature was found to increase polyphenols extraction yield and aid polyphenols in dissolving better into the solvent. Increasing extraction temperature reduces solvent viscosity and thermal conductivity, increases inter-phase surface which improves mass transfer in extraction due to improved sample wetting and solvent penetration into the sample.²² Nevertheless, degradation and oxidation of polyphenols may occur if the extraction temperature is too high, causing flavor loss and darkening of the extract. The findings of this investigation are consistent with those of Le *et al*. (2019), Arina *et al*. (2019) and Vuong *et al.* (2013). 19,23,24

Effect of extraction time

It is very important to determine the appropriate extraction time because this factor impacts the extraction efficiency, energy consumption, and operation cost. When the extraction time is prolonged, polyphenols are oxidized by exposure to air oxygen and ambient light; polyphenols can also be polymerized to form insoluble compounds that lead to reduced extraction efficiency.²⁵ It is also possible that polyphenols react with other substances in the material causing a change in extraction time.²⁶ Figure 4 shows that the polyphenol content and antioxidant capacity increased during 90 minutes of extraction and reached the highest values at 90 minutes. However, the extraction yield and antioxidant capacity were slightly decreased but not significantly different $(p<0.05)$ when the extraction time was extended to 120-150 minutes. These results followed Fick's second law of the dispersion process, which states that the final equilibrium of solute concentration in plant cells and solvent would be reached in a specific time. ²⁷The study of Thao *et al*. (2018) also reported that the polyphenol extraction yield and antioxidant capacity of *Phyllanthus amarus* were not improved when extending the extraction time. 2

Figure 4. Effect of extraction time on TPC, TFC and TEAC

Different uppercase and lowercase letters on the figures represent the difference between means of TFC, TPC and TEAC with p < 0.05

Effect of microwave pretreatment parameters on MAE process

Effect of microwave power

The solvent-material mixture was treated in a microwave oven for 3 minutes at microwave power from 100W to 700W, and the control was untreated with microwave irradiation. Then, the extraction of polyphenol compounds was conducted under the optimal conditions determined in the previous conventional extraction experiments; the results are shown in table 1.

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Microwave power greatly influences the extraction temperature; increasing the microwave power increases the solvent's temperature. At higher temperature, the viscosity and surface tension of the solvent decrease, the solubility of polyphenol improves, resulting in an increase of extraction efficiency.²⁸ The results in table 1 show that the extraction efficiency improved if irradiation power was 250W; the TPC and TFC increased by 22% and 31% respectively, compared to that of the control. Nonetheless, the extracted phenolic and flavonoid compounds, and antioxidant capacity of the extract could sharply decrease when the exceeded irradiation power of microwave was applied. The primary role of microwave assistance is to break plant cells by vapor pressure, which enhances the release of bioactive compounds into the solvent and aids the increase of extraction efficiency. However, the higher microwave power could increase the process temperature. Thus, excessively high temperature can lead to the heat-sensitive compound's thermal degradation in the plant matrix²⁹. In this study, the selected irradiation power falls into low to mild levels of microwave power, which could avoid the super-boiling phenomenon due to extreme irradiation power.²⁹ The results obtained in this study also agree with previous studies, in which microwave was used to support bioactive compounds extraction from blueberry leaves, sweet potato leaves, and lime peel waste.³⁰⁻³²

Effect of irradiation time

The solvent-material mixture was treated at fixed irradiation power (250W) for 1 to 9 minutes to evaluate the effect of irradiation time, and the control was untreated with microwave irradiation. The further extraction process was then performed under the suitable conditions obtained in the previous conventional extraction experiments.

The observed results in Figure 5 show that 3 minutes of irradiation was appropriate for promoting the extraction yield, and extended irradiation time did not improve the extractability. At suitable irradiation and extraction conditions, the TPC, TFC, and TEAC of the *Phyllanthus urinaria* extract reached maximum values of 277.99 \pm 5.47 mgGAE/gDW, 38.90 \pm 0.58 gQE/gDW and 280.08 \pm 0.75 µmolTE/gDW, 22.5%, 36.1%, and 29.4% higher respectively, compared to that of the control without microwave treatment. The same results were reported by Alara et al. (2018), the authors suggested that the extraction yield could be augmented when irradiation time increased. However, prolonged irradiation leaded to excessively high extraction temperature, which reduced the amount of bioactive compounds of the extract.³³

Different uppercase and lowercase letters on the figures represent the difference between means of TFC, TPC and TEAC with p < 0.05

Kinetics of microwave-assisted extraction

The TPC and TFC obtained during 120 minutes of extraction is shown in Figure 6. The linear forms of the second-order model for phenolics and flavonoids extraction from *Phyllanthus urinaria* are illustrated in Figure 7. The kinetic parameters listed in Table 2 were determined by plotting t/Ct against t and extracting the slope and intercept.

Figure 6. TPC and TFC of the extract during 120 minutes of extraction

Figure 7. Extraction rate reciprocal (t/Ct) of TPC (a) and TFC (b) in the extract at different extraction times (t)

Table 2. The second-order kinetic parameters of conventional and microwave-assisted extractions of polyphenol compounds

		The initial extraction rate, h (mg/gDW.min)	Extraction capacity, C_e (mg/gDW)	The extraction rate constant (k)	\mathbb{R}^2
Phenolic extraction Flavonoid extraction	Control	27.53	241.77	0.00047	0.993
	MAE	85.79	287.32	0.00104	0.998
	Control	2.69	31.75	0.0027	0.989
	MAE	6.30	42.12	0.0036	0.995

The results indicated a good fit of the experimental data to the second-order model (\mathbb{R}^2 from 0.989 to 0.998). The extraction rate constant (k) of flavonoids and phenolics by MAE was higher than that of the conventional extraction by 1.3 and 2.2 times, respectively. In addition, the initial extraction rate (h) of MAE was enhanced by 3.1 times for phenolics and 2.3 times for flavonoids. At small-scale production, microwaveassisted extraction (MAE) is a proven method to reduce extraction time and save energy.²⁸ Figure 6 also shows that with MAE, the highest levels of TPC and TFC were achieved after 60 minutes, with no significant change observed with further extraction time (p>0.05). Conversely, for the control without microwave treatment, the optimal extraction time was 90 minutes. Thus, microwave pretreatment saved 30 minutes of further extraction time, resulting in MAE extract with TPC, TFC, and TEAC of 277.47 ± 3.77 mgGAE/gDW, 38.45 ± 0.54 mgQE/gDW, and 281.2 \pm 0.57 µmolTE/gDW, respectively, representing increases of 22.3%, 35.8%, and 29.6% compared to the control after 90 minutes of extraction. This confirms the significant enhancement in polyphenol extraction efficiency from *Phyllanthus urinaria* with microwave-assisted extraction.

Some authors have reported similar results when using microwaves to support the extraction of polyphenol from plant materials. The study of Verma et al. (2016) found that MAE was the favorable method for bioactive compound extraction from *Phyllanthus amarus*; MAE shortened extraction time and increased the extraction yield compared to Soxhlation and maceration methods.⁹ Research by Alara et al. (2019) showed

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the same kinetic effect when extracting phenolic compounds from *Hibiscus sabdariffa* calyces by MAE method.³⁴ Similarly, MAE could improve the extraction yield of flavonoids from *Terminalia bellirica*. ³⁵ It is clear that, compared with conventional solvent extraction, using MAE for a relatively shorter time can avoid oxidation or decomposition of polyphenols due to high temperature during prolonged extraction.²⁸ This is important for temperature-sensitive biological compounds, especially polyphenols.

In our study, the extraction capacity (C_e) of phenolics and flavonoids (TPC and TFC at the time when the extraction reached equilibrium) was calculated based on the second-order kinetic model and achieved levels of 287.32 mgGAE/gDW and 42.12 mgQE/gDW, 18.8% and 32.7% higher than that of control, respectively. To date, there has been no report using MAE to extract polyphenols from *Phyllanthus urinaria*. In this study, the obtained extraction efficiency was higher than that in previous studies when using ultrasound to support the extraction of phenolics from *Phyllanthus urinaria*¹¹, as well as from *Phyllanthus amarus*². Furthermore, as seen in Table 2, the kinetic parameters of phenolics were much higher than that of flavonoids, which shows that phenolics were extracted more effectively than flavonoids. These results also coincide with previous research proving that phenolics were abundant in the *Phyllanthus* plant.³⁶

Could you add the strengths and limitations of this study?

CONCLUSION

Phyllanthus urinaria is a valuable medicinal herb due to its excellent source of polyphenols with high biological activities. In this study, microwave treatment significantly reduced extraction time, improved the extraction efficiency of total phenolics and flavonoids from *Phyllanthus urinaria,* as well as increased the extract's antioxidant capacity. The optimal MAE conditions included 250W of irradiation power, 3 minutes of irradiation time, ethanol concentration of 60% (v/v), material-to-solvent ratio of 1:40 (w/v), extraction time of 60 minutes, and extraction temperature of 50°C. The observed results reflected that the second-order kinetic model was fitted to describe the microwave-assisted extraction of polyphenols from *Phyllanthus urinaria* with high R^2 values $(R^2>0.99)$.

Further studies should be conducted to identify the phenolic composition and biological activities in the extract, as well as the effects of microwaves on their content and activities, to support the use of the extract in health-beneficial food products.

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