

EFFECTS OF EXTRACTION CONDITIONS ON THE PHYTOCHEMICAL PROFILES AND ANTIOXIDANT ACTIVITY OF *EUPHORBIA HIRTA* L. EXTRACT

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ABSTRACT

Euphorbia hirta L. (Lion's milk in Thai) is a medicinal plant recognized for its diverse therapeutic properties, particularly due to the presence of flavonoids and tannins which exhibit potent antioxidant and anti-inflammatory effects. This study aimed to investigate the impact of different preparation and extraction methods on the total flavonoid and tannin contents, as well as the antioxidant activity of the aqueous extracts. First, the plant samples were prepared by sun-drying. For extraction was conducted using a solid-to-liquid ratio of 1:10 (w/v). Two extraction methodologies were compared: boiling and microwave-assisted extraction (MAE) at various durations. The findings indicated that microwave extraction for 3 minutes yielded the highest concentrations of total flavonoids as 34.20 ± 0.12 μg QE/100 mL and total tannins as 274.63 ± 5.65 μg TAE/100 mL. Furthermore, this condition demonstrated superior antioxidant activity in both DPPH scavenging activity as 20.88 ± 0.16 μg Vitamin C/100 mL and percentage of nitric oxide (NO) radical scavenging activity as $87.76\% \pm 2.61$ when compared to other treatments. In conclusion, microwave-assisted extraction is a highly efficient and "green" method for maximizing the recovery of bioactive compounds and enhancing the biological activity of *E. hirta* L.

Keywords: *Euphorbia hirta* L.; Antioxidant activity; Microwave extraction; Phytochemical contents

1. INTRODUCTION

Currently, herbal drinks have emerged as a prominent alternative both domestically and internationally. It is an extract derived from various plant components, including flowers, leaves, roots, fruits, seeds, vegetables, and grains [1, 2, 3]. Fresh or dried herbs are processed by chopping or grinding into powder, followed by brewing or boiling with water and subsequently squeezing to separate the pulp, resulting in a beverage for health purposes [4, 5]. Numerous extraction methods exist to obtain medicinally beneficial active ingredients from herbal plants, including ultrasonic-assisted extraction, enzyme-assisted extraction, pulsed electric field extraction, and microwave-assisted extraction, which are modern techniques often associated with high costs [6, 7]. Notably, microwave extraction can be conducted using a household microwave oven, thereby reducing the need for expensive equipment.

Previous research conducted by Meechai and Baka [8] examined the impact of extraction methods on the concentration of phytochemicals in roselle, revealing that microwave extraction at 100 watts for 30 minutes yielded higher total phenolic and flavonoid contents than alternative methods. Bilhman et al. [9] employed microwave-assisted extraction (MAE) using a glycerol-ethanol mixture to obtain extracts from the unripe banana fruit, which extract demonstrated significant radical scavenging activity in both DPPH and ABTS assays, indicating strong antioxidant properties. Phaisan et al. [10] reported that MAE was used to extract *Centella asiatica* (L.) to provide a high bioactive compound yield and high antioxidant activity. Moreover, preparing herbal samples prior to extraction also influenced phytochemical yields [11].

Lion's milk, scientifically designated as *Euphorbia hirta* L., belongs to the Euphorbiaceae family and serves as a medicinal plant with a variety of therapeutic properties. It has been employed to treat poisoning, facilitate milk expulsion, address rashes, and manage conditions such as acute enteritis, bacterial dysentery, and hematuria [12]. Previous studies have identified significant phytochemical groups, including flavonoids, tannins, terpenoids, and steroids. Additionally, various biological activities have been documented, such as antioxidant, anti-inflammatory, antimicrobial, antidiabetic, and antitumor properties [13].

Recent studies have increasingly focused on *Euphorbia hirta* L. as a rich source of phenolic compounds with notable antioxidant properties. Several studies have demonstrated that both aqueous and organic solvent extractions of *Euphorbia hirta* yield significant phytochemicals and exhibit strong antioxidant activities, particularly due to the presence of phenolic and flavonoid compounds [14, 15]. In particular, aqueous extracts have been reported to possess considerable radical scavenging activity, confirming that water can serve as an effective and practical extraction solvent [16]. Furthermore, microwave-assisted extraction of *E. hirta* has been widely investigated, with extraction parameters such as time and solvent ratio significantly influencing phenolic yield

[17]. Optimization studies have demonstrated that extraction time is a critical factor affecting both yield and antioxidant activity [18]. In addition, studies on the Euphorbiaceae family have emphasized the importance of optimizing extraction conditions to maximize phenolic recovery while minimizing degradation [19, 20].

The objective of this research is to investigate the effects of lion's milk extract preparation by assessing the impacts of extraction methods (boiling and microwave) for varying durations on the concentrations of total flavonoids and tannins, which are crucial substances contributing to antioxidant properties [21]. The findings of this research will inform future efforts in developing health products.

2. MATERIALS AND METHODS

2.1 Sample collection and preparation

In this study, the arial part of the *Euphorbia hirta* L. was collected in November 2023 at the Lalo sub-district, Rusoh district, Narathiwat province, Thailand. The sample was clean and dried in the sun-drying for 5-7 days. Grind the sample finely, then store it in a bag and place it in the desiccator [22].

2.2 Sample extraction

The extraction methods are divided into two methods: boiling and microwaving. Weigh the sample and add distilled water in a ratio of 1:10 (sample weight in g/solvent volume in mL). In the boiling method, the sample is boiled for 10, 20, 30, 40, and 50 mins. Microwave method, the energy is used at 200 watts for 1, 2, 3, 4, and 5 mins-(shown in Table 1). After that, let it cool to room temperature, then centrifuge at 5,000 rpm for 10 mins. Finally, separate the supernatant and store it in plastic bottles before analysis [23].

Table 1 Symbols and condition of extraction methods

Symbol	Methods	Times (mins)
BE-T10	Boiling method	10
BE-T20	Boiling method	20
BE-T30	Boiling method	30
BE-T40	Boiling method	40
BE-T50	Boiling method	50
ME-T1	Microwave method	1
ME-T2	Microwave method	2
ME-T3	Microwave method	3
ME-T4	Microwave method	4
ME-T5	Microwave method	5

2.3 Determination of phytochemical contents

The total flavonoid content was determined using the aluminum chloride colorimetric method, following the protocol described by [24, 25], with quercetin used as a standard. The extract was diluted tenfold with distilled water. Then, 100 μL of the diluted extract was transferred into a 96-well microplate, followed by the addition of 100 μL of 2% AlCl_3 solution. The mixture was incubated at room temperature for 15 minutes, after which the absorbance was measured at a wavelength of 435 nm using a microplate reader. The total flavonoid content was calculated from the standard curve of quercetin and expressed as micrograms of quercetin equivalent (QE) per 100 milliliters of extract ($\mu\text{g QE}/100 \text{ mL extract}$).

The total tannin content was determined using a modified version of the method proposed by [26]. The extract was diluted tenfold with distilled water. A volume of 8.2 μL of the diluted extract was mixed with 10.2 μL of distilled water, 8.2 μL of Folin-Ciocalteu reagent, and 81.6 μL of 7% (w/v) Na_2CO_3 solution. The mixture was shaken and kept in the dark for 90 minutes. Absorbance was then measured at 700 nm. The total tannin content was calculated using a standard curve of tannic acid and expressed as micrograms of tannic acid equivalent (TE) per 100 milliliters of extract ($\mu\text{g TE}/100 \text{ mL extract}$).

2.4 Determination of antioxidant activities

The DPPH (2,2-diphenyl-1-picrylhydrazyl) assay was used to evaluate the antioxidant activity of the extract, following the method described by [24, 27]. Vitamin C was used as the standard. The extract was diluted tenfold with distilled water. A volume of 100 μL of the diluted extract was transferred into a 96-well microplate, followed by the addition of 100 μL of 0.3 mM DPPH solution in ethanol. The mixture was incubated in the dark at room temperature for 15 minutes. Absorbance was then measured at a wavelength of 517 nm using a microplate reader. The antioxidant capacity of the extract was expressed as micrograms of vitamin C equivalent ($\mu\text{g Vitamin C}/100 \text{ mL extract}$).

The nitric oxide scavenging activity was evaluated using the sodium nitroprusside method, as described by [24], with vitamin C used as the standard. The extract was diluted tenfold with distilled water. Then, 100 μL of the diluted extract was added to a 96-well plate, followed by 50 μL of 10 mM sodium nitroprusside in phosphate buffer (pH 7.4). The mixture was incubated in the dark at 25 $^{\circ}\text{C}$ for 90 minutes. After incubation, 100 μL of Griess reagent was added and further incubated for 30 minutes. Absorbance was measured at a wavelength of 577 nm. The results were reported as the percentage of nitric oxide scavenging activity (1).

$$\% \text{ Nitric oxide Scavenging} = \frac{(\text{Abscontrol} - \text{Abssample})}{\text{Abscontrol}} \times 100 \quad (1)$$

2.5 Statistical analysis

The results were averaged ($n=3$) and the standard deviation (SD) was calculated. The data were then analyzed statistically using One-Way ANOVA and Pearson correlation coefficient at a 95 % confidence level ($p<0.05$).

3. RESULTS

The phytochemical contents and antioxidant activities of the microwave extraction method were displayed higher than those of the boiling method. Additionally, extraction takes less time. Microwave extraction for 3 minutes resulted in TFC and TTC that were higher than other durations, with values of 34.20 ± 0.12 μg QE/100 mL extract and 274.63 ± 5.65 μg TAE/100 mL extract, respectively. As for the DPPH scavenging and % nitric oxide scavenging activities, it was found that microwave extraction for 3 minutes yielded the highest values, which were 20.88 ± 0.16 μg Vitamin CE/100 mL extract and 87.76 ± 2.61 %, respectively. A 30-minute extraction interval produced higher phytochemical contents and antioxidant activity than other time periods for the boiling procedure (Table 2 and Figure 1). According to recent studies, the ethanol extract of lion's milk with reflux extraction showed more than 60 % antioxidant activity at a concentration of 0.5 mg/mL [28], DPPH scavenging activity was lower than previously reported, despite the study's findings. It makes use of easier extraction techniques and eco-friendly solvents.

Table 2 Influence of preparation *Euphorbia hirta* L. aqueous extract on the phytochemical contents and antioxidant

Sample	TFC (μg QE/100 mL extract)	TTC (μg TAE/100 mL extract)	DPPH scavenging activity (μg Vitamin CE/100 mL extract)	% NO scavenging activity
BE-T10	27.58 ± 0.27^d	205.98 ± 4.21^e	17.96 ± 0.04^{de}	55.96 ± 4.25^{de}
BE-T20	28.83 ± 0.02^c	211.02 ± 1.90^{de}	18.96 ± 0.24^{cd}	62.36 ± 1.85^{cd}
BE-T30	29.76 ± 0.09^b	211.53 ± 4.16^{de}	19.35 ± 0.14^{bc}	66.38 ± 2.06^c
BE-T40	24.53 ± 0.60^f	187.90 ± 5.64^f	16.55 ± 0.07^f	51.22 ± 4.73^{ef}
BE-T50	21.02 ± 0.85^g	155.14 ± 4.47^g	14.65 ± 0.57^g	46.84 ± 3.18^{fg}
ME-T1	17.89 ± 0.20^h	184.37 ± 6.15^f	17.79 ± 0.34^e	60.53 ± 4.26^{cd}
ME-T2	25.17 ± 0.17^{ef}	241.62 ± 16.12^{bc}	18.53 ± 0.24^{cd}	75.69 ± 0.81^b
ME-T3	34.20 ± 0.12^a	274.63 ± 5.65^a	20.88 ± 0.16^a	87.76 ± 2.61^a
ME-T4	26.46 ± 1.12^{de}	249.35 ± 8.06^b	20.59 ± 0.25^a	43.64 ± 3.47^g
ME-T5	24.09 ± 0.24^f	223.95 ± 2.21^{cd}	20.02 ± 0.21^{ab}	41.81 ± 2.05^g

Note: Values are mean $\pm$ SD and mean values followed by the same letters are not significantly different at ($p<0.05$)

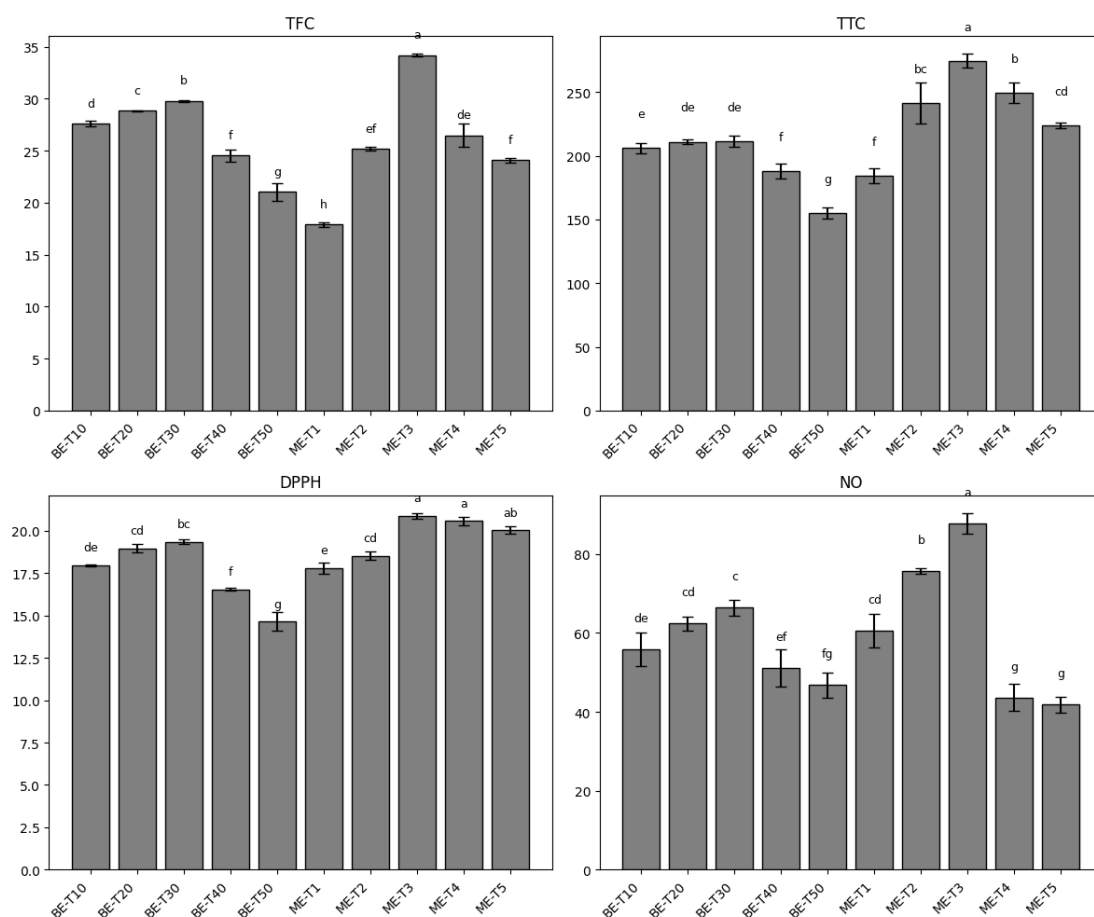


Figure 1 Effects of boiling extraction (BE) and microwave-assisted extraction (ME) on total flavonoid content (TFC), total tannin content (TTC), DPPH radical scavenging activity, and nitric oxide (NO) scavenging activity. Values are expressed as mean $\pm$ standard deviation. Different letters indicate significant differences ($p < 0.05$)

4. DISCUSSION AND SUGGESTION

In the study of extraction methods, both boiling and microwave, presented that the number of active chemicals and bioactivities decreased with increasing extraction time, which may be related to the stability of certain important substances that cannot withstand prolonged heat [8]. Additionally, the microwave extraction method displayed a higher yield of phytochemical contents than boiling method, because electromagnetic waves enable modifications in mass transport, leading to increased efficiency compared to conventional heating techniques [29]. The results indicated that microwave-assisted extraction reached its optimum at 3 min, which is consistent with previous studies reporting that maximum phenolic recovery is typically achieved within a short irradiation time due to rapid cell wall disruption and enhanced mass transfer [30]. Prolonged extraction time (4–5 min) resulted in a significant decrease in antioxidant activity,

particularly NO scavenging activity. This reduction can be attributed to thermal and oxidative degradation of phenolic compounds. Previous studies have demonstrated that excessive microwave exposure leads to the formation of reactive oxygen species such as hydroxyl radicals and hydrogen peroxide, which degrade phenolic structures and reduce antioxidant capacity [31].

Table 3 Correlation between phytochemical contents and antioxidant activities

Boiling method	DPPH scavenging activity	Nitric oxide scavenging activity
TFC	0.998	0.814
TTC	0.975	0.885
Microwave method	DPPH scavenging activity	Nitric oxide scavenging activity
TFC	0.868	0.951
TTC	0.804	0.863

Note: Pearson correlation coefficients (r) were used to evaluate the relationships between total flavonoid content (TFC), total tannin content (TTC), and antioxidant activities, including DPPH radical scavenging activity and nitric oxide (NO) scavenging activity. All correlations were statistically significant at the 95% confidence level ($p < 0.05$).

The correlation of phytochemical contents and antioxidant activities is shown in Table 3. The correlation showed a strong positive relationship for all data. These data indicated that the yield of phytochemicals might be affected by the potential on the DPPH scavenging and Nitric oxide scavenging activities. The previous study reported that the strong correlation of total flavonoid content and total tannin content with antioxidant capacity is due to flavonoid compounds and tannins contributing to the antioxidant of extracts [32]. Furthermore, this might suggest that the extract's flavonoid and tannin structures have a favorable activity profile for DPPH and Nitric oxide scavenging [24, 32].

This study's results highlight the extraction methods utilizing water as a solvent. The results suggest that the microwave extraction method, which is a rapid technique, has high flavonoid and tannin contents and antioxidant activities. Compared to conventional heating methods, the microwave extraction technique is more efficient. With a total tannin content (TTC) of 274.63 ± 5.65 $\mu\text{g TAE}/100$ mL and a total phenolic content (TFC) of 34.20 ± 0.12 $\mu\text{g QE}/100$ mL, the microwave extraction method proved to be more effective than boiling. Three minutes was the ideal microwave extraction duration for antioxidant activity and phytochemical content. This involved eliminating nitric oxide ($87.76 \pm 2.61\%$) and DPPH free radicals (20.88 ± 0.16 $\mu\text{g Vitamin CE}/100$ mL). However, the efficiency decreases as the extraction time increases. This might be

related to the stability of important materials that cannot withstand high temperatures. Furthermore, the phytochemical concentration and antioxidant activity were positively correlated. Potentialities are associated with the structure of flavonoids and tannins. Thus, the microwave method of extracting lion's milk with water as a solvent is an intriguing way to make herbal drinks and conduct additional research in other areas. This method offers high concentrations of significant chemicals, is environmentally benign, and shortens the extraction time.

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