The extraction procedure of a pluthiophenol-rich extract from the leaves of *Pluchea indica* L. collected in Hanoi

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ABSTRACT

In this study, we evaluated some conditions affecting the extraction of pluthiophenol rich in pluthiophenol from the leaves of Pluchea indica collected in Hanoi. The investigated factors included extraction method, extraction solvent, medicinal plant size, material mass/solvent volume ratio, extraction time, and number of extraction times. Additionally, several methods of refining and enriching pluthiophenol in the Pluchea indica extract were also investigated. The criteria used to evaluate and select extraction conditions were the pluthiophenol content by the HPLC method. The best results included using hot extraction at 80 °C, extraction solvent of 60% ethanol, material size of 1 mm, extraction three times and 2 hours each time, with material/solvent ratios of 1/8, 1/6, and 1/6 (g/mL). Refining and enriching the pluthiophenol component was done using a liquid-liquid distribution and column chromatography (Amberlite XAD4 polymer adsorbent). The obtained extract after refining had a pluthiophenol content of 6.12%, and the pluthiophenol recovery efficiency of the whole process was 79.74% on a laboratory scale (50 g). In the 1 kg scale extraction procedure, the obtained extract had a pluthiophenol content of 6.29%.

Keywords: Pluthiophenol; Extraction; *Pluchea indica*.

1. INTRODUCTION

Pluchea indica (L.) Less is a widely distributed plant species in Vietnam and several Asian countries. It possesses significant potential for applications in both traditional and modern medicine due to its biologically active compounds [1]. Previous studies have identified thiophenes as the major chemical constituents responsible for the plant's antibacterial, anti-inflammatory, antioxidant, and anticancer activities [2]. Notably, pluthiophenol - a compound isolated from *Pluchea indica* - has exhibited moderate cytotoxicity activity against several cancer cell lines, including K562, HepG2, SW626 [3]. Despite these promising properties, an optimized extraction process for pluthiophenol from locally sourced plant materials, particularly leaves collected in the Hanoi region, has not yet been established. This study aims to evaluate the effects of extraction methods and key technological parameters on the efficiency of pluthiophenol extraction from *Pluchea indica* leaves. The pluthiophenol content in the resulting extracts was quantified using high-performance liquid chromatography (HPLC) [4]. The findings provide a scientific foundation for future applied research and the development of medicinal products derived from *Pluchea indica*.

2. MATERIALS AND METHODS

2.1. Research subject

The research material consisted of *P. indica* L. leaves collected from Canh Dien, Hanoi, Vietnam. The plant samples (Moisture content 8.15%) were dried and preserved in sealed plastic bags for use in the research process. The specimen was re-identified by Dr. Nguyễn Thị Kim Thanh from the University of Science in 2023.

2.2. Solvents and chemicals

Reference substance: Pluthiophenol (purity 98.20% based on peak area).

Solvents: 95% ethanol (food grade), methanol, double-distilled water.

Chemicals and reagents: Phosphoric acid (Merck), methanol, acetonitrile (Merck), polyme Amberlite XAD4 (is a nonionic (neutral) polymer resin made from crosslinked polystyrene-divinylbenzene)

2.3. Research methods

2.3.1. HPLC method for quantification of Pluthiophenol in the extract of Pluchea indica leaves

Reference solution: A stock solution of pluthiophenol was prepared by dissolving the compound in methanol to a final concentration of 1 mg/mL. Working standard solutions were prepared by a serial dilution of the stock solution with water to obtain the desired concentrations.

Test solution: Extract: Approximately 10 mg of the dried extract was accurately weighed, dissolved in an appropriate solvent, and diluted to a final volume of 10 mL to obtain the test solution.

Sample preparation: All solutions were filtered through a 0.45 μm PTFE membrane filter prior to HPLC analysis.

Chromatographic conditions: Column: Agilent Eclipse Plus C18 (4.6 × 250 mm, 5 μm); Mobile phase: Acetonitrile – 0.1% phosphoric acid in water (40:60, v/v); Flow rate: 1.0 mL/min; Column temperature: 40 ºC; Injection volume: 20 μL; Detection wavelength: 320 nm.

Quantification of the Test Sample Inject the test solutions and determine the content in the test sample using the following formula:

$$X (\%) = \frac{C \times V \times 100}{m \times 1000000} \times \frac{100}{100 - x}$$

X: Content of the analyte in the herbal material/extract (%); C: Concentration of the analyte in the test solution (μg/mL); V: Volume of the test solution (mL); m: Weight of the herbal material/extract (g); x: Moisture content of the herbal material/extract (%)

2.3.2. Development of an Extraction Procedure at a 50 g/Batch Scale

Extraction scale: 50 g of herbal material per batch

Investigated factors: Extraction solvent: 50% ethanol, 60% ethanol, 70% ethanol, 80% ethanol, 90% ethanol, 50% methanol, 70% methanol, methanol,... Extraction time: 1 hour, 2 hours, 3 hours, 4 hours. Number of extractions: 1, 2, 3 times. Solvent-to-material ratio (mL/g): corresponding to two extractions as 7/1 and 5/1; 9/1 and 7/1; 10/1 and 8/1; 12/1 and 10/1; or corresponding to three extractions as 6/1, 6/1, and 4/1 (denoted as 6/6/4) or 8/1, 6/1, and 4/1 (denoted as 8/6/4)

Evaluation Criteria:

- High extraction yield: determined based on the following formula:

$$H_1 (\%) = \frac{m_{cao} \times 100}{m_{dl}} \times \frac{100 - a_{cao}}{100 - a_{dl}}$$

m_{cao}: Weight of the extract (g); m_{dl}: weight of the herbal material (g); a_{cao}: moisture content of the extract (%); a_{dl}: moisture content of the herbal material (%)

- Active compound extraction yield in the extract:

The pluthiophenol content was determined based on the method described in section 2.3.1.

Absolute pluthiophenol extraction yield from the herbal material:

$$H_2 (\%) = \frac{C \times V \times 100}{m_{dl} \times 1000000} \times \frac{100}{100 - a_{dl}}$$

C: pluthiophenol content in the extract (μg/mL); V: volume of the extract (mL); m_{dl}: weight of

the herbal material (g); a_{dl}: moisture content of the herbal material (%)

Relative pluthiophenol extraction yield:

$$H_3 (\%) = \frac{H_2 \times 100}{X}$$

H_2: Absolute pluthiophenol extraction yield from the herbal material (%); X: Pluthiophenol content in the input herbal material (%)

2.3.3. Investigation of Impurity Removal Method

Survey scale: 10 g of extract per batch. Method: Investigation of impurity removal methods: precipitation with water, liquid-liquid extraction, etc. Evaluation criteria: Active compound content in the extract after impurity removal:

Active compound recovery yield: determined based on the following formula:

$$\text{Active ingredient recovery efficiency (\%)} = \frac{M_s \times (100 - a_s) \times X_s \times 100\%}{M_t \times (100 - a_t) \times X_t}$$

m_s, a_s, X_s: weight (g), moisture content (%) and active compound content (%) of the extract after impurity removal; m_t, a_t, X_t: weight (g), moisture content (%) and active compound content (%) of the extract before impurity removal.

2.3.4. Upgrading the extraction process to a 1 kg/batch scale

Based on the optimized conditions identified at the 50 g/batch scale, the extraction process was scaled up to a 1 kg/batch scale. A total of six batches were processed under these conditions. The process was evaluated for consistency, efficency and reproducibility at the larger scale. When necessary, adjustments to extraction parameters were made to ensure scalability and maintain extration efficiency.

3. RESEARCH RESULTS

3.1. Investigation of various factors on the extraction process

Dried extracts were prepared from 50 g batches of *P. indica* leaves with an initial moisture content of 8.15% and a pluthiophenol content of 0.04 1% in the raw material.

3.1.1. Investigation of extraction methods

To determine optimal extraction conditions and better understand the extraction behavior of *P.indica* leaves, two extraction methods were investigated. Reflux extraction: conducted at the boiling temperature of the solvent. Maceration (soaking): performed at room temperature (~ 30 ºC at the time of experiment)

The investigation method was as follows:

Sample 1 (Reflux extration): Herbal material was extracted with methanol by refluxing at 70 ºC, in two successive cycles, each lasting 2 hours. Solvent-to-material ratios were 9/1 and 7/1 (mL/g). Sample 2 (Room temperature soaking): Herbal material was extracted with methanol by soaking at room temperature for 24 hours per cycle, in two extraction cycles. Solvent-to-material ratios were 9/1 and 7/1 (mL/g).

***Table 1.** Results of the extraction method investigation.*

Extraction Method	Average extraction efficiency (%)	Absolute pluthiophenol extraction efficiency (%)	Pluthiophenol extraction efficiency (%)
Soaking	7.38	0.030	73.91
Reflux	11.06	0.036	87.20

The results demonstrated that the reflux extraction method at 70 ºC using methanol yielded a

higher overall extraction efficiency and significantly enhanced pluthiophenol recovery compared to the maceration method. Specifically, the reflux method achieved an extraction yield of 11.06% and a pluthiophenol recovery of 87.20% after two extractions cycles. In contrast, room temperature soaking yielded only 7.38% and 73.91% respectively. In addition to improved efficiency, reflux extraction required substantially less time and utilized readily available and easy-to-operate equipment. Based on these advantages, reflux extraction at 70ºC using methanol as the solvent was selected for further experiments.

3.1.2. Investigation of extraction solvents

To evalutate the influence of solvent composition on extraction efficiency, several solvents were tested: 50%, 60%, 70%, 80%, and 90% ethanol, as well as 70% methanol. Experimental procedure: Approximately 50.0 g of *P.indica* leaf material (moisture content of 8.15%) was weighed for each trial. The herbal material was refluxed using the specified solvent at 70 ºC for methanol-containing solutions and 80 ºC for ethanol-containing solutions. Each sample underwent two extraction cycles, with solvent-to-material ratios of 9/1 and 7/1 (mL/g), and each extraction lasting 2 hours.

Table 2. *Results of the solvent investigation.*

Solvent	Average extraction efficiency (%)	Absolute pluthiophenol extraction efficiency (%)	Pluthiophenol extraction efficiency (%)
Methanol	11.06	0.036	87.20
Methanol 70%	16.56	0.029	70.87
Ethanol 90%	12.79	0.030	74.10
Ethanol 80%	15.38	0.034	82.49
Ethanol 70%	18.70	0.035	85.32
Ethanol 60%	22.78	0.036	88.98
Ethanol 50%	23.77	0.035	85.80

The results indicated that 50% ethanol and 60% ethanol provided the highest overall extraction yields, achieving 23.77% and 22.78% respectively, after two extraction cycles. However, in terms of pluthiophenol recovery, methanol and 60% ethanol were the most effective, with recovery rates of 87.20% and 88.98%, respectively. Although methanol yielded a higher pluthiophenol concentration in the extract (11.06% extraction yield), its total extract mass was significantly lower than that obtained with 60% ethanol. This suggested that methanol extracts a smaller total amount of material but concentrates pluthiophenol more effectively. However, due to its high toxicity and unsuitability for pharmaceutical and food related applications, methanol is rarely used in practical large-scale production. Therefore, ethanol 60% was selected as the optimal solvent for subsequent studies, offering a favorable balance of safety, extraction efficiency and pluthiopenol content.

3.1.3. Investigation of the number of extractions and solvent-to-material ratio

To determine the influence of the number of extractions and the solvent-to-material ratio on the extraction of pluthiophenol from *P. indica* leaves, experiments were conducted with the following solvent-to-material volume/weight ratios (mL/g). Two extractions: 7/1 and 5/1 (denoted as 7/5); 9/1 and 7/1 (denoted as 9/7); 10/1 and 8/1 (denoted as 10/8); 12/1 and 10/1 (denoted as 12/10); And three extractions: 7/1, 7/1, and 5/1 (denoted as 7/7/5) or 8/1, 6/1, and 6/1 (denoted as 8/6/6).

Table 3. *Results of the investigation of solvent-to-material Ratio and number of extractions.*

Solvent Ratio	Average extraction efficiency (%)	Absolute pluthiophenol extraction efficiency (%)	Pluthiophenol extraction efficiency (%)
7/5	21.34	0.036	89.82
9/7	22.78	0.037	90.64
10/8	24.29	0.038	92.32

12/10	25.49	0.038	93.27
7/5/5	24.62	0.038	92.34
8/6/6	27.31	0.039	95.57

The results showed that increasing the solvent–to-material ratio during two extraction cycles improved both extraction yield and pluthiophenol recovery. Specifically, the dried extract increased from 21.34% to 25.49%, and pluthiophenol extraction yield rose from 89.82% to 93.27% as the solvent-to-material ratio increased from 7/1 and 5/1 to 12/1 and 10/1. When the material was extracted three times, both the dry extract yield and the pluthiophenol recovery continued to increase with higher solvent volumes. The best results were obtained with solvent-to-material ratios of 8/1, 6/1, and 6/1, yielding a dry extract of 27.31% and a pluthiophenol extraction yield of 95.57%. This total solvent volume (20 mL/g) was more efficient than the 22 mL/g used in the two extraction scheme (12/1 and 10/1), both in terms of extract yield and pluthiophenol recovery. In conclusion, extracting the herbal material three times with solvent-to-material ratios of 8/1, 6/1, and 6/1 (mL/g) provided the best balance between solvent usage and extraction efficiency and was selected as the optimal condition for subsequent experiments.

3.1.4. Investigation of extraction time

To identify the optimal extraction time per cycle, experiments were conducted with durations of 1, 2, 3, and 4 hours per extraction. The experiments procedure: Approximately 50 g of herbal material was subjected to reflux at 80 ºC using 60% ethanol as the solvent. The extraction was performed three times per batch, using solvent-to-material ratios of 8/1, 6/1, and 6/1 (v/w) for each cycle. The only variable was the duration of each extraction (1- 4 hours), which was kept constant across all three cycles within a given trial.

Table 4. *Results of the investigation of the extraction time.*

Time	Average extraction efficiency (%)	Absolute pluthiophenol extraction efficiency (%)	Pluthiophenol extraction efficiency (%)
1 hour	20.97	0.036	87.36
2 hour	27.31	0.039	95.57
3 hour	27.87	0.040	95.80
4 hour	27.99	0.039	95.65

The yield increased significantly from 20.97% to ~27.31% when the extraction time was set to 2 hours per cycle over three extractions. Extending the time to 3 or 4 hours showed only slight improvements. Thus, three 2-hour extractions are sufficient. The optimal conditions for extracting pluthiophenol from *P. indica* leaves are: 60% ethanol as solvent, reflux at 80 °C, three sequential extractions (solvent-to-material ratios: 8:1, 6:1, 6:1 mL/g), and 2 hours per extraction. Extracts are combined and concentrated under reduced pressure

3.2. Investigation of impurity removal method

Survey scale: 10 g of extract per trial. Evaluation criteria: Pluthiophenol content in the extract after impurity removal, Recovery efficiency of pluthiophenol, Feasibility of the method.

3.2.1. Investigation of impurity removal method

To identify an effective method for purifying the extract, the following methods were evaluated:

- Method 1: Weigh approximately 10 g of extract, add 25 mL of distilled water, and sonicate for 30 minutes to ensure uniform dispersion. Centrifuge the mixture, collect the precipitate, and dry it under suitable conditions.

- Method 2, 3: Weigh approximately 10 g of extract, add 50 mL of distilled water, sonicate for 10 minutes to achieve uniform dispersion. Perform liquid-liquid extraction with *n*-hexane or

dichloromethane solvent at a ratio of 1/1 (mL/mL), repeated 3 times. Combine the extracts, concentrate to remove the solvent, and collect the *n*-hexane or dichloromethan fraction.

***Table 5.** Results of the investigation of impurity removal method.*

Method	Extract mass (g)	Extract content (%)	Extract mass after purification (g)	Pluthiophenol content after purification (%)	Pluthiophenol recovery efficiency (%)
Method 1	10.62	0.14	0.71	0.92	43.50
Method 2	10.12	0.14	0.38	2.27	59.74
Method 3	10.3	0.14	0.92	0.85	54.67

The results of the investigation demonstrated that the impurity removal by liquid-liquid extraction with *n*-hexane (Method 2) resulted in the highest pluthiophenol content in the extract, reaching 2.27%. This was significantly higher than the content obtained using dichloromethane (Method 3) and water precipitation (Method 1), which yielded pluthiophenol contents of 0.85% and 0.92%, respectively. Moreover, *n*-hexane extraction also showed the highest recovery efficiency of pluthiophenol among the three methods evaluated. Therefore, liquid-liquid extraction with *n*-hexane was selected as the preferred method for purifying the extract from *P. indica* leaves.

3.2.2. Investigation of the number of liquid-liquid extractions

To determine the optimal number of *n*-hexane extractions, approximately 10 g of the crude extract was accurately weighed and dispersed in 50 mL of water. The mixture was sonicated for 10 minutes to ensure uniform dispersion. Liquid-liquid extraction was then performed using *n*-hexane, at a 1/1 ratio (mL/mL). This extraction process was repeated six times. After each extraction, the *n*-hexane layer was collected and concentrated under reduced pressure to remove the solvent and obtain the corresponding extract fraction..

***Table 6.** Results of the investigation on the number of liquid-liquid extractions with n-hexane*

Extraction No.	Mass of Extract (g)	Pluthiophenol Content (%)	Extraction No.	Mass of Extract (g)	Pluthiophenol Content (%)
1	0.15	2.16	4	0.03	2.56
2	0.11	2.31	5	0.02	2.61
3	0.06	2.30	6	0.01	2.20

The results of the investigation showed that, starting from 10 g of extract with a pluthiophenol content of 0.14%, liquid-liquid extraction with *n*-hexane yielded 0.15 g and 0.11 g of extract after the first and second extractions, with pluthiophenol contents of 2.16% and 2.31%, respectively. From the third extraction onwards, although the pluthiophenol content in the extract remained relatively high (between 2.20% and 2.61%), the mass of the recovered extract decreased significantly - from 0.06 g down to 0.01 g. At the sixth extraction, the extract mass was only 7% of that obtained in the first extraction. Based on these results, performing five rounds of *n*-hexane was considered optimal. The *n*-hexane extracts from five rounds were combined, and the solvent was removed under reduced pressure to yield the final *n*-hexane extract.

3.2.3. Investigation of chlorophyll removal methods from n-hexane extract of P. indica leaves

- Method 1: Accurately weigh approximately 2g of the *n*-hexane extract. Add 25 mL of 96% ethanol, and sonicate for 30 minutes to ensure uniform dispersion. Filter the mixture through filter paper to remove solid particles. Add 1 mL of $Cu(OAc)_2$ 0.1 M solution. Stir on a magnetic stirrer at 25 °C for 30 minutes. Adjust the pH to 8, allow the solution to settle, and filter again to collect a colorless solution. Evaporate to dryness to obtain the purified extract.

- Method 2: Accurately weigh approximately 2g of the *n*-hexane extract. Add 25 mL of ethanol,

and sonicate for 30 minutes. Filter through filter paper to remove solid particles. Add 1 mL of $Pb(OAc)_2$ 0.1 M solution. Stir at 25 °C for 30 minutes, adjust the pH to 8, allow the solution to settle for 30 minutes, and filter the solid to obtain a colorless solution. Evaporate the colorless solution to dryness to obtain the final extract.

- Method 3: Two grams of the *n*-hexane extract were subjected to liquid–liquid partitioning using a biphasic solvent system of methanol–acetone–*n*-hexane–water (26:8.5:15:7.5, v/v). Seven successive extractions were performed with 6 mL of the solvent mixture per cycle. The combined extracts were concentrated under reduced pressure to yield 0.85 g of a dark brown, pluthiophenol-enriched fraction. This fraction was further purified by column chromatography on Amberlite XAD-4 resin (100 g; extract-to-resin ratio 1:50; column dimensions: 27 cm height × 3 cm radius). Elution was carried out sequentially with $MeOH/H_2O$ (20%, 40%, 60%, 80%) followed by 1.19 L of MeOH. Methanol eluates (5 mL per fraction) were pooled based on TLC analysis (DCM/EtOAc, 9:1, v/v) into two groups: MeOH1 (pluthiophenol-rich) and MeOH2 (chlorophyll-containing). The MeOH1 and aqueous MeOH fractions were combined and evaporated under reduced pressure to constant weight, yielding a dry, chlorophyll-free, pluthiophenol-rich extract

Table 7. *Results of the chlorophyll removal method.*

Method	Initial mass (g)	Initial content (%)	Mass after removal (g)	Chlorophyll content after removal (%)	Recovery efficiency of pluthiophenol (%)
Method 1	2.32	2.61	0.75	4.86	61.79
Method 2	2.15	2.61	0.67	4.56	55.82
Method 3	2.41	2.61	0.8	6.12	79.74

The results of the investigation indicate that Method 3, involving chlorophyll removal via liquid-liquid extraction with a methanol-acetone-*n*-hexane-water solvent system, yielded the highest pluthiophenol content in the final extract, reaching 6.12%. This value was significantly higher than those obtained using the copper complexation method (Method 1, 4.86%) and the lead complexation method (Method 2, 4.56%). In addition to providing the highest pluthiophenol content, method 3 also demonstrated superior pluthiophenol recovery efficiency compared to methods 1 and 2. Therefore, method 3 is selected as the optimal approach for chlorophyll removal from the *n*-hexane extract of *Pluchea indica* leaves. The process includes: 1. Liquid-liquid extraction using methanol-acetone-*n*-hexane-water solvent system; 2. Solvent removal under reduced pressure; and 3. Column chromatography on Amberlite XAD4 adsorbent using gradient elution with $MeOH/H_2O$ solvent systems. This combined method effectively eliminates chlorophyll and yields a pluthiophenol rich dry extract suitable for further analysis or application.

3.3. Upgrading the extraction process of dry extract from P. indica leaves at a 1 kg/batch scale

A batch of 1 kg of powdered *P. indica* leaves (sieved to a particle size of 0.2 cm) was subjected to reflux extraction using 60% ethanol at 80 °C. The extraction was carried out in three successive 2-hour cycles, with solvent-to-material ratios of 8:1, 6:1, and 6:1 (v/w), respectively. The combined ethanol extracts were concentrated under reduced pressure to approximately half of the original volume and further evaporated in a 70 °C water bath to yield a viscous paste. This concentrate was dispersed in approximately 1 L of water and extracted five times with *n*-hexane (1:1, v/v). The *n*-hexane layers were pooled and concentrated under reduced pressure to a constant weight. The resulting residue was further purified by liquid–liquid extraction using a methanol–acetone–*n*-hexane–water solvent system. The pluthiophenol-rich fraction obtained was subjected to column chromatography on Amberlite XAD4 resin (20–60 mesh) at an extract-to-adsorbent ratio of 1:50 (w/w). Elution was performed with a gradient of methanol–water mixtures (20%, 40%, 60%, 80%, and 100% MeOH). Fractions containing chlorophyll were excluded based on

chromatographic profilling, and the final dry pluthiophenol-enriched extract was collected for further analysis.

***Table 8.** Results of scale 1 kg/batch.*

	Mdl (g)	Extract mass (g)	Moisture content of extract (%)	Extraction yield (%)	Extract mass after PIHS (g)	Pluthiophenol content (%)
1	1102,00	336.30	18.32	27.14	7.94	6.54
2	1003,00	303.12	15.05	27.95	7.67	6.50
3	1025,00	311.05	15.00	28.08	7.93	6.30
4	1080,00	320.90	14.89	27.53	7.76	6.22
5	1010,00	311.56	16.26	28.12	7.55	6.26
6	1003,00	307.82	18.11	27.36	7.03	6.24

Based on experimental results, the following extraction conditions were selected as optimal: The *P. indica* leaves were ground into a fine powder and subjected to reflux extraction at 80 °C using 60% ethanol. The extraction was performed three times (2 hours/ time), with solvent-to-material ratios of 8/1, 6/1, and 6/1, respectively. The combined extracts were concentrated under reduced pressure to half of their original volume, followed by further evaporation in a 70 °C water bath until a viscous raw extract was obtained. This raw extract was dispersed in approximately 1L of water and extracted five times with *n*-hexane at a 1/1 ratio. The *n*-hexane fractions were pooled and concentrated under reduced pressure to a constant weight. The resulting *n*-hexane extract was further purified by liquid-liquid extraction using a methanol-acetone-*n*-hexane-water solvent system. The combined extract was again concentrated under reduced pressure to yield a pluthiophenol extract-enriched. Final purification was achieved by column chromatography using Amberlite XAD4 polymer adsorbent at an extract-to-adsorbent ratio of 1/50 (w/w). Elution was performed with a methanol-water gradient of decreasing polarity. Fractions containing chlorophyll were excluded to yield the final dry extract, which was rich in pluthiophenol.

Thiophenes are the primary group of active compounds found in the leaves of *Pluchea indica,* among which pluthiophenol is the major constituent in samples collected from Hanoi. Based on our research and previous studies, pluthiophenol has been demonstrated to possess notable anticancer activity [3]. Therefore, selecting pluthiophenol as the target marker compound for developing a bioactive extract from *P. indica* leaves is both appropriate and scientifically justified. The extraction process was designed using three key evaluation criteria: extract yield, pluthiophenol content, and pluthiophenol recovery efficiency. Starting with *P. indica* leaves containing 0.41% pluthiophenol, the final extract achieved a pluthiophenol concentration of 6.12%. This represents a 14.9-fold increase, with an overall pluthiophenol recovery efficiency of 79.49%. The process was initially developed on a laboratory scale and successfully scaled up to 1 kg/batch level, demonstrating reproducibility, feasibility and potential for further scale-up to pilot production. The pluthiophenol-rich extract from *P. indica* leaves shows promise as a raw material for the development of functional products aimed at supporting cancer treatment and related diseases.

4. CONCLUSIONS

This study successfully developed an extraction and purification process for obtaining a pluthiophenol-rich extract from *Pluchea indica* leaves collected in Hanoi, Vietnam, at laboratory scale. The optimized extraction conditions included hot reflux extraction at 80 °C using 60% ethanol as the solvent, with finely ground plant material. The extraction was carried out three times, each for 2 hours, using material-to-solvent ratios of 1/8, 1/6, and 1/6 (g/mL), respectively. The combined crude extract was dispersed in approximately 1 L of water and

subjected to five sequential liquid-liquid extractions with *n*-hexane (1:1, v/v). The *n*-hexane extract was the further purified using a methanol–acetone–*n*-hexane–water solvent system and concentrated under reduced pressure to yield a pluthiophenol-enriched fraction. This enriched fraction was subjected to column chromatography using Amberlite XAD4 resin [5] (20–60 mesh) at an extract-to-resin ratio of 1:50 (w/w). Gradient elution was performed with methanol/water mixtures of increasing polarity (20%, 40%, 60%, 80%, and 100% MeOH). Chlorophyll-containing fractions were excluded to yield the final dried extract. At laboratory scale (50g of raw material per batch), the final extract contained 6.12% pluthiophenol, with an overall pluthiophenol recovery efficiency of 79.74%. When the process was scaled up to 1 kg batch, the final extract achieved a comparable pluthiophenol content of 6.24%, demonstrating the reproducibility and scalability of the developed method.

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TÓM TẮT

Quy trình điều chế cao chiết giàu pluthiophenol từ lá cây cúc tần vùng Hà Nội

Nghiên cứu này được thực hiện nhằm khảo sát, đánh giá ảnh hưởng của phương pháp chiết xuất cũng như một số điều kiện công nghệ đến quá trình chiết xuất nhằm thu được cao chiết giàu hợp chất pluthiophenol từ lá cúc tần (Pluchea indica) thu tại Hà Nội. Các yếu tố được khảo sát gồm có dung môi chiết, kích thước dược liệu, tỷ lệ khối lượng dược liệu/thể tích dung môi, số lần chiết và thời gian chiết. Ngoài ra, một số phương pháp tinh chế, làm giàu pluthiophenol trong cao cúc tần cũng được khảo sát. Tiêu chí dùng để đánh giá và lựa chọn điều kiện chiết xuất là hàm lượng pluthiophenol bằng phương pháp HPLC. Kết quả nghiên cứu cho thấy phương pháp chiết nóng ở 80 °C là phù hợp nhất để chiết xuất cúc tần. Các điều kiện công nghệ chiết xuất phù hợp nhất cả về yếu tố kỹ thuật và kinh tế gồm: dung môi chiết là ethanol 60%, kích thước nguyên liệu 1 mm, chiết 3 lần, mỗi lần 2 giờ, với tỷ lệ nguyên liệu/dung môi lần lượt là 1/8, 1/6 và 1/6 (g/mL). Quá trình tinh chế làm giàu hợp chất pluthiophenol được thực hiện bằng phương pháp chiết phân bố lỏng - lỏng và sắc ký cột với chất hấp phụ thích hợp. Ở quy mô 50 g dược liệu, cao chiết thu được có hàm lượng pluthiophenol trung bình đạt 6,12%, hiệu suất thu hồi pluthiophenol của cả quy trình đạt 79,74%. Ở quy mô 1 kg dược liệu/mẻ, cao chiết thu được có hàm lượng pluthiophenol đạt 6,24%.

Từ khóa: Pluthiophenol; Chiết xuất; Cúc tần.