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Study on the causal relationship and optimise the extraction procedure of herbal preparation from *Herba Mimosae pudicae*, *Folium Erythrinae variegatae*, *Cortex Cinnamomi iners*, and *Herba et radix Scopariae*

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Abstract:

Herbal preparations from four herbs - *Mimosa pudica*, *Erythrina variegata*, *Scoparia dulcis*, and *Cinnamomum iners* - exhibit sedative effects, treat insomnia, and effectively reduce anxiety and depression. The chemical components present in these medicinal herbs, such as mimosine and coixol, are biologically active substances. This study investigates the causal relationship and optimises the extraction procedure to achieve higher extraction efficiency. Fourteen experiments were designed using Design-Expert software to evaluate the influence of three independent variables (number of extractions, extraction time, and solvent-to-material ratio) on four dependent variables (extraction efficiency of mimosine, extraction efficiency of coixol, total extraction efficiency, and production cost). The content of mimosine and coixol was determined using the high-performance liquid chromatography (HPLC) method. Data were utilised as input for Phasolpro RD 1.0 software to model and optimise the extraction procedure for the medicinal herbs in the formulation. The extraction process was optimised with a solvent-to-material ratio of 21:1, two extractions, and an extraction time of 2.03 hours. The optimised procedure provides a useful foundation for improving extract quality and applying it as a semi-finished material for medicinal product production.

Keywords: **coixol, *herba et radix Scopariae*, *herba Mimosae pudicae*, high-performance liquid chromatography, mimosine.**

Classification numbers: **3.2, 3.3, 3.5**

1. Introduction

Insomnia is a common sleep disorder that poses significant public health concerns due to its physical and mental health impacts. Several studies report a strong correlation between symptoms of depression, anxiety, stress, and insomnia [1]. Current research focuses on identifying medicinal herbs with sedative effects to treat insomnia, thereby improving patients' quality of life. The remedy studied includes four herbal medicines, each playing a specific role in the formulation: Shameplant (*Herba Mimosae pudicae*; Monarch), Coraltree (*Folium Erythrinae variegatae*; Minister), Licorice Weed (*Herba et radix Scopariae*; Minister and Guide), and Wild cinnamon (*Cortex Cinnamomi iners*; Assistant). These herbs have been effectively used by physician Vo Dinh Hung to treat insomnia and related conditions.

Studies highlight the effects of Shameplant (*Mimosa pudica*) in reducing anxiety and depression, enhancing memory, and providing anticonvulsant properties [2, 3]. The plant's roots contain flavonoids, phytosterols, alkaloids, amino acids, tannins, glycosides, and fatty acids [4]. Preliminary analyses of leaf extracts revealed compounds such as terpenoids, flavonoids, glycosides, alkaloids, quinines, phenols, tannins, saponins, and coumarins [5]. Among these, the alkaloid mimosine has been identified as a marker compound.

Herbal medicine Licorice weed (*Scoparia dulcis*) contains flavones, terpenes, steroids, phenols, tannins, saponins, amino acids, coumarins, and carbohydrates. Its primary active substances include scopadulcic acid A and B, scopadiol, scopadulciol, scopadulin, and betulinic acid. Additional compounds, such as amyrin, apigenin, coixol,

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and coumaric acid, have also been identified [6]. Studies demonstrate that *S. dulcis* has sedative, anxiolytic, and antioxidant effects [7-9].

Coraltree (*Erythrina variegata*) is widely cultivated in Vietnam, India, China, Laos, and Thailand. Its chemical constituents include alkaloids, flavonoids, pterocarpans, triterpenes, steroids, proteins, and lecithin [10, 11]. Total alkaloids from *E. variegata* exhibit neuromuscular blockade, central nervous system depression, and anticonvulsant effects, along with anxiolytic and antidepressant properties [12,13].

Cinnamomum iners (Wild cinnamon) is traditionally used for its antimicrobial, antioxidant, anti-inflammatory, and analgesic activities. Its bark contains terpenes, phenylpropanoids, lignans, flavonoids, and aromatic compounds, all of which contribute to its therapeutic efficacy. These properties make it an effective "assistant" herb, supporting the primary herbs and enhancing the remedy's overall efficacy [14].

The biologically active substances in the remedy-mimosine from *M. pudica* and coixol from *S. dulcis*-are used as markers for quality control. Optimising the extraction process is thus essential to obtain extracts with high marker content and optimal efficiency at a reasonable cost. This study aims to investigate the causal relationships and optimise the extraction process for the mixture of *M. pudica*, *S. dulcis*, *E. variegata*, and *C. iners*.

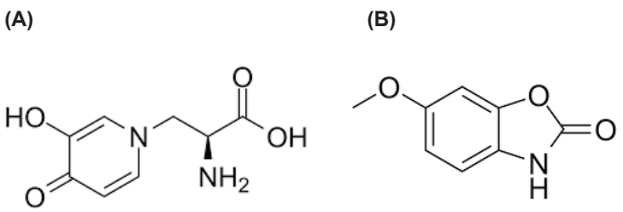

Fig. 1. Chemical structure of (A) mimosine and (B) coixol.

2. Materials and methods

2.1. Materials

Herba Mimosae pudicae, Herba et radix Scopariae, Folium Erythrinae variegatae, and *Cortex Cinnamomi iners* were provided by the Hospital of Traditional Chinese Medicine, Ho Chi Minh City, and met in-house specifications. The identity of these medicinal herbs was confirmed using DNA methods at the University of Science, Vietnam National University - Ho Chi Minh City. The materials were ground and sieved to ensure herb particle sizes ranged between 3-5 mm.

Reference standards for mimosine (purity: 98.16%) and coixol (purity: 99.90%) were obtained from Shanghai Aladdin Biochemical Technology and Chengdu Biopurify Phytochemicals, respectively. Methanol of analytical grade was purchased from Merck (Germany), while acetonitrile was sourced from Scharlau (Spain). Distilled water of HPLC grade was used throughout the study.

2.2. Chromatographic conditions

The HPLC system consisted of a Chromaster CM5000 coupled with a PDA detector (Hitachi, Japan). The column used was a Shimadzu Shim-pack GIST C18 (250×4.6 mm; 5 µm) with an HQ 105 C18 pre-column (10×4.6 mm; 5 µm) from Thermo Scientific (USA). The column temperature was maintained at 40°C, with detection carried out at a wavelength of 277 nm. The injection volume was set to 20 µl, and the flow rate was maintained at 1 ml/min.

The mobile phase comprised acetonitrile (A) and sodium heptanesulfonate solution (2 g/l) adjusted to pH 1.9 with ortho-phosphoric acid (B). The mobile phase gradient programme is shown in Table 1.

Table 1. Gradient programme.

Time (min)	A (%)	B (%)
0	5	95
3	5	95
5	8	92
15	10	90
25	20	80
49	20	80
50	5	95
55	5	95

2.3. Extraction of herbal preparation

Dried samples of each herb were weighed according to the quantities specified in Table 2, ground into small pieces (3-5 mm), and extracted using the reflux method at 100°C with water. The number of extractions, the extraction time, and the solvent-to-material ratio were varied based on the experimental design.

Table 2. Composition of herbal preparation.

Materials	Proportion (w/w%)
Herba Mimosae pudicae	40
Herba et radix Scopariae	32
Cortex Cinnamomi iners	16
Folium Erythrinae variegatae	12

2.4. Experimental design and optimisation

Fourteen experiments were designed using Design-Expert 6.0.6 software (Stat-Ease Inc., USA) based on the D-optimal model, with three independent variables and four dependent variables, as detailed in Table 3.

Table 3. Coded and uncoded variables.

Independent variables	Level 1	Level 2	Level 3
X_1: Number of extractions (times)	1	2	3
X_2: Extraction time (hours)	2	3	-
X_3: Solvent-to-material ratio (ml/g)	14	20	24
Dependent variables	*Optimum conditions*		
Y_1: Mimosine yield (%)	Maximum		
Y_2: Coixol yield (%)	Maximum		
Y_3: Total extraction yield (%)	Maximum		
Y_4: Production cost (VND)	Minimum		

Each experiment was conducted in triplicate, and the average result of the three replicates was used for analysis. Phasolpro RD 1.0 software (University of Medicine and Pharmacy at Ho Chi Minh City) was employed to investigate causal relationships and optimise the extraction process. The optimised extraction procedure was experimentally verified with three replicates. A one-sample t-test was applied using SPSS software to compare the experimental and predicted results.

2.5. Determination of the mimosine/coixol yield

The extracts obtained under identical experimental conditions were combined, mixed, and filtered through a 0.22 µm membrane filter. The mimosine and coixol content (mg/ml) in the filtrate was determined using HPLC. The yields of mimosine (Y_1) and coixol (Y_2) were calculated using the following equation:

$$Y_{1/2} = \frac{C \times V \times 100}{M \times 1000}$$

where M is the mass of the raw material (*Mimosa pudica*, *Scoparia dulcis*) used to prepare the filtrate (grams, on dried basis) and V is the total volume of filtrate (ml); C is the concentration of mimosine/coixol (mg/ml) in the extract.

2.6. Determination of the total extraction efficiency

Extracts obtained under identical experimental conditions were combined, mixed, and filtered to obtain a filtrate volume (V) (ml). The filtrate was evaporated to dryness in a water bath and dried in an oven at 105°C to a constant mass. The residue mass (m) (mg) was recorded. Total extraction efficiency (Y_3) (%) was calculated as follows:

$$Y_3 = \frac{m \times 100}{M \times 1000}$$

where M is the mass of the raw material (*Mimosa pudica*, *Scoparia dulcis*) used to prepare the extraction (grams, dried basis).

2.7. Determination of production cost

The total duration of extraction and concentration (30-40% moisture content) was recorded. Electricity consumption cost (E) was calculated using the equation:

$$E = \frac{400 \times t \times 1700}{1000}$$

where t is the total extraction and concentration time (hours), 400 is the wattage of the heating device (W), and 1700 is the electricity unit price of 1 kWh (VND).

The aqueous solvent used for the extraction was investigated experimentally. The water cost (W) was calculated using the following equation:

$$W = \frac{V' \times 12000 \times X_1}{1000 \times 1000}$$

where V' is the water volume used to prepare the extract (ml), X_1 is the number of extractions, and 12000 is the unit price of water (VND).

In addition, the labour involved in extracting and concentrating the extract was investigated experimentally. The labour wage (L) was calculated using the following equation:

$$L = t \times 16619$$

where t is the total extraction and concentration time (hours) and 16619 is the hourly wage (VND).

Therefore, the total production cost (Y_4) was calculated as (E+W+L) (VND).

3. Results and discussion

3.1. Experimental data and model fitting

The experimental data obtained from 14 experiments (Table 4) were used as input for Phasolpro RD 1.0 software to analyse the causal relationships and optimise the extraction procedure of the medicinal herbs in the formulation.

Table 4. Experimental results.

No.	Independent variables			Dependent variables			
	X_1	X_2	X_3	Y_1 (%)	Y_2 (%)	Y_3 (%)	Y_4 (VND)
1	2	2	14	0.0057	0.0271	11.3980	75644
2	3	3	20	0.0061	0.0233	12.8520	169148
3	2	3	24	0.0070	0.0205	11.6000	112774
4	1	2	24	0.0055	0.0201	8.8780	39326
5	1	3	24	0.0056	0.0105	8.8280	55471
6	2	2	20	0.0075	0.0282	11.0500	78647
7	1	2	14	0.0032	0.0122	6.4140	38421
8	2	3	20	0.0075	0.0191	11.7568	110938
9	3	2	24	0.0079	0.0260	12.5970	117781
10	1	3	20	0.0053	0.0125	8.3034	55384
11	3	3	14	0.0058	0.0173	12.0130	165541
12	2	2	24	0.0077	0.0241	11.9900	76414
13	3	2	20	0.0085	0.0199	12.8790	117553
14	1	3	14	0.0052	0.0085	7.7660	55081

X_1: Number of extractions; X_2: Extraction time; X_3: Solvent-to-material ratio; Y_1: Extraction efficiency of mimosine; Y_2: Extraction efficiency of coixol; Y_3: Extraction efficiency of herbal preparation; Y_4: Production cost.

Model fitting was performed using the backpropagation learning algorithm under two experimental conditions, corresponding to test groups 9 and 14.

The regression and correlation results of the optimisation method, illustrating the degree of causal relationships, are presented in Table 5. These results confirm that the model is suitable for analysing causality, optimisation, and prediction of dependent variables.

Table 5. Correlation coefficients of the training and test sets.

Dependent variables	Test R^2 (%)	Training R^2 (%)
Y_1	87.97	90.00
Y_2	77.90	90.00
Y_3	96.01	90.37
Y_4	96.03	90.01

3.2. Causality relationship analysis

The law of causality related to mimosine extraction efficiency:

The results of the optimisation, as shown in Fig. 2, indicate the following: when the number of extractions (X_1), the extraction time (X_2), and the solvent-to-material ratio (X_3) are low, the mimosine yield (Y_1) is low (p=0.92). Increasing the extraction time (X_2) to a moderate level while keeping the other parameters low does not significantly affect the mimosine yield (Y_1) (p=0.85). When both the extraction time (X_2), and the number of extractions (X_1) or the extraction time (X_2), and the solvent-to-material ratio (X_3) are increased to high levels, the mimosine yield (Y_1) reaches a moderate level (p=0.93). The solvent-to-material ratio (X_3) has the highest impact on the mimosine yield (Y_1). Specifically, increasing the solvent-to-material ratio (X_3) alone to a high level results in a moderate mimosine yield (Y_1). Additionally, when both the solvent-to-material ratio (X_3), and the number of extractions (X_1) are high while the extraction time (X_2) is low or medium, the mimosine yield (Y_1) is high in both scenarios (p=0.81).

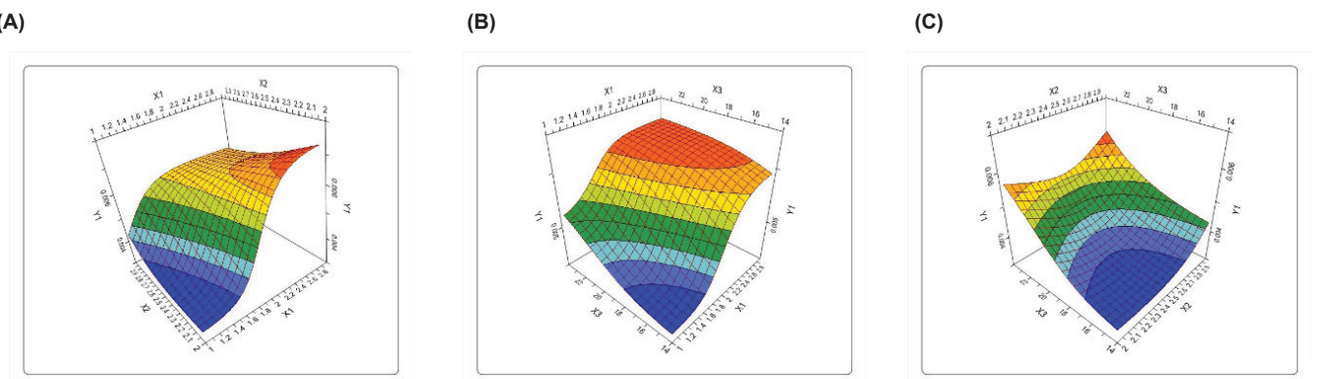

Fig. 2. Effect of (A) extraction number (X_1) and extraction time (X_2); (B) extraction number (X_1) and solvent-to-material ratio (X_3), and (C) extraction time (X_2) and solvent-to-material ratio (X_3) on mimosine yield (Y_1).

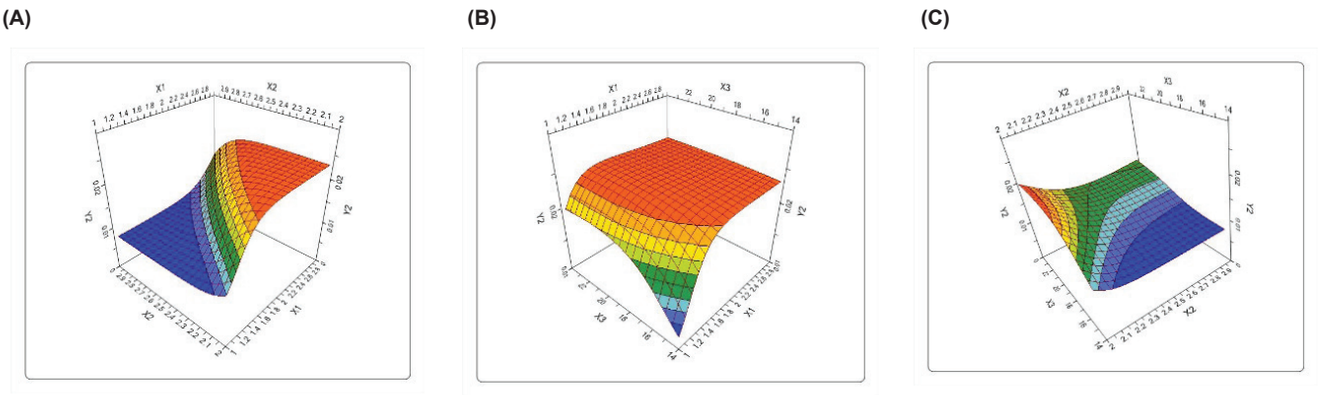

Fig. 3. Effect of (A) extraction number (X_1) and extraction time (X_2), (B) extraction number (X_1) and solvent-to-material ratio (X_3), and (C) extraction time (X_2) and solvent-to-material ratio (X_3) on coixol yield (Y_2).

The law of causality related to coixol extraction efficiency:

When the extraction time (X_2) is moderate or high and the other parameters (X_1 and X_3) are low, the coixol yield (Y_2) is low (p=0.95) in both cases. However, when the solvent-to-material ratio (X_3) is high, and the other parameters (X_1 and X_2) are low, the coixol yield (Y_2) is moderate (p=0.83). Additionally, when both the number of extractions (X_1) and the extraction time (X_2) are high while the solvent-to-material ratio (X_3) is low, the coixol yield (Y_2) is moderate (p=0.87) (Fig. 3).

The extraction process involves solvent permeation through the medicinal herbs, dissolving and transferring solutes from the herbs to the extract. This process relies on the concentration gradient of solutes between the extraction solvent and the raw material. Increasing the number of extractions (X_1), the extraction time (X_2), or the solvent-to-material ratio (X_3) enhances the concentration gradient of mimosine and coixol between the medicinal herbs and the extracts, thereby increasing the yields of mimosine (Y_1) and coixol (Y_2). The experimental results support this assumption.

The law of causality related to total extraction efficiency:

When the number of extractions (X_1) is high, the total extraction yield (Y_3) is high, regardless of whether the solvent-to-material ratio (X_3) is low or high (p=0.79; p=0.81, respectively). However, when the number of extractions (X_1) is low but the extraction time (X_2) and the solvent-to-material ratio (X_3) are high, the total extraction yield (Y_3) is moderate (p=0.78) (Fig. 4).

As with the yields of mimosine and coixol, increasing the number of extractions (X_1), the extraction time (X_2), or the solvent-to-material ratio (X_3) enhances the concentration gradient of solutes between the medicinal herbs and the extracts. This increase in the concentration gradient leads to a higher total extraction efficiency (Y_3).

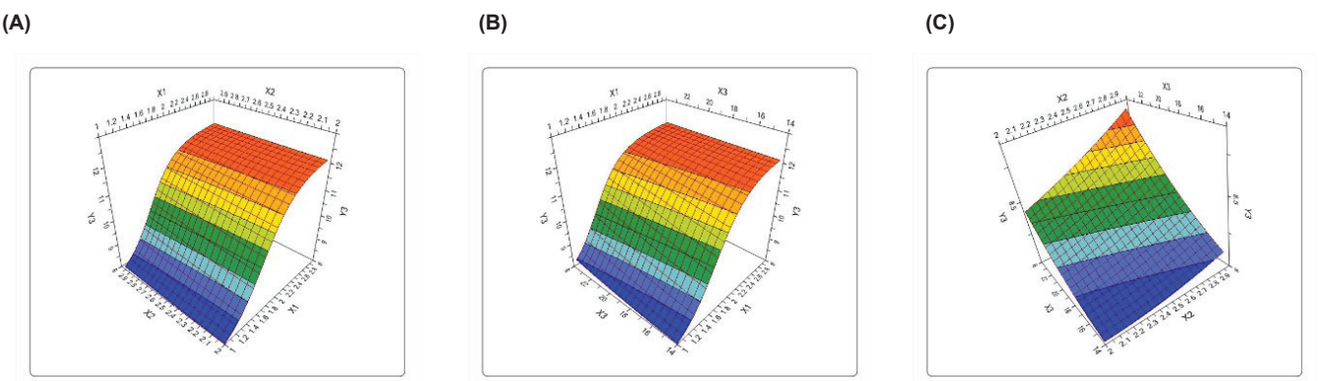

Fig. 4. Effect of (A) extraction number (X_1) and extraction time (X_2), (B) extraction number (X_1) and solvent-to-material ratio (X_3), and (C) extraction time (X_2) and solvent-to-material ratio (X_3) on total yield (Y_3).

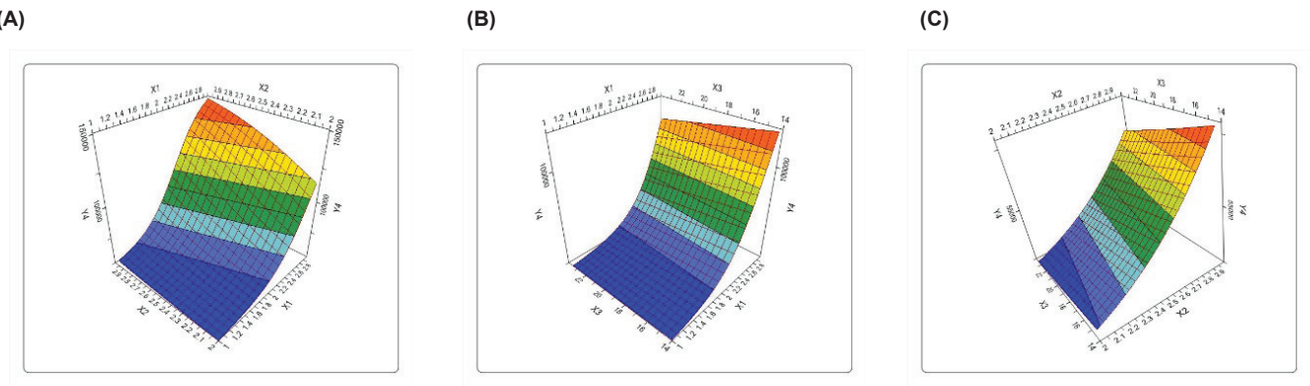

Fig. 5. Effect of (A) extraction number (X_1) and extraction time (X_2), (B) extraction number (X_1) and solvent-to-material ratio (X_3), and (C) extraction time (X_2) and solvent-to-material ratio (X_3) on production cost (Y_4).

The law of causality related to production cost:

When all three parameters (X_1, X_2, X_3) are high, the production cost (Y_4) is high (p=0.72). When the number of extractions (X_1) and the extraction time (X_2) are high, but the solvent-to-material ratio (X_3) is low, the production cost (Y_4) remains high (p=0.76). Conversely, when the number of extractions (X_1) and the solvent-to-material ratio (X_3) are high, but the extraction time (X_2) is low, the production cost (Y_4) is medium (p=0.84). When the number of extractions (X_1) is low, regardless of whether the solvent-to-material ratio (X_3) is low or high, the production cost (Y_4) is low in both cases (p=0.77; p=0.78, respectively) (Fig. 5).

Particularly, an increase in the solvent-to-material ratio (X_3) significantly raises the production cost (Y_4). The extraction process involves two primary stages: heating and concentrating the extract to achieve a specific moisture content (30-40%). As the solvent-to-material ratio (X_3) increases, the time required for concentration also increases significantly, especially when water is used as the extraction solvent. Therefore, increasing the solvent-to-material ratio (X_3) leads to a substantial rise in production cost (Y_4).

3.3. Optimisation of extraction parameters

Condition:

Independent variables (X_i):

- $1 \leq X_1 \leq 3$
- $2 \leq X_2 \leq 3$
- $14 \leq X_3 \leq 24$

Dependent variables (Y_i):

- Maximum Y_1, Y_2, Y_3
- Minimum Y_4

Optimum extraction parameters and values of Y_i predicted by Phasolpro RD1 software are presented in Table 6.

Table 6. Optimised extraction parameters and predictive values of Y_i.

Variables	Optimum values			Predictive values			
	X_1	X_2	X_3	Y_1 (%)	Y_2 (%)	Y_3 (%)	Y_4 (VND)
Value	2	2.03	21	0.0079	0.0237	11.6300	62282

3.4. Experimental verification of the optimised results

The optimal extraction procedure was conducted three times to verify the results. Statistical analysis was performed using SPSS 20.0.0 to compare the experimental data with the predicted outcomes. The statistical results are summarised in Table 7.

Table 7. Comparison of predicted and experimental results (n=3).

	Y_1(%)	Y_2(%)	Y_3(%)	Y_4(VND)
Predictive values	0.0079	0.0237	11.6300	62282
Experimental values				
Sample no. 1	0.0075	0.0235	11.6960	63948
Sample no. 2	0.0079	0.0232	11.2000	62851
Sample no. 3	0.0078	0.0237	11.1000	63248
Average ± SD	0.0077±0.0002	0.0235±0.0003	11.3320±0.3192	63349±555.4300
P-value	0.300	0.250	0.247	0.080

The t-test (Table 7) indicated no significant differences between the predicted and experimental outcomes (p>0.05). This finding confirms that the experimental results obtained under the optimal extraction conditions are consistent with the predictions made by the Phasolpro RD 1.0 software.

4. Conclusions

This study successfully established a causal relationship between extraction conditions - number of extractions, solvent-to-material ratio, and extraction time - and the extraction efficiencies of mimosine, coixol, and the total

yield from a herbal preparation containing *Mimosa pudica*, *Scoparia dulcis*, *Erythrina variegata*, and *Cinnamomum iners*. By optimising these parameters, we achieved higher extraction efficiency and reduced production costs.

The optimised extraction procedure, validated through experimental verification, demonstrated consistency with the predictions made by the Phasolpro RD 1.0 software, thereby confirming the reliability and robustness of the model.

The findings of this study contribute significantly to the field of herbal medicine by enhancing the quality of extracts used in medicinal products. The optimised extraction process provides a strong foundation for future research and the development of herbal formulations, ensuring improved efficacy and cost-effectiveness. Future studies can expand upon this work by exploring additional marker compounds and further refining extraction techniques, ultimately enhancing the therapeutic potential of herbal remedies.

CRediT author statement

Oanh - Hoang Hua: Conceptualisation, Writing, Reviewing, Editing; Van- Thi- Thanh Nguyen: Data curation, Writing - Original draft preparation; Nam- Van- Ho Phan: Methodology, Software; Kiet- Khang Chung: Software, Validation; Quyen- Hong- To Duong: Supervision, Visualisation.

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COMPETING INTERESTS

The authors declare that there is no conflict of interest regarding the publication of this article.

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