ISSN 1859 - 4735

JOURNAL OF MEDICINAL MATERIALS

No. 3 - Vol. 30
6/2025

NATIONAL INSTITUTE OF MEDICINAL MATERIALS

3B QUANG TRUNG - HOAN KIEM - HANOI - VIETNAM
TEL: (+84-24) 38247058 FAX: (+84-24) 39348740
Email: tapchiduoclieu@nimm.org.vn; tapchiduoclieu@gmail.com

JOURNAL OF MEDICINAL MATERIALS (Print and Electronic Journals)

EDITORIAL BOARD (Term 2025-2030)

(Attached to Decision No. 666/QD-VDL dated April 24, 2025 signed by the Director of Vietnam National Institute of Medicinal Materials)

EDITOR-IN-CHIEF

Assoc. Prof. PhD. Do Thi Ha

I. Subcommittee of Pharmacognosy - Traditional Medicine

- **Assoc. Prof. Dr. Sci. Nguyen Minh Khoi - Head of Subcommittee**
- Assoc. Prof. PhD. Nguyen Thuong Dong - Member
- Prof. PhD. Nguyen Minh Duc - Member
- Prof. PhD. Tran Cong Luan - Member
- Prof. PhD. Won Keun Oh - Member
- Assoc. Prof. PhD. Tran Hung - Member
- Assoc. Prof. PhD. Nguyen Thi Bich Thu - Member
- Assoc. Prof. PhD. Phuong Thien Thuong - Member
- Assoc. Prof. PhD. Phan Minh Giang - Member
- Assoc. Prof. PhD. Le Nguyen Thanh - Member
- Assoc. Prof. PhD. Le Viet Dung - Member
- Assoc. Prof. PhD. Nguyen Quoc Huy - Member
- Assoc. Prof. PhD. Nguyen Thu Hang - Member
- PhD. Tran Minh Ngoc - Member
- PhD. Nguyen Van Tai - Member
- Assoc. Prof. PhD. Le Dinh Chi - Member
- PhD. Nguyen Tuan Hiep - Member.

II. Subcommittee of Pharmacology - Clinical Pharmacy

- **Assoc. Prof. PhD. Pham Thi Nguyet Hang - Head of Subcommittee**
- Assoc. Prof. Dr.Sci. Do Trung Dam - Member
- Assoc. Prof. PhD. Nguyen Thi Thu Huong - Member
- Assoc. PhD. William R. Folk - Member
- Assoc. Prof. PhD. Pham Thi Van Anh - Member
- Assoc. Prof. PhD. Nguyen Thuy Duong - Member
- Assoc. Prof. PhD. Le Van Minh - Member
- PhD. Tran Thi Hien - Member.

III. Subcommittee of Agriculture - Biology

- **PhD. Nguyen Van Khiem - Head of Subcommittee**
- PhD. Phan Thuy Hien - Member
- Assoc. Prof. PhD. Nguyen Van Tap - Member
- Assoc. Prof. PhD. Pham Thanh Huyen - Member
- Prof. PhD. Tran The Bach - Member.

IV. Subcommittee of Secretariat Administration

- **Assoc. Prof. PhD. Le Nguyen Thanh - Head of Subcommittee**
- PhD. Le Thi Xoan - Academic Secretary
- BSc. Nguyen Thi Minh Loc - Administrative Secretary.

CONTENTS VOL. 30(3), 2025
(Pages 145 - 224)

Page	SCIENTIFIC RESEARCH

Journal of Medicinal Materials, 2025, Vol. 30, No.3 (pp. 179 - 184)

OPTIMIZATION OF DAPHNORETIN EXTRACTION FROM *WIKSTROEMIA INDICA* (L.) C.A.MEY TWIGS AND LEAVES USING RESPONSE SURFACE METHODOLOGY

Luong Le Uyen Trang[1], Pham Van Tan[1], Nguyen Tra My[2], Vu Thi Diep[2], Nguyen Thi Thu[2], Nguyen Van Lieu[2,3], Nguyen Hoang Tuan[4], Do Thi Ha[2,*]

[1]*Hanoi Medical College, Vietnam;*

[2]*National Institute of Medicinal Materials (NIMM), Hanoi, 11022, Vietnam;*

[3]*Institute of Pharmaceutical Training, Vietnam Military Medical University, Vietnam;*

[4]*Faculty of Pharmacognosy and Traditional Medicine, Hanoi University of Pharmacy, Vietnam*

*Corresponding author: hado.nimms@gmail.com

Received April 26th, 2025 Accepted May 12th, 2025

Summary

This study developed an optimized process for extracting and preparing a daphnoretin-rich extract from *Wikstroemia indica* (L.) C.A.Mey twigs and leaves using the One-Factor-At-A-Time (OFAT) method combined with Response Surface Methodology (RSM). Extraction was performed by simple soaking at room temperature. The optimal conditions were as follows: 1-2 mm material size, single extraction, 80% ethanol as the solvent, 26-hour extraction time, and a solvent-to-material ratio of 12:1. At a batch scale of 1 kg, the optimized process yielded an extract containing 2.481% daphnoretin with an extraction efficiency of 10.15%. This study highlights the feasibility of scaling up the process for industrial applications, offering a simple, efficient, and cost-effective method to produce high-quality daphnoretin-rich extracts.

Keywords: *Wikstroemia indica; Daphnoretin; Extraction optimization; Response surface methodology; One factor at a time.*

1. Introduction

Wikstroemia indica (L.) C.A.Mey, a member of the Thymelaeaceae family, has been traditionally utilized for the treatment of lymphadenitis, asthma, pertussis, tonsillitis, and mumps. Recent studies in Vietnam and globally have explored the chemical composition and biological activities of *W. indica*, highlighting its immunomodulatory potential and its efficacy against HIV-1, hepatitis B, malaria, and cancer [1],[2]. Daphnoretin, a bioactive coumarin isolated from *W. indica*, has demonstrated significant pharmacological properties, including tumor growth inhibition, antiviral activity, protein kinase C activation, coronary vasodilation, and anti-anginal effects. This compound is considered a key contributor to the plant's immunomodulatory, antitumor, and antiviral properties [3],[4].

This study optimized the extraction process of daphnoretin from *W. indica* twigs and leaves using Response Surface Methodology (RSM) combined with the One-Factor-at-A-Time (OFAT) approach. Commonly investigated factors include extraction technique, particle size, solvent-to-sample ratio, solvent concentration, extraction duration, and the number of extraction cycles, all of which influence active compound content and extraction efficiency. The OFAT method was utilized to determine optimal values for discrete factors, including medicinal material size and the number of extraction cycles. Meanwhile, RSM was applied to continuous variables such as ethanol concentration, extraction time, and solvent-to-material ratio. This integrated methodology enabled a comprehensive evaluation of key extraction parameters, facilitating the development of an optimized extraction procedure to achieve the desired extract composition. Based on these findings, we developed a process for producing daphnoretin-rich concentrated extracts.

2. Materials and methods

2.1. Materials

Twigs and leaves of *Wikstroemia indica* (L.) C.A. Mey (Thymelaeaceae) were collected from Co To, Quang Ninh province in October 2022. The plant was taxonomically identified, and a voucher specimen (DL-021020) was deposited at the Department of Medicinal Material Resources, National Institute of Medicinal Materials. The samples were cleaned, oven-dried at 60°C, and ground into powder before extraction.

2.2. Methods

2.2.1. Preparation of concentrated extract

Twigs and leaves of *W. indica* were broken into appropriate sizes, moistened with 1/10 of the extraction solvent for 2 hours, and then extracted under specified conditions. The extract was

concentrated at low temperature and pressure. The obtained extract had a moisture content of less than 20%.

2.2.2. Daphnoretin quantification method

Daphnoretin content in medicinal herbs and concentrated extracts was determined using high performance liquid chromatography (HPLC). The quantitative method was validated for specificity, system suitability, limit of detection, limit of quantification, linearity, repeatability, and precision [5,6].

2.2.3. Investigation of factors affecting daphnoretin extraction

Factors influencing the extraction of daphnoretin-rich extracts from *W. indica* twigs and leaves were examined using the OFAT method combined with RSM. The study was conducted on 20-gram sample batches, evaluating six key parameters: extraction methods (soaking and reflux), particle size (>3 mm, 2-3 mm, 1-2 mm, and <1 mm), solvent-to-sample ratio (ranging from 20:1 to 3:1), extraction solvent concentration (water and ethanol at 30-90%), extraction time (6-48 hours for soaking), and the number of extraction (1, 2, or 3 times).

The response values:

- The percent daphnoretin in concentrated extract (D%)

$$D\ (\%) = \frac{C \times V}{m_{extract} \times (100 - a_{extract}) \times 10^6} \times 100$$

- The extraction rate (H%)

$$H\ (\%) = \frac{m_{extract} \times (100 - a_{extract})}{m_{material} \times (100 - a_{material})} \times 100$$

In which: C: the concentration of analyzed compound in the sample solution from the calibration curve equation (µg/mL), m: weight of tested sample taken to prepare the sample solution (g), a: water content of test sample (%), V: volume of solvent to dissolve the test sample (mL)

3. Results and discussion

3.1. OFAT experiments for extraction

3.1.1. Evaluation of the extraction method

To determine the optimal extraction method, this study compared soaking extraction at room temperature (30°C at the time of the study) with reflux extraction at the solvent's boiling point. The material was extracted by soaking with 80% ethanol at 30°C for 24 hours (solvent-to-material ratio 20:1, single extraction). In parallel, a similar procedure was performed by reflux extraction for 2 hours at 80°C (single extraction). The daphnoretin content and extraction yield obtained by soaking and reflux extraction were 8.42, 8.18 (%) and 2.453, 0.852 (%), respectively. The results (**Fig. 1A**) showed that soaking at room temperature yielded a significantly higher daphnoretin content and extraction efficiency than the 2-hour reflux method. Given its advantages in scalability, simplicity, and minimal reliance on specialized equipment, the soaking method was selected for further investigations.

3.1.2. Evaluation of particle size

The material was divided into different particle sizes for investigation and extracted by soaking with 80% ethanol at room temperature for 24 hours (solvent-to-material ratio 20:1, single extraction). The raw material was classified into four particle sizes: >3 mm, 2-3 mm, 1-2 mm, and <1 mm (**Fig. 1B**). Among them, the 1-2 mm size yielded the highest daphnoretin content (2.429%) and extraction yield (9.42%), making it the optimal choice. Larger particles (>3 mm, 2-3 mm) limited solvent penetration, reducing extraction efficiency. In contrast, excessively fine particles (<1 mm) increased impurity dissolution, leading to lower daphnoretin content despite a higher extraction yield.

3.1.3. Evaluation of solvent-to-sample ratio

The material was extracted by soaking at room temperature for 24 hours with 80% ethanol at the investigated solvent-to-material ratios, with a single extraction. Solvent-to-material ratios ranging from 3:1 to 20:1 were investigated (**Fig. 1C**). Both extraction yield and daphnoretin content increased significantly up to a ratio of 15:1 but showed minimal improvement beyond this point (17:1 and 20:1), despite greater solvent usage. Therefore, 15:1 was selected as the optimal ratio for further studies, with a range of 8:1 to 15:1 used for process optimization via RSM.

3.1.4. Evaluation of extraction solvent concentration

The solvent to be investigated was added to the material with a solvent-to-material ratio of 15:1, and the extraction was performed by soaking for 24 hours with a single extraction. Different extraction solvents, including water and ethanol (30-90%), were evaluated (**Fig. 1D**). Water-extracted samples contained minimal daphnoretin, confirming ethanol as the preferred solvent. Daphnoretin content increased with ethanol

concentration, peaking at 80%. Extraction yield followed a similar trend, reaching its highest values at ethanol concentrations between 50% and 90%. Based on these findings, 80% ethanol was selected for further investigations, with a 50-90% range used for RSM optimization.

3.1.5. Evaluation of extraction time

Soaking extraction was conducted over 6-48 hours (**Fig. 1E**). The material was soaked at room temperature with 80% ethanol for various time intervals (6-48 hours), with a single extraction and a solvent-to-material ratio of 15:1. Both daphnoretin content and extraction yield increased with longer extraction times, reaching optimal levels at 24-30 hours. However, beyond 42 hours, these values declined. This may be because after about 36 hours of extraction, most compounds in the material, including daphnoretin, reached equilibrium, and continuing the extraction would cause the solutes to be reabsorbed into the residue. Thus, 24 hours was chosen for subsequent experiments, while a range of 12-36 hours was used for RSM optimization.

3.1.6. Evaluation of the number of extractions

The optimal number of extractions was assessed by soaking *W. indica* samples in 80% ethanol for 24 hours at a 15:1 solvent-to-material ratio (**Fig. 1F**). Increasing extractions from one to two or three led to higher solvent consumption and longer processing times, yet the improvement in yield and daphnoretin content was negligible. Therefore, a single-extraction method was chosen to maintain efficiency while minimizing solvent usage.

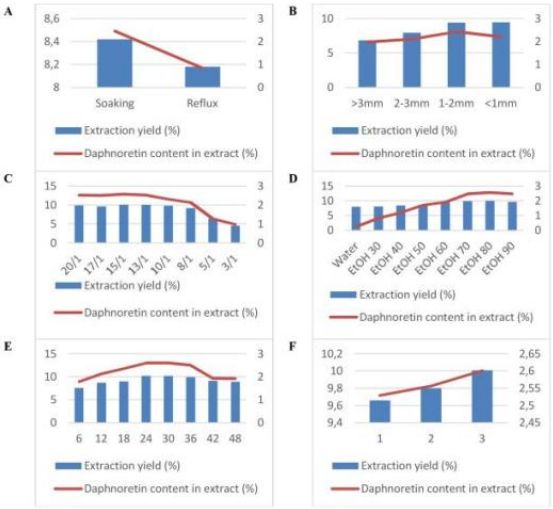

Fig. 1. Results of One-Factor-at-a-Time experiments for extraction

A. Extraction methods; B. Material size; C. Solvent-to-material ratio; D. Extraction solvents; E. Extraction time; F. Number of extractions.

3.2. Optimization of extraction conditions using RSM

Based on the OFAT results, three key factors [ethanol concentration (50-90%), solvent-to-sample ratio (8:1-15:1 mL/g), and extraction time (12-36 hours)] were selected for RSM optimization. The percentage of daphnoretin in the concentrated extract and the extraction yield were used as response variables. The extraction process was standardized to soaking at room temperature, with a single extraction and a material size of 1-2 mm. A central composite design (CCD) in a face-centered model (α = 1) was employed using *Design Expert 11* software, generating 20 experimental runs under different conditions. The detailed experimental design and results are presented in **Table 1**.

Table 1. Results of the experimental design and implementation

Run	Input variable (actual value)			Input variable (encoded value)			Daphnoretin content in extract (%)	Extraction yield (%)
	EtOH conc. (%)	Time (hours)	SMR (mL/g)	EtOH conc.	Time	SMR		
1	50	12	8	-1	-1	-1	1.079	4.97
2	90	12	8	1	-1	-1	1.659	7.86
3	50	36	8	-1	1	-1	1.010	5.02
4	90	36	8	1	1	-1	1.948	8.86
5	50	12	15	-1	-1	1	1.395	5.51
6	90	12	15	1	-1	1	2.014	8.05
7	50	36	15	-1	1	1	1.543	7.87
8	90	36	15	1	1	1	2.496	10.32
9	50	24	11.5	-1	0	0	1.558	7.01
10	90	24	11.5	1	0	0	2.420	9.81
11	70	12	11.5	0	-1	0	2.016	9.07
12	70	36	11.5	0	1	0	2.154	9.69

Run	Input variable (actual value)			Input variable (encoded value)			Daphnoretin content in extract (%)	Extraction yield (%)
	EtOH conc. (%)	Time (hours)	SMR (mL/g)	EtOH conc.	Time	SMR		
13	70	24	8	0	0	-1	1.990	8.79
14	70	24	15	0	0	1	2.525	10.01
15	70	24	11.5	0	0	0	2.402	9.74
16	70	24	11.5	0	0	0	2.295	9.97
17	70	24	11.5	0	0	0	2.398	9.94
18	70	24	11.5	0	0	0	2.298	9.97
19	70	24	11.5	0	0	0	2.398	9.94
20	70	24	11.5	0	0	0	2.410	9.45

(EtOH conc.: ethanol concentration, SMR: solvent-to-material ratio).

The predicted values of the responses were obtained from a quartic model by fitting the experimental data to the following equations:

Coded independent variable:

$Y1 = 2.36 + 0.3952X1 + 0.0988X2 + 0.2287X3 + 0.0865X1X2 + 0.0513X2X3 - 0.3614X1^2 - 0.2654X2^2 - 0.0929X3^2$ **(1)**

$Y2 = 9.87 + 1.45X1 + 0.63X2 + 0.626X3 - 0.2175X1X3 + 0.4475X2X3 - 1.5X1^2 - 0.5323X2^2 - 0.5123X3^2$ **(2)**

Independent variable:

$Y1 = -5.44401 + 0.136494X1 + 0.057441X2 + 0.203749X3 + 0.00036X1X2 + 0.00122X2X3 - 0.000904X1^2 - 0.001843X2^2 - 0.007584X3^2$ **(3)**

$Y2 = -23.40337 + 0.623378X1 + 0.076040X2 + 1.10246X3 - 0.003107X1X3 + 0.010655X2X3 - 0.003756X1^2 - 0.003696X2^2 - 0.041818X3^2$ **(4)**

ANOVA analysis was conducted to evaluate the significance and adequacy of the quadratic model. The significance of each coefficient was assessed using an F-test and corresponding *p*-values (**Table 2**), which indicate the strength of interactions between independent variables.

Table 2. Analysis of variance (ANOVA) for the fitted quadratic polynomial model for optimization of extraction parameters based on the percent daphnoretin in concentrated extract and the extraction rate

The percent daphnoretin in concentrated extract (D%)					
	Sum of squares	**df**	**Mean square**	**F-value**	***p*-value**
Model	4.4200	9	0.4708	187.630	<0.0001
Residual	0.0251	10	0.0025		
Lack of fit	0.0101	5	0.0020	0.6793	0.6591
Pure Error	0.0149	5	0.0030		
Cor Total	4.2600	19			
$R^2 = 0.9941$		$R^2_{adj} = 0.9888$		$R^2_{pre} = 0.9775$	
The extraction rate (H%)					
	Sum of squares	**df**	**Mean square**	**F-value**	***p*-value**
Model	56.1100	9	6.2300	98.32	<0.0001
Residual	0.6341	10	0.0634		
Lack of fit	0.4183	5	0.0837	1.94	0.2425
Pure Error	0.2157	5	0.0431		
Cor Total	56.7400	19			
$R^2 = 0.9888$		$R^2_{adj} = 0.9788$		$R^2_{pre} = 0.9019$	

In the Fisher test, the model's *p*-value (<0.0001) indicated an extremely low probability (<0.01%) that the observed F-value variations were due to random noise, confirming its high reliability (99.99%). The lack-of-fit test yielded *p*-values >0.05 for both models (Y1 and Y2), suggesting no significant variance differences and supporting the model's predictive suitability. The adjusted R^2 values (0.9888 for Y1 and 0.9788 for Y2) closely aligned with the R^2 values, indicating strong model robustness. Similarly, the predictive R^2 values were high and differed from the adjusted R^2 by less than 0.2, further confirming the model's predictive capability. These results validate the model's accuracy, robustness, and reliability.

When assessing the fit between observed and predicted values, the models showed minimal deviation, with mean errors of 1.56% for daphnoretin content and 1.67% for extraction yield, confirming their high accuracy and reliability. The models effectively describe the effects of ethanol concentration, extraction time, and solvent-to-material ratio, exhibiting high predictive accuracy, low error, and strong experimental fit. They meet criteria for linearity, robustness, and predictive capability, allowing for precise analysis of factor influences on extraction outcomes.

For daphnoretin content (Y1), regression equation (1) indicates that increasing ethanol concentration, extraction time, and solvent-to-material ratio proportionally enhances daphnoretin content. Among these, ethanol concentration (X1) and solvent-to-material ratio (X3) have the most significant positive effects.

For extraction yield (Y2), regression equation (2) identifies ethanol concentration (X1) as the key determinant. The interaction between extraction time and solvent-to-material ratio (X2X3) has a greater impact than that between ethanol concentration and extraction time (X1X2).

Using RSM in *Design Expert 11*, multiple optimization solutions were generated to maximize daphnoretin content and extraction yield. However, for large-scale applications, minimizing solvent use and energy consumption was prioritized. Thus, the selected optimal conditions (**Table 3**) balance efficiency and economic feasibility.

Table 3. The optimal extraction process parameters

		Variable Name	Unit	Predicted optimal values
Independent variables	**X1**	Ethanol concentration	%	81.241
	X2	Extraction time	hour	26.452
	X3	Solvent-to-material ratio	mL/g	12.099
Dependent variables	**Y1**	Daphnoretin content	%	2.526
	Y2	Extraction efficiency	%	10.410

To align with practical laboratory conditions, the optimal extraction parameters were set as follows: 80% ethanol as the solvent, 26-hour extraction time, and a solvent-to-material ratio of 12:1. The model's predictions were experimentally validated, with the results presented in **Table 4**.

Table 4. Results of model validation through experimentation

	Daphnoretin content in extract (D, %)	Extraction yield (H, %)
Actual	2.493 ± 0.009	10.330 ± 0.080
Predicted	2.526	10.410
Accuracy (%)	98.690	99.260

The predicted values for daphnoretin content in the extract (%) and extraction yield (%) closely matched the experimental values under optimal conditions, confirming the high predictive accuracy of the models. Specifically, the prediction accuracy for daphnoretin content and extraction yield reached 98.69% and 99.26%, respectively. Based on the analysis of factors influencing the extraction and preparation of daphnoretin-enriched extracts from *W. indica* twigs and leaves using a combination of OFAT and RSM, the optimal extraction conditions were determined as follows: soaking extraction at room temperature, material size of 1-2 mm, single extraction, 80% ethanol as the solvent, extraction time of 26 hours, and a solvent-to-material ratio of 12:1. The obtained extract was filtered and concentrated under reduced pressure at 40-45°C to recover the solvent. This optimized extraction process was successfully applied on a scale of 1 kg of medicinal material per batch, with results presented in **Table 5**.

Table 5. Results of process implementation on a 1 kg/batch scale

	Daphnoretin content in extract (%)	Extraction yield (%)
Scale: 1 kg/batch (n=3)	2.481±0.004	10.15±0.15
Scale: 20 g/batch	2.493±0.009	10.33±0.08
Similarity (%)	99.52	98.19

The results confirmed the high stability of the process when scaled up to 1 kg per batch, yielding a daphnoretin content of 2.481 ± 0.004% and an extraction efficiency of 10.15 ± 0.15%. The strong consistency between the large-scale and small-scale processes suggests that this extraction method is feasible for further industrial scaling.

4. Discussions

This study combined two common methods (OFAT and RSM) to investigate factors affecting the extraction process. OFAT was used for discrete factors with limited values (e.g., material size, number of extraction), while RSM was applied to continuous variables (ethanol concentration, extraction time, and solvent-to-material ratio). This approach provided a comprehensive assessment, optimizing extraction conditions and ensuring efficient daphnoretin extraction. Compared to reflux extraction, room-temperature soaking yielded higher daphnoretin content and extraction efficiency. This indicates that the extraction temperature affects the daphnoretin content in the material, leading to a decrease in daphnoretin levels in the extract at

higher temperatures. Extraction at room temperature is suitable to prevent daphnoretin degradation during the extraction process. This method was selected for its simplicity, ease of implementation, and minimal equipment requirements. Based on optimization results, the material size was 1-2 mm. With more extraction times, the daphnoretin content and extraction yield increase because the material is more fully extracted. However, for the soaking extraction method, multiple extraction times take more time and use more solvent, with only a small increase in efficiency. Therefore, the process was designed to use a single extraction time with optimal conditions. Extraction time and solvent-to-material ratio were optimized to minimize solvent use while maintaining daphnoretin enrichment. The influence of ethanol concentration, extraction time, and solvent-to-material ratio was first examined using OFAT to determine suitable ranges for RSM optimization. Ethanol concentrations of 70-90% yielded the highest daphnoretin content, while lower concentrations significantly reduced extraction efficiency. Ethanol concentration (X1) positively influenced both daphnoretin content and extraction efficiency, with a notable interaction between ethanol concentration and extraction time (X1X2). The RSM-derived optimal ethanol concentration (81.241%) closely matched the 80% identified in OFAT. Daphnoretin content peaked at 24-36 hours but declined at 42-48 hours, likely due to equilibrium and reverse absorption. The solvent-to-material ratio positively affected extraction up to 15:1, beyond which further increases had negligible benefits and led to inefficiencies. RSM optimization determined an extraction time of 26.452 hours and a solvent-to-material ratio of 12.099:1. These conditions aligned with OFAT results while balancing solvent efficiency. The extraction process initially developed at a 20 g/batch scale was successfully scaled up to 1 kg/batch, maintaining over 98% similarity in daphnoretin content and extraction efficiency. These findings support the feasibility of large-scale industrial production. In comparison, Guo J. Y. et al. extracted daphnoretin from *W. indica* roots using 75% ethanol, overnight soaking, and four times of 2-hour reflux extraction, followed by hot filtration and concentration. Their extract contained 0.403% daphnoretin, which was lower than that obtained from *W. indica* twigs and leaves in this study [7]. Lu C. L. et al. optimized daphnoretin extraction from *W. indica* root bark using RSM, determining optimal conditions as 67.44% ethanol, 49.44-minute extraction time, and 60.19°C, with a solvent-to-material ratio of 23.49:1. The maximum daphnoretin content obtained was 0.218% [3]. While ultrasonic extraction significantly reduced extraction time, it required a substantially higher solvent-to-material ratio than the soaking method in this study. The soaking extraction method developed in this study offers a practical, cost-effective approach that minimizes solvent use and does not require complex equipment. Moreover, it is easily scalable for large-scale production.

5. Conclusion

A daphnoretin-rich extract from *W. indica* twigs and leaves was developed using the One-Factor-At-A-Time method combined with Response Surface Methodology. The optimized conditions include room-temperature soaking, a material size of 1-2 mm, a single extraction, 80% ethanol as the solvent, a 26-hour extraction time, and a solvent-to-material ratio of 12:1. After filtration, the solvent is recovered under reduced pressure. At a 1 kg batch scale, the process yields a concentrated extract (8.5% moisture) with 2.481% daphnoretin and an extraction efficiency of 10.15%.

Acknowledgments: *This study was financially supported by the project "Study on anticancer and immune modulation of some Vietnamese medicinal plants", code NĐT.85.KR/20.*

References

1. Chen C. A., Liu C. K., Hsu M. L., Chi C. W., Ko C. K., Chen J. S., Lai C. T., Chang H, H., Lee T. Y., Lai Y. L., Chen Y. L. (2017), Daphnoretin modulates differentiation and maturation of human dendritic cells through down-regulation of c-Jun N-terminal kinase, *International Immunopharmacology*, 51, 25-30.

2. Hu K., Kobayashi H., Dong A., Iwasaki S., Yao X. (2000), Antifungal, antimitotic and anti-HIV-1 agents from the roots of *Wikstroemia indica*, *Planta Medica* 66(6), 564-567.

3. Lu C. L., Li Y. M., Fu G., Yang L., Jiang J. G., Zhu L., Lin F. L., Chen J., Lin Q. S. (2011), Extraction optimisation of daphnoretin from root bark of *Wikstroemia indica* (L.) C.A. and its anti-tumor activity tests, *Food Chemistry*, 124(4), 1500-1506.

4. Peng J., Dong F., Xu Q., Xu Y., Qi Y., Han X., Xu L., Fan G., Liu K. (2006), Orthogonal test design for optimization of supercritical fluid extraction of daphnoretin, 7-methoxy-daphnoretin and 1,5-diphenyl-1-pentanone from *Stellera chamaejasme* L. and subsequent isolation by high-speed counter-current chromatography, *Journal of Chromatography A*, 1135(2), 151-157.

5. *HongKong Chinese Materia Medica Standard* (2002), *Wikstroemiae Radix*, Vol.9, 235-376.

6. Pham T. H., Nguyen t. H. L., Nguyen V. L., Vu T. D., Ho H. T., Nguyen C. L., Do T. H. (2023), Quantitative determination of daphnoretin in aerial parts of *Wikstroemia indica* L. (Thymelaeaceae) by HPLC-DAD, *Journal of Medicinal Materials*, 28(2), 93-98.

7. Wang D., Chen Y., Xu Z., Yu W., Liu X. Y. (2011), Method for extracting daphnoretin from *Wikstroemia indica*, CN102311415A.