



Ultrasound-assisted ethanol extraction of mountainous *Millettia dielsiana* from Vietnam: Effects on flavonoid yield and anti-inflammatory potential

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Abstract

This study evaluated the effects of temperature, ultrasound intensity, and extraction time on flavonoid yield and nitric oxide (NO) inhibitory activity of the extract from *Millettia dielsiana* (a rare mountainous medicinal plant of Vietnam) using 70% ethanol. Extractions were performed at 60-70 °C for a total duration of 60 min and 75 min, with and without ultrasound assistance at 30 W/cm² for 10-15 min. Flavonoid content was quantified, and NO production inhibition was assessed by determining IC₅₀ values. At 70°C without ultrasound, flavonoid yield was 68.5% with an IC₅₀ of 205.48 µg/mL. Application of ultrasound (30 W/cm²) for 10 or 15 min at the same temperature significantly increased yields to 74.9-75.1% and improved NO inhibition (IC₅₀: 176.15-169.28 µg/mL). At 60 °C with ultrasound for 15 min, the yield remained high (74.0%) with the strongest NO inhibition observed (IC₅₀: 150.89 µg/mL). Results indicate that moderate temperature combined with short ultrasound pre-treatment enhances both flavonoid extraction efficiency and bioactivity. These findings support the potential of ultrasound-assisted extraction as a green and effective method for producing bioactive flavonoid-rich extracts from *Millettia dielsiana* with implications for functional food and nutraceutical applications.

Keywords: Flavonoid extract, Mountain blood vine plant, Nitric oxide radical scavenging, Ultrasound-assisted extraction

1. Introduction

Millettia dielsiana, commonly known as the mountain blood vine plant, is a leguminous plant traditionally used in folk medicine across several regions of Southeast Asia. Phytochemical investigations have revealed that this species is particularly rich in flavonoids, especially iso-flavonoids such as daidzein, genistein, and formononetin; which are known for their diverse pharmacological properties including antioxidant, antimicrobial, anti-inflammatory, and estrogenic effects [1]. The increasing interest in natural antioxidants and bioactive compounds from medicinal plants has driven extensive research into efficient extraction methods for flavonoid-rich materials. Conventional extraction techniques, such as maceration and Soxhlet extraction, are characterized by significant limitations including prolonged processing times typically ranging from 24 to 72 hours for maceration and 3 to 24 hours for Soxhlet [2]. Furthermore, extended thermal exposure and long processing periods lead to the degradation of thermolabile compounds and loss of bioactivity [3-4]. In this context, ultrasound-assisted extraction (UAE) has emerged as a promising green extraction technique that offers several advantages including reduced solvent consumption, significantly shorter extraction times achieving reductions of 87% to 90% relative to traditional techniques, and enhanced yield of target compounds [5]. This enhanced yield can be up to 6.1 times higher than traditional solvent extraction. For instance, in the extraction of *Urtica dioica*, UAE recovers 680.4 mg of bioactive matter in only 60 minutes, whereas traditional maceration requires 72 hours to reach a significantly lower yield of 303.37 mg [2]. Furthermore, UAE facilitates high-purity recovery with high reproducibility in just a few minutes while dramatically lowering both energy and solvent requirements compared to conventional processing cycles. The cavitation effect generated by ultrasonic waves disrupts plant cell walls, thereby improving mass transfer and releasing intracellular constituents such as flavonoids more efficiently [3, 6].

Although *Millettia* species have been investigated for their phytochemical profiles and pharmacological properties, there remains a significant gap in optimizing extraction techniques to maximize flavonoid yield and preserve their bioactivity. Conventional methods often suffer from prolonged processing times, low recovery rates, and potential degradation of thermolabile compounds [7]. UAE offers a promising alternative due to its ability to generate acoustic cavitation to disrupts plant cell walls, enhance solvent penetration, and accelerate mass transfer. This not only improves the release of intracellular flavonoids but can also help preserve their structural integrity and antioxidant activity by operating under milder thermal conditions [8]. Phytochemical investigations of *M. dielsiana* so far have historically focused on identifying its rich profile of flavonoid, primarily utilizing conventional extraction methods like maceration or Soxhlet extraction to evaluate their structural diversity, extraction, isolation, and pharmacological properties [1, 9, 10]. To date, no systematic study has examined the influence of ultrasound parameters on the extraction efficiency and bioactivity of flavonoids from *M. dielsiana*.

This study aims to fill this gap by investigating the effects of UAE on flavonoid yield and activity, assessing variables such as solvent type, ultrasound power, extraction time, and solvent-to-material ratio. The results will contribute to the development of an efficient, green, and high-yield method for producing bioactive flavonoid-rich extracts from this valuable medicinal plant.

2. Materials and methods

2.1 Materials

The stems of *Millettia dielsiana* were collected from Hoa Binh, Hai Phong, and Lao Cai provinces, Vietnam. The plant materials were identified by macroscopic and microscopic characteristics in accordance with the criteria described in the Vietnamese Pharmacopoeia, 5th Edition [11]. Voucher specimens (BK_KTTP_HBMDie; BK_KTTP_HPMiDie; BK_KTTP_LCMiDie) were deposited at Food Engineering Department, Hanoi University of Science and Technology. The plant materials (leaves and stems) were thoroughly washed, air-dried at room temperature (~25 °C) in the shade to preserve phenolic compounds, and then ground into fine powder using IKA M 20 universal grinding mill. The powder was stored in airtight containers at 4 °C until extraction.

All the solvents used were of analytical grade and purchased from Merck.

2.2 Effect of ethanol concentration, temperature, and extraction time on flavonoid extraction

To investigate the effect of ethanol concentration, temperature, and extraction time on flavonoid extraction from *Millettia dielsiana*, a series of single-factor experiments were conducted. Sample was extracted using ethanol 60%, 70%, 80%, and 90% in flasks under continuous stirring at a constant temperature of 50 °C for 60 minutes. To assess the influence of temperature, extractions were performed at 70% ethanol for 60 minutes at different temperatures (50, 60, 70, and 80 °C). Similarly, the effect of extraction time was evaluated by extracting with 70% ethanol at fixed temperatures 60 °C and 70 °C for various durations (45, 60, 75, and 90 minutes). After the extraction, the total flavonoid content was determined spectrophotometrically with results expressed as percentage of yield [12].

2.3 Ultrasound-assisted extraction (UAE) procedure

Ultrasound-assisted extraction (UAE) of flavonoids from *Millettia dielsiana* was conducted to evaluate the effects of ultrasonic intensity and duration under two conditions. The solvent extraction (SE) was performed at 70% ethanol and 70 °C, with total extraction time fixed at 60 or 75 minutes. For each condition, UAE was applied for 5, 10, or 15 minutes at the beginning of the extraction, followed by conventional maceration for the remaining time. Ultrasound intensities tested were 20, 25, 30, and 35 W/cm², with a non-ultrasound extraction used as control. The extracts were filtered and the total flavonoid content was determined spectrophotometrically [12].

2.4 Physicochemical analyses

Moisture content: Moisture content was determined according to TCVN 10788:2015. Ground samples were dried at 105 ± 1 °C until constant weight. Moisture (%) was calculated based on the difference in sample mass before and after drying.

Crude fiber content: Crude fiber was analyzed following TCVN 5714:2007. Approximately 2-3 g of dried sample was sequentially digested with dilute sulfuric acid and sodium hydroxide. The sample was then incinerated at 550°C, and crude fiber content was calculated based on mass loss.

Total ash content: Ash content was measured according to TCVN 10761:2015. Samples were incinerated in a muffle furnace at $525 \pm 25^\circ\text{C}$ until a constant mass was reached. Ash was expressed as a percentage of dry mass after correcting for moisture content.

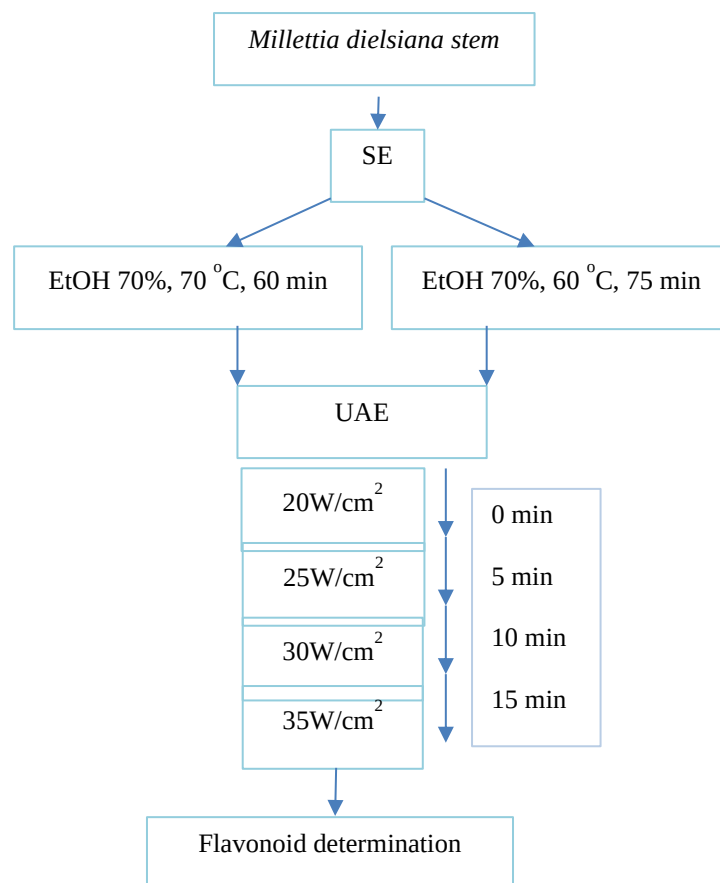


Figure 1 UAE investigation

2.5 Determination of total flavonoid content (TFC)

TFC was determined using the aluminum chloride colorimetric method described by Chang et al. (2002) with quercetin as the standard. Briefly, 0.5 mL of extract or quercetin solution was reacted with ethanol, AlCl_3 , potassium acetate, and water, incubated for 30 min, and absorbance was measured at 415 nm. Results were expressed in mg of quercetin equivalents per g of dry sample (mg QE/g) [13].

2.6 Nitric oxide radical scavenging assay (Griess reaction method)



Figure 2 Scheme for NO radical scavenging assay [14]

2.7 Statistical Analysis

All the experiments were conducted in triplicate, and results were expressed as mean $\pm$ standard deviation (SD). One-way analysis of variance (ANOVA) was performed using Statgraphics Plus 5.1 software. Differences were considered statistically significant at $p < 0.05$.

3. Results and discussion

3.1 Physicochemical properties of *M. dielsiana*

The physicochemical analysis of *M. dielsiana* samples from Hai Phong, Hoa Binh, and Lao Cai revealed notable regional variations. The Hoa Binh sample showed the highest total flavonoid content ($3.40 \pm 0.03\%$) and the lowest ash ($4.32 \pm 0.03\%$), suggesting a potentially superior pharmacological profile (Table 1).

Table 1 Physicochemical properties of *M. dielsiana*

Parameter	Hai Phong	Hoa Binh	Lao Cai
Total ash content (% dry mass)	$5.34^a \pm 0.04$	$4.32^b \pm 0.03$	$5.20^a \pm 0.05$
Crude fiber content (% dry mass)	$86.20^a \pm 0.02$	$80.88^b \pm 0.05$	$89.27^c \pm 0.02$
Total flavonoid content (% dry mass)	$2.82^a \pm 0.02$	$3.40^b \pm 0.03$	$3.07^c \pm 0.05$

In each row, values marked with different superscripts letters differ significantly from one another ($p < 0.05$), while values marked with the same letters are not statistically different.

In contrast, the Lao Cai sample exhibited the highest crude fibre content ($89.27 \pm 0.02\%$), which may contribute to functional health benefits. These differences highlight the influence of geographic and environmental factors on the chemical composition of *M. dielsiana*. The variation in flavonoid content among samples is primarily driven by an environmental gradient across Lao Cai, Hoa Binh, and Hai Phong. The synthesis of protective flavonoids as antioxidant shields is higher in regions at high altitudes and low temperatures, like Lao Cai ($>1,500$ m, $\sim 23^\circ\text{C}$) in comparison with regions at lower altitudes and with higher temperatures, like Hoa Binh (200 m, 25°C) and Hai Phong (20 m, 28°C) [15, 16]. On the other hand, the maritime humidity of Hai Phong and the unique limestone soils of Hoa Binh create distinct ecological stresses that modulate the specific secondary metabolite profiles of plants in general [17]. The sample from Hoa Binh was selected for further analyses of extraction.

3.2 Effect of solvent concentration, temperature and time on flavonoid yield

The Hoa Binh sample was subjected to ethanol extraction in further analysis. The extraction yield of flavonoids from *M. dielsiana* was significantly influenced by ethanol concentration, temperature, and extraction time (Table 2). At a temperature of 50°C for 60 minutes, increasing ethanol concentration from 60% to 70% resulted in a notable rise in flavonoid yield (60.8%); whereas further increases in concentration to 80% and 90% did not significantly improve extraction efficiency because the low water content hindered plant matrix swelling and mass transfer. This resulted from a polarity mismatch of the extraction mixture [18].

At a fixed ethanol concentration of 70%, increasing the temperature from 50°C to 70°C enhanced flavonoid recovery, reaching a maximum yield of 68.5% at 70°C , before declining slightly at 80°C . Regarding extraction time, both at 60°C and 70°C , prolonging the duration from 45 to 75 minutes improved yield but extending beyond 75 minutes led to a decline likely due to thermal degradation or saturation effects. These results suggest that high yield of extraction occurs at 70% ethanol, $60\text{--}70^\circ\text{C}$, and 60–75 minutes balancing efficient solubilization with minimal degradation. These conditions were subjected to UAE investigation.

3.3 Effect of ultrasound on flavonoid extraction

To further assess the potential of UAE in enhancing flavonoid recovery from *M. dielsiana*, experiments were conducted under two extraction conditions: 70% ethanol at 70°C for a total of 60 minutes, and 70% ethanol at 60°C for a total of 75 minutes. In each case, ultrasound was applied at varying intensities ($20\text{--}35\text{ W/cm}^2$) for 5, 10, or 15 minutes at the start of extraction, followed by conventional maceration for the remaining time.

Table 2 Effect of ethanol concentration, temperature and time on flavonoid extraction. (Values marked with different superscript letters in each column differ significantly from one another ($p < 0.05$), while values marked with the same letters are not statistically different).

a. Effect of EtOH%		b. Effect of temperature		c. Effect of time	
EtOH (%) @50 °C, 60 min	Flavonoid yield (%)	Temp. @70%, 60 min	Flavonoid yield (%)	Time @70%, 60 °C	Flavonoid yield (%)
60	55.3 ^a ± 1.8	50 °C	60.8 ^b ± 2.1	45 min	61.1 ^b ± 1.1
70	60.8 ^b ± 2.1	60 °C	65.1 ^d ± 1.7	60 min	65.1 ^d ± 1.0
80	59.7 ^b ± 1.5	70 °C	68.5 ^f ± 1.0	75 min	66.7 ^e ± 1.3
90	60.2 ^b ± 1.9	80 °C	65.9 ^d ± 1.6	90 min	65.9 ^d ± 1.6
				Time @70%, 70 °C	Flavonoid yield (%)
				45 min	63.2 ^c ± 1.2
				60 min	68.5 ^f ± 1.3
				75 min	68.2 ^f ± 1.1
				90 min	60.9 ^b ± 0.9

Table 3 shows the influence of ultrasound power and duration on flavonoid yield under moderate and high temperatures. Next, the evaluation of nitric oxide (NO) inhibitory activity was carried out in conditions yielding the highest flavonoid content.

Table 3 UAE at different ultrasonic intensities. (Values marked with different superscript letters differ significantly from one another ($p < 0.05$), while values marked with the same letters are not statistically different) @ (70%, 70 °C, 60 min) → @ (70%, 70 °C, X min UAE + 60 min - X no UAE)

Ultrasonic intensities (W/cm ²)	UAE time + Conventional extraction time	Flavonoid yield (%)
Control	0 + 60	68.5 ^b ± 1.3
20	5 + 55	69.1 ^{bc} ± 1.0
	10 + 50	70.3 ^c ± 0.9
	15 + 45	70.0 ^c ± 1.2
	5 + 55	70.6 ^c ± 1.1
25	10 + 50	72.4 ^d ± 1.0
	15 + 45	73.1 ^e ± 1.2
	5 + 55	72.0 ^d ± 1.0
	10 + 50	74.9 ^f ± 0.8
30	15 + 45	75.1 ^f ± 1.1
	5 + 55	73.0 ^e ± 0.9
	10 + 50	75.2 ^f ± 0.8
	15 + 45	74.8 ^f ± 1.2

@ (70%, 60 °C, 75 min) → @ (70%, 60 °C, X min UAE + 75min - X no UAE)		
Ultrasonic intensities (W/cm ²)	UAE time + Conventional extraction time	Flavonoid Yield (%)
Control	0 + 75	66.7 ^a ± 1.3
20	5 + 70	67.4 ^b ± 1.0
	10 + 65	68.9 ^b ± 0.9
	15 + 60	69.2 ^c ± 1.1
	5 + 70	69.8 ^c ± 1.0
25	10 + 65	71.6 ^d ± 1.1
	15 + 60	72.4 ^d ± 1.0
	5 + 70	71.0 ^d ± 0.9
	10 + 65	73.6 ^e ± 1.0
30	15 + 60	74.0 ^f ± 1.2
	5 + 70	67.8 ^b ± 1.0
	10 + 65	68.9 ^c ± 0.9
	15 + 60	69.2 ^c ± 1.1

The data demonstrate the effect of ultrasonic-assisted extraction (UAE) on flavonoid yield from *Milletia dielsiana* under the conditions of 70% ethanol and 70 °C with a total extraction time of 60 minutes (UAE + conventional). Compared to the control (68.5 ± 1.3%), the application of ultrasound significantly improved flavonoid yield. At an intensity of 20 W/cm², the yield increased slightly with longer UAE time, reaching a maximum of 70.3 ± 0.9% after 10 minutes, and then stabilized. More notably, higher intensities of 25 and 35 W/cm² resulted in a substantial increase in yield, with peak values of 74.0–75.2% observed after 10–15 minutes of UAE, suggesting a combined effect of ultrasonic energy and extraction time.

The trend indicates that short UAE exposure (5 minutes) provided modest enhancement, while 10–15 minutes resulted in more pronounced increases. However, after 15 minutes, no further significant increase was observed, suggesting that ultrasonic efficiency reached a saturation point or potential degradation of sensitive compounds occurred. Interestingly, extraction at 35 W/cm² for 10 minutes produced the highest yield (75.2 ± 0.8%)

emphasizing that both intensity and time must be optimized. These results confirm that UAE effectively enhances flavonoid extraction, especially at moderate-to-high intensity and optimized UAE duration, offering a more efficient and greener alternative to conventional methods.

The presented data show that ultrasonic-assisted extraction (UAE) significantly enhanced flavonoid yields compared to conventional extraction alone, particularly at higher intensities of UAE and optimized extraction durations. Under both tested conditions, 70% ethanol at 70 °C for 60 min, and 70% ethanol at 60 °C for 75 min, the flavonoid yield increased as the ultrasonic intensity increased, and the sonication time was optimized.

At 70 °C, the highest yield was observed at 35 W/cm² after 10 minutes of sonication followed by 50 minutes of conventional extraction (75.2 ± 0.8%); similar yield was obtained after 15 min of UAE with 45 min of conventional extraction (74.8 ± 1.2%). Similarly, at 60 °C, the maximum yield was obtained at 30 W/cm² after 15 minutes (74.0 ± 1.2%). Notably, lower UAE intensities (20-25 W/cm²) also improved yields compared to control but to a lesser extent, and further increases in sonication time beyond 10-15 minutes did not yield any significant benefits, indicating the occurrence of a saturation effect.

These findings highlight the fact that flavonoid extraction efficiency does not depend solely on intensity or time, but rather on the combination of both factors. This underscores that maximizing flavonoid extraction requires a synergistic balance between ultrasound intensity and duration. Specifically, intensities of 30 - 35 W/cm² applied for 10-15 minutes yielded the highest flavonoid recovery, which is consistent with previous studies, where increasing both the ultrasound amplitude and time significantly enhanced flavonoid and phenolic yields in garlic leaves, up to an optimal point beyond which degradation occurred [19]. Similarly, investigations on *Celastrus hindsii* leaves demonstrated that optimizing collectively extraction variables via response surface methodology achieved maximal flavonoid content and strong antioxidant activity [20]. The best-performing conditions, 30 -35 W/cm² for 10 - 15 minutes, were thus selected for further analyses of nitric oxide (NO) inhibitory activities.

3.4 NO scavenging activity

The values of NO production inhibition (IC₅₀) indicate that ultrasound-assisted extraction improved the bioactivity of flavonoid extracts. Without ultrasound at 70 °C, the IC₅₀ value was highest (205.48 µg/mL), reflecting lower NO inhibitory activity. The application of ultrasound (30 W/cm²) at 70 °C reduced IC₅₀ values to 176.15 µg/mL (10-min ultrasound) and 169.28 µg/mL (15-min ultrasound), suggesting enhanced antioxidant/anti-inflammatory potential. This marked improvement in anti-inflammatory potential, despite relatively similar flavonoid yields, can be attributed to the mechanism of acoustic cavitation. The implosion of cavitation bubbles generates intense local pressure and microjets that effectively disrupt plant cell walls, facilitating the release of highly potent, often sequestered, bioactive sub-fractions that remain trapped under conventional thermal diffusion [16]. At 60 °C with 15 min ultrasound, the IC₅₀ further decreased to 150.89 µg/mL, indicating the strongest NO inhibition. For instance, Ratananikom & Premprayoon (2022) found that UAE significantly enhanced flavonoid yield and antioxidant potency in dill extracts. Additionally, Pham et al. (2020) demonstrated that UAE conditions optimized via response surface methodology raised both flavonoid recovery and functional bioactivity (antioxidant, antitumor) in *Celastrus hindsii* [20-21]. Table 4 shows that although similar flavonoid yields were obtained under different extraction conditions, their NO inhibitory activities varied. This is because total flavonoid content does not reflect differences in flavonoid composition. Individual compounds differ in bioactivity depending on their structure (e.g., aglycones vs. glycosides) [22-23]. Extraction conditions also affect selectivity and stability. Higher temperatures may degrade thermolabile flavonoids or alter their structure, reducing biological activity despite similar yields [8]. This trend implies that moderate temperature combined with sufficient ultrasound time enhances the recovery of bioactive flavonoids with higher NO inhibitory activity.

Table 4 NO production inhibition of flavonoid extract

Temperature °C	Extraction condition			Flavonoid yield (%)	NO production inhibition, IC ₅₀ , ug/mL
	With ultrasound intensities, W/cm ²	Time			
		With ultrasound	No ultrasound		
70	No	No	60	68.5±1.3 ^a	205.48±5.12
70	30	10	50	74.9±1.8 ^{bc}	176.15±4.36
70	30	15	45	75.1±2.1 ^b	169.28±3.98
60	30	15	60	74.0±1.6 ^c	150.89±3.06

Values marked with different superscript letters in each column differ significantly from one another ($p < 0.05$)

4. Conclusion

This study demonstrates that ultrasound-assisted extraction (UAE) significantly enhances flavonoid yield from *Millettia dielsiana* when optimal combinations of intensity and duration are applied. The highest yields were achieved at 30–35 W/cm² for 10–15 minutes, confirming that extraction efficiency depends on the combination

of these parameters rather than on either factor alone. Compared to conventional methods, UAE offers shorter extraction times, improved recovery, and potential preservation of bioactivity. The conditions that produced the highest flavonoid content were selected for further assessment of nitric oxide inhibitory activity, linking extraction optimization to potential therapeutic applications.

5. Author Contributions

Nguyen Thi Minh Tu: Experimental design, Collecting material and data, Writing methodology, Reviewing the draft; Hoang Thi Le Hang: Collecting data, Analysing and interpreting data.

6. References

- [1] Desta KT, Abd El-Aty AM. Millettia isoflavonoids: a comprehensive review of structural diversity, extraction, isolation, and pharmacological properties. *Phytochem Rev.* 2023;22(1):275-308.
- [2] Haido MH, Matti AH, Taher SM. Optimization of extraction conditions of bioactive compounds from Kurdistan species *Urtica dioica*. *Cureus.* 2024;16(5):e61146.
- [3] Zhang QW, Lin LG, Ye WC. Techniques for extraction and isolation of natural products: a comprehensive review. *Chin Med.* 2018;13:20.
- [4] Chávez-González ML, Sepúlveda L, Verma DK, Luna-García HA, Rodríguez-Durán LV, Iliana A, Aguilar CN. Conventional and emerging extraction processes of flavonoids. *Processes.* 2020;8(4):434.
- [5] Shen L, Pang S, Zhong M, Sun Y, Qayum A, Liu Y, et al. A comprehensive review of ultrasonic assisted extraction (UAE) for bioactive components: Principles, advantages, equipment, and combined technologies. *Ultrason Sonochem.* 2023;101:106646.
- [6] Chen X, Jia X, Yang S, Zhang G, Li A, Du P, Liu L, Li C. Optimization of ultrasonic-assisted extraction of flavonoids, polysaccharides, and eleutherosides from *Acanthopanax senticosus* using response surface methodology in development of health wine. *LWT.* 2022;165:113725.
- [7] Azmir J, Zaidul ISM, Rahman MM, Sharif KM, Mohamed A, Sahena F, et al. Techniques for extraction of bioactive compounds from plant materials: A review. *J Food Eng.* 2013;117(4):426-436.
- [8] Chemat F, Rombaut N, Sicaire AG, Meullemiestre A, Fabiano-Tixier AS, Abert-Vian M. Ultrasound assisted extraction of food and natural products: mechanisms, techniques, combinations, protocols and applications – a review. *Ultrason Sonochem.* 2017;34:540-560.
- [9] Jena R, Rath D, Rout SS, Kar DM. A review on genus *Millettia*: traditional uses, phytochemicals and pharmacological activities. *Saudi Pharm J.* 2020;28(12):1686-1703.
- [10] Hung DV, Ha DTN, Duong TTT, Hue PT, Le VTT. Antioxidant and cytotoxic activities of *Millettia dielsiana* in Vietnam. *TNU J Sci Technol.* 2022;227(08):558-564.
- [11] Dat LD, Minh Tu NT, Duc NV, Luyen BTT, Huyen CTT, Jang HJ, Thu DT, Huong TT, Tram LH, Thong NV, Hung ND, Kim YH, Thao NP. Anti-inflammatory secondary metabolites from the stems of *Millettia dielsiana* Harms ex Diels. *Carbohydr Res.* 2019; 484:107778.
- [12] Zeng X, Tan H, Liu B, Zhang R, Li J, Wang Z, et al. Optimization of ultrasonic-assisted extraction and purification of total flavonoids with biological activities from *Radix puerariae*. *Biomass Convers Biorefin.* 2024;14:31533-1545.
- [13] Chang CC, Yang MH, Wen HM, Chern JC. Estimation of total flavonoid content in propolis by two complementary colorimetric methods. *J Food Drug Anal.* 2002;10(3):178-182.
- [14] Bang YJ, Yun HY, Yoo SR, Kim HS. Nitric oxide radical scavenging activity of selected plant extracts using Griess reagent method. *J Ethnopharmacol.* 2004;92(2-3):513-518.
- [15] National Statistics Office. Administrative unit, land and climate [Internet]. [cited 2026 Mar 30]. Available from: <https://www.nso.gov.vn/en/administrative-unit-land-and-climate/>.
- [16] Osorio-Tobón JF. Recent advances and comparisons of conventional and alternative extraction techniques of phenolic compounds. *J Food Sci Technol.* 2020;57(2):4299-4315.
- [17] Pant P, Pandey S, Dall'Acqua S. The influence of environmental conditions on secondary metabolites in medicinal plants: a literature review. *Chem Biodivers.* 2021;18(11):e2100345.
- [18] Wu EY, Sun WJ, Wang Y, Zhang GY, Xu BC, Chen XG, et al. Optimization of ultrasonic-assisted extraction of total flavonoids from *Abrus cantoniensis* (Abriherba) by response surface methodology and evaluation of its anti-inflammatory effect. *Molecules.* 2022;27(7):2036.
- [19] Shekhar S, Prakash P, Singha P, Prasad K, Singh SK. Modeling and optimization of ultrasound-assisted extraction of bioactive compounds from *Allium sativum* leaves using response surface methodology and artificial neural network coupled with genetic algorithm. *Foods.* 2023;12(9):1925.
- [20] Pham DC, Nguyen HC, Nguyen TL, Ho HL, Trinh TK, Riyaphan J, et al. Optimization of ultrasound-assisted extraction of flavonoids from *Celastrus hindsii* leaves using response surface methodology and evaluation of their antioxidant and antitumor activities. *Biomed Res Int.* 2020;2020:3497107.

- [21] Dzah CS, Duan Y, Zhang H, Wen C, Zhang J, Chen G, et al. The effects of ultrasound assisted extraction on yield, antioxidant, anticancer and antimicrobial activity of polyphenol extracts: a review. *Food Biosci.* 2020;35:100547.
- [22] Middleton EJ, Kandaswami C, Theoharides TC. The effects of plant flavonoids on mammalian cells: implications for inflammation, heart disease, and cancer. *Pharmacol Rev.* 2000;52(4):673-751.
- [23] Nijveldt RJ, van Nood E, van Hoorn DE, Boelens PG, van Norren K, van Leeuwen PA. Flavonoids: a review of probable mechanisms of action and potential applications. *Am J Clin Nutr.* 2001;74(4):418-425.