

THE INFLUENCE OF SEVERAL TECHNOLOGICAL FACTORS ON THE EXTRACTION EFFICIENCY OF ALKALOIDS AND MAACKIAIN FROM *MILLETTIA SPECIOSA* CHAMP. TUBERS

Do Tat Thuy¹, Nguyen Tat Thang¹, Le Thu Hang, Bui Thanh Toan¹
Chu Le Trang², Nguyen Thi Thuan², Vu Thi Huong²

ABSTRACT

This study was conducted to evaluate the influence of several technological factors on the extraction process of alkaloid bioactive compounds and maackiain from *Millettia speciosa* Champ. (*M. speciosa*) rhizomes, including the influence of: extraction solvent (distilled water, 70% ethanol, 70% methanol; 99% ethyl acetate); extraction solvent concentrations (ethanol solvent at concentrations of 65, 70, 75, 80, 85, 90%); ratio of raw material/extraction solvent (1/10, 1/15, 1/20, 1/25, 1/30); extraction temperatures (30, 35, 40, 45, 50°C); extraction time (30, 40, 50, 60, 70 minutes); Number of extractions (1 time, 2 times, 3 times). Ingredients: *M. speciosa* Champ., grown and cared for according to GACP-WHO standards (in Tam Dao commune, Phu Tho province). Harvest the *M. speciosa* roots in the winter season (November 2025) after 2 years of planting. The raw material contains 1,12% alkaloids and 2,5 mg of maackiain/100g dry matter.

The results show that the extraction technology for *M. speciosa* extract includes the following parameters: Ethanol solvent, ethanol concentration 80%; material/solvent ratio 1/20; extraction temperature 40°C; extraction time: 60 minutes; extraction performed twice (first extraction: 40 minutes, second extraction: 20 minutes); ultrasonic extraction (500W power, 20 kHz ultrasonic frequency) yields the highest extraction results, in which case: The alkaloid content reached 1,01% and maackiain reached 2,25 mg/100g dry matter; The extraction efficiency reached 90,1%. After harvesting, cleaning, and concentrating the extract, the resulting *M. speciosa* extract yielded an alkaloid content of 4,04% dry matter and maackiain of 9,0 mg/100g dry matter.

The product, made from *M. speciosa* extract, has a moisture content of 4,52%; The alkaloid content is 4,04% dry matter and maackiain is 9,0 mg/100g dry matter, pale yellow color, characteristic aroma. Microbiological indicators: *E. coli*, *Salmonella*: not detected; Total aerobic bacteria $1,0 \times 10^2$ Cf/g; Total number of yeast and mold spores 12 Cf/25g. The product meets food safety requirements as regulated by the Ministry of Health.

Keywords: Alkaloids, Maackiain, *Millettia speciosa*, Southern ginseng, Sam paddles the cat,.

1. INTRODUCTION

Millettia speciosa Champ. (*M. speciosa* Champ.) belongs to the genus *Millettia*, family Fabaceae. It has other local names such as Southern Ginseng, Cat's Paw Ginseng, Mouse Ginseng, etc. The genus *Millettia* has more than 200 species worldwide; in Vietnam, there are 25 species.

In Vietnam, *M. speciosa* is scattered in many provinces in the lowlands and midlands such as Nghe An, Thanh Hoa, Bac Ninh, Vinh Phuc, Phu Tho, Quang Ninh, Hoa Binh, etc. [3].

In the world, *M. speciosa* is found in many countries in Asia, Africa, Northern Australia, South and Southeast Asia (China, Indonesia, Japan, Thailand, etc.). Besides growing wild,

this plant is now also widely cultivated for medicinal purposes [3].

M. speciosa is a precious medicinal plant. According to domestic and foreign studies, *M. speciosa* root has the following effects: reducing cough and fever [3]; protecting the liver, treating anemia [11]; reducing bone and joint pain [3, 11]. *M. speciosa* contains many active substances such as alkaloids, terpenoids, phenolics, and saccharides that have the effect of protecting the liver, preventing cough, fighting asthma, and strengthening immunity [7, 8]. In addition, some substances in *M. speciosa* also show anti-inflammatory, antioxidant, anti-thrombotic, anti-tumor activity [9, 10]; nourishing and strengthening health [12]. Especially, *M. speciosa* root has the effect of

¹ Institute of Agricultural Engineering and Post-Harvest Technology

² Hanoi University of Pharmacy

combating fatigue and is a potential raw material for the production of health-protecting foods [12].

Currently, the application of scientific and technological advancements in the cultivation of *M. speciosa* by farmers, as well as the number of processing and trading facilities for *M. speciosa*, are limited, and production remains small-scale and fragmented. Farmers cultivating *M. speciosa* have not yet focused on organizing production and improving efficiency through local farmers' associations and representative organizations. The lack of linkages with businesses supplying inputs and consuming the product leads to high costs and low selling prices. Farmers also face many obstacles in *M. speciosa* production due to the high cost of seeds and supplies.

Products processed from *M. speciosa* are still limited in variety, lacking high quality and failing to meet food safety standards. Most *M. speciosa* products are produced manually on a small scale, resulting in products that lack commercial appeal, do not

2. MATERIALS AND METHODS OF RESEARCH

2.1. Research materials

Millettia speciosa Champ. (*M. speciosa* Champ.) is grown and cared for according to GACP-WHO standards in Tam Dao commune, Phu Tho province. The *M. speciosa* tubers are harvested in the winter season (November, 2025).

Harvest the *M. speciosa* roots two years after planting, in the early morning (7-9 am) or late evening (4-6:30 pm); harvest when the weather is cool, dry, and without rain.

After harvesting, the *M. speciosa* roots undergo preliminary processing and selection (removing roots that are diseased, damaged, or rotten). The *M. speciosa* roots are washed thoroughly with clean water to remove all soil, sand, and dirt. They are then soaked in an anolyte solution for 5 minutes, drained, and the roots are trimmed.

guarantee food safety, are difficult to preserve, and have a short shelf life. Overall, the added value of these products remains low.

The aim of this study is to determine the extraction method (extraction solvent, material/extraction solvent ratio, extraction temperature, extraction time, number of extractions), the technology for obtaining the extract of *M. speciosa* (recovery, purification, concentration of the extract, extraction of the extract) and the quality of the *M. speciosa* extract. The quality of the extracted *M. speciosa* extract with the parameters selected from the results of this study will also be evaluated through physicochemical indicators (moisture content, alkaloid content, maackiain), sensory (color, odor) and microbiological indicators. The success of this research will contribute to improving the livelihoods of local *M. speciosa* growers as well as businesses processing products from *M. speciosa*.

The *M. speciosa* root is dried to a moisture content of $\leq 10\%$. Dried *M. speciosa* roots are stored in vacuum-sealed PE bags and kept at a cool temperature ($25 \pm 2^\circ\text{C}$).

M. speciosa roots should be stored in a dry, cool place, away from direct sunlight.

2.2. Research Materials

- Chemicals: Ethanol ($\text{C}_2\text{H}_5\text{OH}$); Ethyl acetate ($\text{C}_4\text{H}_8\text{O}_2$), Acetonitrile (CH_3CN), Chloroform, Sodium hydroxide, Potassium chloride, Potassium phosphate monobasic, HCl, n-butanol, NH_3Cl , MgSO_4 , Magnesium sulfate, Maackain standard, TLC thin-layer chromatography plates, Silica gel 60 F₂₅₄ 25, Plastic sheets (20 x 20 cm), chromatographic packing material silica gel 60 (40-63 μm), reversed-phase silica gel LiChroprep® RP-18 (40-63 μm) 500 mg 3 ml, and other analytical chemicals.

- Equipment and instruments: Material grinder (Vietnam). Vacuum drying oven

(Memmert, Germany). Vacuum rotary evaporator (Buechi R-210). Sonic vibra cell ultrasonic device (USA, ultrasonic frequency 20KHz, power 500W, extraction capacity 200-5000ml).

High-performance liquid chromatography (HPLC) system (Shimadzu, Kyoto, Japan). UV 254 nm - 366 nm device (Camag, Switzerland). Thin-layer chromatography chamber (Chromatography tank, Switzerland). Water bath (Memmert, Germany). Plastic pipettes, rubber bulb for chemical aspiration, scissors, gloves, masks and protective clothing,...

2.3. Research Methods

2.3.1. Extraction process of *M. speciosa*

M. speciosa extract is obtained through a specific process:

Raw material selection → Pre-processing, drying, and grinding of raw materials → Extraction of *M. speciosa* extract → Recovery of the extract → Purification of the extract → Concentration of the extract to obtain *M. speciosa* extract → *M. speciosa* extract → Preservation of *M. speciosa* extract.

Fresh *M. speciosa* roots are cleaned of impurities (processed mechanically and washed), then sliced. The roots are dried by sun drying or oven drying (moisture content <10%). The roots are then ground into a fine powder (then sieved through a sieve with a mesh size of 2 mm), resulting in a powder size of 1-2 mm. Quality of dried *M. speciosa* rhizome: alkaloid 1,12% CK; maackiain 2,5 mg/100g CK. The finely ground *M. speciosa* rhizome was subjected to extraction: solvent 80% ethanol; material/solvent ratio 1/20; extraction temperature 40°C; extraction time 60 minutes; extraction twice (first extraction 40 minutes, second extraction 20 minutes), obtaining *M. speciosa* extract (recovery: coarse filtration with filter cloth to remove some impurities; cleaning: using an RO water purifier; concentration of the extract: using a vacuum concentrator, to a dry matter

concentration > 40%; obtaining *M. speciosa* extract: the extract was stored at a cool temperature (20±2°C) or dried to obtain *M. speciosa* extract). *M. speciosa* extract is packaged in glass jars or vacuum-packed in tin containers, store in a dry place, away from direct sunlight.

Within the scope of this paper, we will only discuss the study of the influence of certain technological factors in some key stages such as extraction and harvesting on the quality of high-quality *M. speciosa* extract.

2.3.2. Experimental Method

Fresh *M. speciosa* tubers were sliced and dried (moisture content <10%). The dried *M. speciosa* tubers were ground into a fine powder (sieved through a sieve with a mesh size of 2 mm, resulting in a powder size of 1-2 mm), stored at a cool, dry, and well-ventilated place for the experiment.

a) *The effect of solvent type on the extraction efficiency of M. speciosa*

The experiment was designed with 4 treatments: distilled water, 70% ethanol, 70% methanol, and 99% ethyl acetate.

Monitored parameters: alkaloid content, maackiain, and extraction efficiency.

b) *The effect of solvent concentration on the extraction efficiency of M. speciosa*

The experiment was designed with 6 treatments: using ethanol as the solvent at concentrations of 65, 70, 75, 80, 85 and 90%.

Monitoring parameters: Alkaloid content, maackiain, and extraction efficiency.

c) *The effect of the material/solvent ratio on the extraction efficiency of M. speciosa*

Experimental design included 5 treatments: with material/solvent ratios of 1/10, 1/15, 1/20, 1/25, 1/30.

Monitoring parameters: Alkaloid content, maackiain, and extraction efficiency.

d) *The effect of extraction temperature on the extraction efficiency of M. speciosa*

Experimental design included 5 treatments: at extraction temperatures of 30, 35, 40, 45, and 50°C.

Monitoring parameters: Alkaloid content, maackiain, and extraction efficiency.

d) The effect of extraction time on the high extraction efficiency of M. speciosa

Experimental design included 6 treatments: at extraction times of 30, 40, 50, 60, and 70 minutes.

Monitoring parameters: Alkaloid content, maackiain, and extraction efficiency.

e) The effect of the number of extractions on the extraction efficiency of M. speciosa

The experimental design consisted of 3 formulas, with the number of extraction cycles being: 1, 2, and 3 (cycle 1: 40 minutes, cycle 2: 20 minutes, cycle 3: 10 minutes).

Monitored parameters: Alkaloid content, maackiain content, and extraction efficiency.

f) Recover the extracted liquid, concentrate the extracted liquid, and obtain the M. speciosa extract.

- *Recovery of the extracted solution:* The extract containing the active ingredients alkaloid and maackiain was coarsely filtered using a filter cloth to remove some impurities, and the liquid portion was collected.

- *Clean the extracted solution:* Use an RO water purifier with a capacity of 32 liters/hour, modified for liquid purification. The Kangaroo 32-liter/hour water purifier has 6 different filter cartridges. This device has been modified to suit the purification of the extracted liquid by removing the RO filter and other unnecessary cartridges. Specifically, only 4 column cartridges are used. Filter 1: Made of PP fiber with a 5-micron pore size. Its function is to remove coarse impurities, heavy metals, etc., larger than 5 microns. Filter 2: Made of granular activated carbon. Its function is to adsorb heavy metals, organic substances, detergents, and various toxic chemicals. Filter 3: Made of PP fiber with a 1-micron pore size. Its

function is to remove coarse impurities, heavy metals, etc., larger than 1 micron. Filter core 4: The material is compressed porous activated carbon integrated with silver nanoparticles.

- *Concentrate the extracted liquid to obtain the M. speciosa extract:*

+ Concentration of the extract: using a vacuum concentrator, to a dry matter concentration of > 40%.

+ Extraction of *M. speciosa* extract: After vacuum concentration, the extract is stored at a cool temperature (20±2°C) or dried to obtain *M. speciosa* extract containing bioactive compounds. Evaluation of the quality of *M. speciosa* extract containing bioactive compounds (color, smell, bioactive compound content, physicochemical and microbiological indicators, etc.).

- *M. speciosa extract:* Light yellow color, characteristic aroma. The content of bioactive compounds alkaloid and maackiain is 1,01% dry matter and 2,25 mg/100g dry matter, respectively.

- *How to preserve M. speciosa extract:* in various types of packaging such as glass jars, sealed PE bags, etc. The product should be stored in a dry, cool place, away from sunlight.

2.3.3. Analytical methods and techniques used

- Analysis of major saponin components in *M. speciosa* using thin-layer chromatography.

- Analysis of the main saponin components in *M. speciosa* using high-performance liquid chromatography (HPLC).

- Determination of total alkaloid content: according to NIFC.05.M.111 (Gravimetric method [6] and the Vietnamese Pharmacopoeia V (Volume 2, 2018) [16].

- Determination of maackiain content: according to Nguyen Thi Hang *et al.* (2019) [17].

- Extraction efficiency (%): $H(\%) = (m / M) \times 100$

- TCVN 6846:2007, microorganisms in food and animal feed - Method for detection and quantification of presumptive *Escherichia coli* - Most probable number counting technique [18].

- TCVN 4884-1:2015, microorganisms in the food chain - Methods for quantifying microorganisms - Part 1: Colony counting at 30°C by plate pouring technique [19].

- TCVN 8275-2:2010, microorganisms in food and animal feed - Methods for

3. RESULTS AND DISCUSSION

3.1. Quality of *Codonopsis pilosula* raw material

Millettia speciosa Champ. (*M. speciosa* Champ.) was grown and cared for according

quantifying yeasts and molds - Part 2: Colony counting technique in products with water activity less than or equal to 0.95 [20].

2.3.4. Statistical Analysis

Experimental results were analyzed using ANOVA and LSD (5%) to compare the mean differences between treatments and the variability between replicates within the same treatment over time. Statistical analyses were performed using SPSS 20 software.

to GACP-WHO standards. *M. speciosa* tubers were harvested in the winter season (November 2025) in Tam Dao commune, Phu Tho province. The quality of the dried *M. speciosa* tubers obtained is shown in table 1.

Table 1. Quality of dried *M. speciosa* root raw material

TT	Target name	Unit of calculation	Result
A	Sensory		
1	Color of the <i>M. speciosa</i> root		Natural ivory white
2	Scent		Natural fragrance
3	Taste		Bland
B	Physicochemical properties		
1	Moisture content	%	7,8
2	Alkaloid	% dry matter	1,12
3	Maackiain	mg/100g dry matter	2,5

The quality of the dried *M. speciosa* root material is as follows: The root's interior color is naturally ivory white, with a natural aroma; moisture content is 7,8%; alkaloid content is 1,12% dry matter; maackiain content is 2,5 mg/100g dry matter.

3.2. Influence of solvent type on the extraction process of *M. speciosa* extract

The efficiency of three types of extraction solvents was compared under the following conditions: water, 70% ethanol, 70% methanol; 99% ethyl acetate. With a material/solvent ratio of 1/15, extraction temperature of 35°C, and extraction time of 40 minutes, the alkaloid and maackiain content were determined. The experimental results are shown in table 2 and chart 1.

Table 2. Effect of solvent type on extraction efficiency high bioactive compound content of *M. speciosa*

Solvent type	Content		Extraction efficiency (%)
	Alkaloid (%dry matter)	Maackiain (mg/100g dry matter)	
Water	0,31 ^a ±0,06	0,67 ^a ±0,04	26,9 ^a ±0,08
Ethanol	0,57 ^b ±0,05	1,26 ^b ±0,06	50,5 ^b ±0,19
Methanol	0,59 ^b ±0,04	1,32 ^b ±0,07	52,9 ^c ±0,78
Ethyl acetate	0,61 ^b ±0,05	1,35 ^b ±0,03	54,1 ^d ±0,41

*Note: Data in the same column with different capital letters (a, b, c, d) are significantly different at the 5% significance level. Values represent the mean of 3 replicates $\pm$ standard deviation.

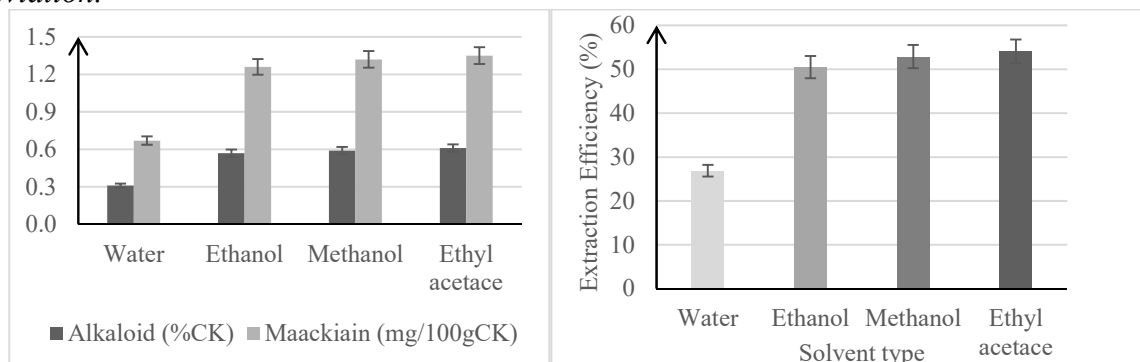


Chart 1. The effect of solvent type on the extraction efficiency of *M. speciosa*

The results in table 2 and chart 1 show that: Different solvents used to extract alkaloids and maackiain from *M. speciosa* rhizomes yield different extraction efficiencies. Water was the solvent with the lowest extraction efficiency of alkaloids and maackiain from *M. speciosa* roots (26,9%). This was followed by ethanol at 50,45% and ethyl acetate at the highest extraction efficiency (54,1%). However, methanol and ethyl acetate are highly toxic organic solvents and are therefore unsuitable for extracting bioactive compounds for use in food processing.

Ethanol provides a much higher extraction efficiency than water. This is because the alkaloids and maackiain in the extract of *M. speciosa* root, although containing many mobile glycoside groups, have large molecular structures, so their polarity is close to that of ethanol, and therefore they dissolve better in ethanol. Thus, ethanol is chosen as the suitable solvent for extracting the active compounds alkaloids and maackiain.

The results of the study are consistent with the research of author Nguyen Tan Thanh and associates (2024) [5] which also selected 80% ethanol solvent to produce total saponin extract powder from the roots of *M. speciosa*, with the saponin content in the extract powder reaching 37,52%. Zhenyu Zhao and associates (2017) when studying the optimization of extracting Flavonoids from associates by ultrasound also used 80% ethanol as the solvent for extraction [13].

3.3. The effect of solvent concentration on the extraction process of bioactively rich *M. speciosa* extract.

The extraction efficiency was compared at 6 solvent concentrations: 65, 70, 75, 80, 85, and 90%. The material/solvent ratio was 1/15, the extraction temperature was 35°C, and the extraction time was 40 minutes. The alkaloid and maackiain content were determined. The research results are presented in table 3 and chart 2.

Table 3. Effect of solvent concentration on the extraction process of bioactively rich *M. speciosa* extract

Solvent concentration (%)	Content		Extraction efficiency (%)
	Alkaloid (% dry matter)	Maackiain (mg/100g dry matter)	
65	0,47 ^a $\pm$ 0,04	1,05 ^a $\pm$ 0,08	42,3 ^a $\pm$ 0,40
70	0,57 ^b $\pm$ 0,02	1,26 ^b $\pm$ 0,07	50,5 ^b $\pm$ 0,57
75	0,63 ^{bc} $\pm$ 0,04	1,40 ^c $\pm$ 0,06	56,2 ^c $\pm$ 0,82

80	0,75 ^d ±0,04	1,68 ^d ±0,09	67,2 ^d ±0,25
85	0,76 ^d ±0,03	1,70 ^d ±0,04	68,0 ^d ±0,06
90	0,65 ^c ±0,05	1,44 ^c ±0,09	57,7 ^e ±0,63

*Note: Data in the same column with different capital letters (a, b, c, d) are significantly different at the 5% significance level. Values represent the mean of 3 replicates ± standard deviation.

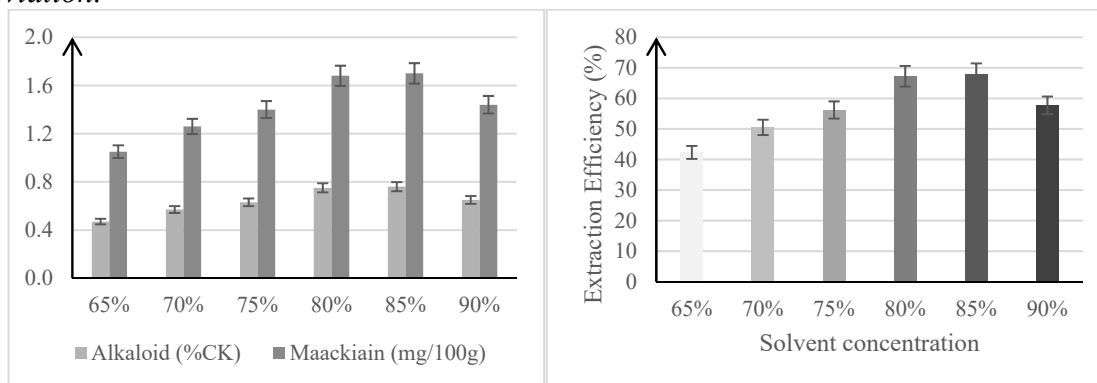


Chart 2. The effect of solvent concentration on the extraction process of *M. speciosa* extract

The results in table 3 and chart 2 show that: At different solvent concentrations, the extraction efficiency of bioactive compounds varies.

The alkaloid content increased from 0,57% dry matter to 0,63% dry matter and maackiain increased from 1,26 mg/100g dry matter to 1,4 mg/100g dry matter when the solvent concentration was 70-75% and stabilized at a solvent concentration of 80-85%; then gradually decreased at a concentration of 90%, with alkaloid at 0,65% dry matter and maackiain at 1,44 mg/100g dry matter. The alkaloid and maackiain content reached their highest levels at an ethanol solvent concentration of 85%, reaching 0,76% dry matter and 1,7 mg/100g dry matter respectively. However, there was no significant difference between ethanol solvent concentrations of 80% and 85%. Therefore, to save solvent and ensure

economic efficiency in the extraction process of ginseng extract, the solvent concentration will be chosen as 80% Ethanol.

The results of the study are similar to the study of Zhenyu Zhao *et al.* (2017) when studying the optimization of extracting flavonoids from *M. speciosa* by ultrasound with 80% ethanol as the solvent, yielding optimal results [13].

3.4. The influence of the raw material/solvent ratio on the extraction process of bioactively rich *M. speciosa* extract

The extraction efficiency was compared at 5 material/solvent ratios: 1/10, 1/15, 1/20, 1/25 and 1/30. Solvent: 80% ethanol, extraction temperature: 35°C, extraction time: 40 minutes. The alkaloid and maackiain content were determined. The experimental results are shown in table 4 and chart 3.

Table 4. The effect of the raw material/solvent ratio on the extraction process of bioactively rich *M. speciosa* extract

Raw material ratio/solvent	Content		Extraction efficiency (%)
	Alkaloid (% dry matter)	Maackiain (mg/100g dry matter)	
1/10	0,57 ^a ±0,03	1,27 ^a ±0,07	50,8 ^a ±0,96
1/15	0,75 ^b ±0,02	1,68 ^b ±0,03	67,2 ^b ±1,90

1/20	0,81 ^c ±0,04	1,81 ^c ±0,04	72,5 ^c ±0,92
1/25	0,82 ^c ±0,02	1,82 ^c ±0,02	72,8 ^c ±1,32
1/30	0,81 ^c ±0,01	1,83 ^c ±0,02	73,1 ^c ±1,66

*Note: Data in the same column with different capital letters (a, b, c, d) are significantly different at the 5% significance level. Values represent the mean of 3 replicates ± standard deviation.

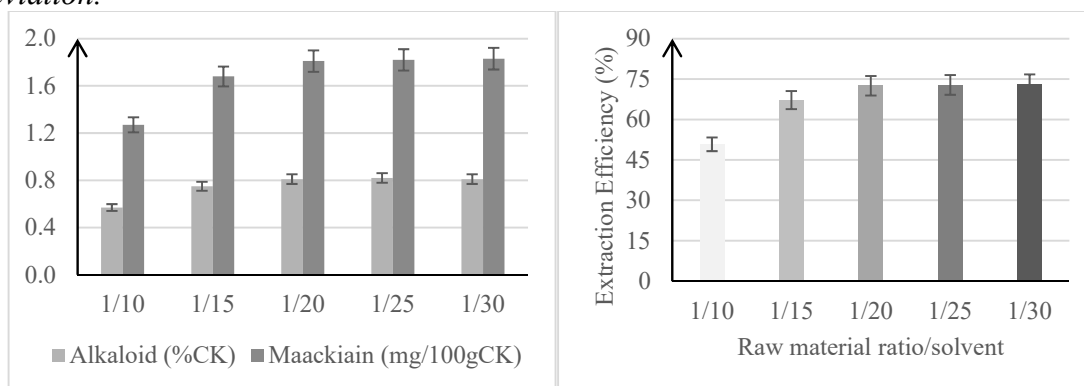


Chart 3. The influence of the raw material/solvent ratio on the extraction process of *M. speciosa* extract

The results in table 4 and chart 3 show that different material/solvent ratios yielded different extraction efficiencies of *M. speciosa*.

The material/solvent ratio of 1/10 resulted in the lowest extraction efficiency (50,8%). The highest extraction efficiency was achieved at ratios from 1/20 to 1/30 (72,5 - 73,1%). However, at ratios from 1/25 to 1/30, the extraction efficiency increased only slightly compared to the material/solvent ratio of 1/20 (only increasing from 0,33 to 0,52%). Furthermore, statistical analysis showed that the extraction efficiency between the 1/20 ratio and the 1/55 and 1/30 ratios did not differ significantly at the 5% level. Therefore, a material/solvent ratio of 1/20 was selected for further research.

Nguyen Tan Thanh and associates (2023) in the process of studying the ultrasonic

extraction conditions of total triterpenoid saponins and polysaccharides from the roots of *M. speciosa* determined the optimal conditions for extraction as follows: the solvent used for extraction is water, the water/material ratio is 38 mL/g, the extract obtained has a total triterpenoid saponin content of 1,25±0,02 mg/g and polysaccharide of 7,86±0,03 mg/g [4].

3.5. The effect of extraction temperature on the extraction process of bioactively rich *M. speciosa* extract

The extraction efficiency was compared at 5 different temperatures: 30, 35, 40, 45, and 50°C. The solvent was 80% ethanol; the material/solvent ratio was 1/20; and the extraction time was 40 minutes. The alkaloid and maackiain content were determined. The experimental results are shown in table 5 and chart 4.

Table 5. The effect of temperature on the extraction process of bioactively rich *M. speciosa* extract

Extraction temperature (°C)	Content		Extraction efficiency (%)
	Alkaloid (% dry matter)	Maackiain (mg/100g dry matter)	
30	0,71 ^a ±0,03	1,58 ^a ±0,05	63,3 ^a ±1,31
35	0,81 ^b ±0,04	1,33 ^b ±0,07	72,5 ^b ±0,92

40	0,87 ^c ±0,05	1,92 ^c ±0,04	76,8 ^c ±1,86
45	0,81 ^b ±0,04	1,81 ^b ±0,06	72,2 ^b ±1,29
50	0,79 ^b ±0,04	1,76 ^b ±0,03	70,2 ^b ±0,52

*Note: Data in the same column with different capital letters (a, b, c, d) are significantly different at the 5% significance level. Values represent the mean of 3 replicates ± standard deviation.

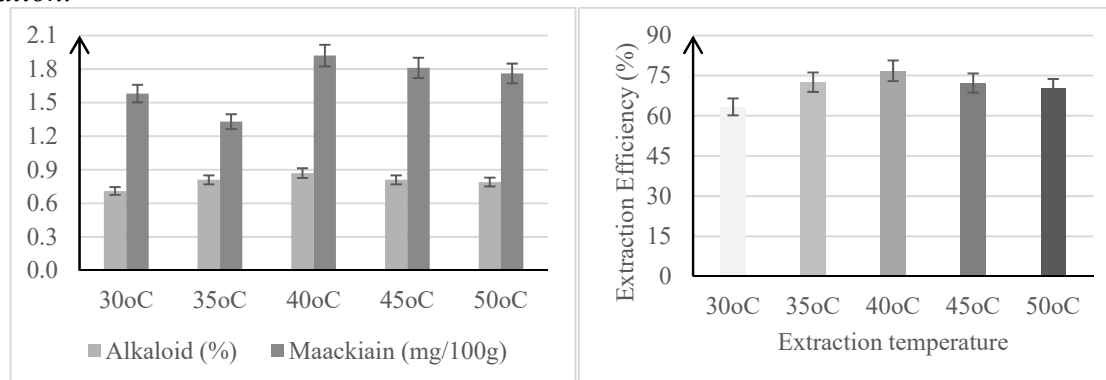


Chart 4. The effect of temperature on the extraction process of *M. speciosa* extract

The results in table 5 and chart 4 show that: As temperature increases, the movement of molecules increases, the solvent permeates quickly into the cell membrane, increasing intracellular pressure. At a certain point, the cell wall ruptures, releasing substances into the external environment, leading to increased extraction efficiency. When the temperature is increased from 35-40°C, the extraction efficiency of the bioactive alkaloid reaches 72,5-76,8%.

The optimal temperature for the extraction process is 40°C with an extraction efficiency of 76,8%. This result is also similar to the research results of Zhenyu Zhao *et al.* (2017)

when studying the optimization of extracting flavonoids from *M. speciosa* by ultrasound at 40°C, which is the optimal temperature for the extraction process [13].

3.6. The effect of extraction time on the extraction process of bioactively rich *M. speciosa* extract

The extraction efficiency was compared at 5 different time intervals: 30; 40; 50; 60, 70 minutes. Solvent: 80% ethanol; material/solvent ratio: 1/20; extraction temperature: 40°C. The alkaloid and maackiain content were determined. The experimental results are shown in table 6 and chart 5.

Table 6. The effect of time on the extraction process of bioactively rich *M. speciosa* extract

Extraction time (minutes)	Content		Extraction efficiency (%)
	Alkaloid (% dry matter)	Maackiain (mg/100g dry matter)	
30	0,70 ^a ±0,05	1,56 ^a ±0,05	62,3 ^a ±1,11
40	0,87 ^b ±0,07	1,92 ^b ±0,07	76,8 ^b ±0,97
50	0,90 ^b ±0,04	2,01 ^{bc} ±0,12	80,3 ^d ±1,75
60	0,93 ^b ±0,02	2,1 ^{bc} ±0,11	83,4 ^c ±1,81
70	0,92 ^b ±0,04	2,07 ^{bc} ±0,07	82,9 ^c ±0,46

*Note: Data in the same column with different capital letters (a, b, c, d) are significantly different at the 5% significance level. Values represent the mean of 3 replicates ± standard deviation.

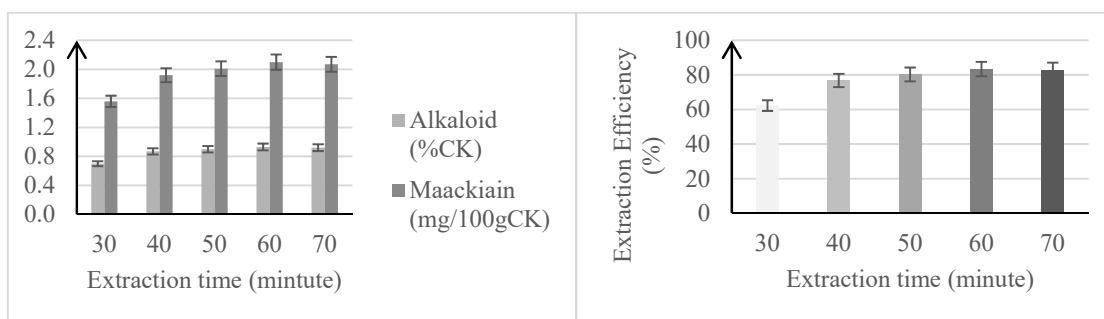


Chart 5. The effect of time on the extraction process of *M. speciosa* extract

The results in table 6 and chart 5 show that the alkaloid and maackiain content increased with increasing sonication time from 30 to 40 minutes. The highest alkaloid and maackiain content reached 0,93% and 2,1 mg/100g dry matter at 60 minutes; the extraction efficiency was 83,4%. Further increasing the sonication time to 70 minutes, the alkaloid and maackiain content tended to decrease, reaching only 0,92% dry matter and 2,07 mg/100g dry matter. The longer the sonication time, the more profound the changes in the raw material caused by the ultrasound waves. Alkaloids and maackiain are easily decomposed at high temperatures over extended periods.

Nguyen Tan Thanh and associates (2023) during the study of ultrasonic extraction conditions of total triterpenoid saponins and polysaccharides from the roots of *M.*

speciosa, determined that the optimal extraction time was 65 minutes, which yielded an extract with a total triterpenoid saponin content of 1,25±0,02 mg/g and a polysaccharide content of 7,86±0,03 mg/g [4].

3.7. The effect of the number of extractions on the extraction process of bioactively rich *M. speciosa* extract

The extraction efficiency was compared at three different extraction times: 1, 2, and 3 (1st extraction time: 40 minutes, 2nd extraction time: 20 minutes, 3rd extraction time: 10 minutes). Solvent: 80% ethanol; material/solvent ratio: 1/20; temperature: 40°C. The alkaloid and maackiain content were determined. The experimental results are shown in table 7 and chart 6.

Table 7. The effect of the number of extractions on the extraction process of bioactively rich *M. speciosa* extract

Number of extraction cycles (times)	Content		Extraction efficiency (%)
	Alkaloid (%dry matter)	Maackiain (mg/100g dry matter)	
1	0,93 ^a ±0,02	2,1 ^a ±0,09	83,4 ^a ±1,46
2	1,01 ^b ±0,07	2,25 ^b ±0,08	90,1 ^b ±1,10
3	0,98 ^b ±0,01	2,19 ^b ±0,11	87,7 ^c ±0,93

*Note: Data in the same column with different capital letters (a, b, c, d) are significantly different at the 5% significance level. Values represent the mean of 3 replicates ± standard deviation.

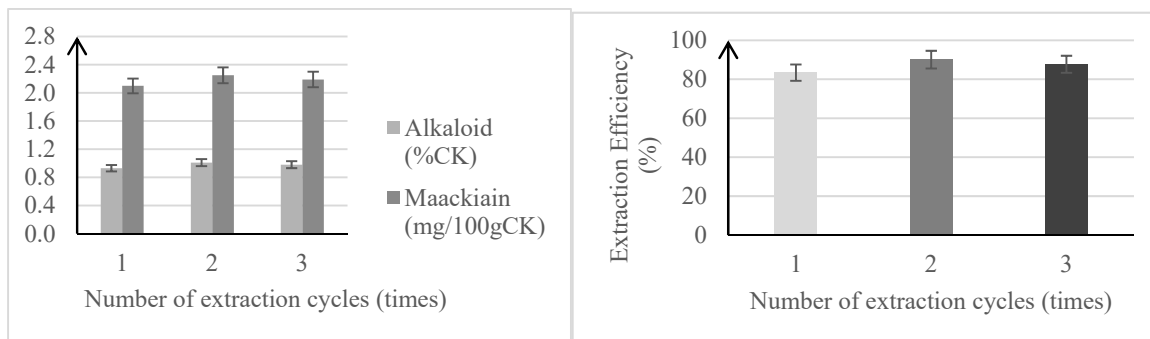


Chart 6. Effect of the number of extractions on the extraction process of *M. speciosa* extract

Table 7 and chart 6 shows that different extraction cycles resulted in different extraction efficiencies of the bioactive compounds alkaloids and maackiain.

With 1 extraction cycle, the alkaloid and maackiain content was 0,93% dry matter and 2,1 mg/100g dry matter, respectively; the extraction efficiency was 83,4%. With 2 extraction cycles, the alkaloid and maackiain content was higher, at 1,01% and 2,25 mg/100g dry matter; the extraction efficiency was 90,1%. However, when the number of extraction cycles increased to 3, the alkaloid and maackiain content did not increase but tended to decrease.

Wu *et al.* (2001) reported that when extracting saponins from ginseng (*Panax ginseng*) using ultrasonic extraction, the recovery efficiency reached > 90% of total saponins after 2 extraction cycles. Lin *et al.* (2013) also showed that ultrasonic extraction accelerates the diffusion rate of solutes, so only 1-2 extraction cycles are needed to recover most of the ginsenosides, while reflux extraction requires 3-4 extraction cycles.

3.8. Product quality assessment *M. speciosa* extract

Table 8. Some product quality indicators *M. speciosa* extract

TT	Target name	Unit of calculation	Result
A Sensory indicators			
1	Color		Light yellow
2	Smell		Characteristic aroma
B Physicochemical indicators			
1	Moisture content	%	4,52

The extracted *M. speciosa* root is purified and concentrated to obtain *M. speciosa* extract.

The extract containing the bioactive substances is coarsely filtered through a filter cloth to remove some impurities, and the liquid portion is collected. The water is then purified using an RO water purifier (with 4 filter cartridges). The extracted solution is passed sequentially through four columns with four filter cores to purify the solution.

The extracted solution was concentrated using a vacuum concentrator, with a dry matter concentration of > 40%. The concentrated extract is stored at a cool temperature (20±2°C) or dried to obtain a *M. speciosa* extract containing bioactive compounds.

M. speciosa extract is preserved in various types of packaging such as glass jars, sealed PE bags, etc. The product should be stored in a cool, dry place, away from direct sunlight.

The quality of *M. speciosa* extract was evaluated through the analysis of sensory, physicochemical and microbiological indicators. The results are shown in Table 8.

2	Alkaloid	% dry matter	4,04
3	Maackiain	mg/100g dry matter	9,0
C	Microbiological indicators		
1	<i>E. coli</i>	Cfu/g	Not detected
2	<i>Salmonella</i>	Cfu/g	Not detected
3	Total aerobic bacteria	Cfu/g	$1,0 \times 10^2$
4	Total number of yeast and mold spores	Cfu/25g	12

The high-quality *M. speciosa* extract has a low moisture content of 4,52%; a pale yellow color, and a characteristic aroma. The alkaloid content is 4,04% dry matter, and maackiain is 9,0 mg/100g dry matter.

Microbiological analysis results show that all microbiological indicators meet food safety standards as prescribed by the Ministry

4. CONCLUSION

This study investigated several technological factors in the extraction process of alkaloid bioactive compounds, maackiain, from dried *M. speciosa* rhizomes. The extraction technology for *M. speciosa* extract included the following parameters: 80% ethanol solvent; material/solvent ratio 1/20; extraction temperature 40°C; extraction time 60 minutes; double extraction (first extraction 40 minutes, second extraction 20

REFERENCES

1. Ministry of Health. *Vietnamese Pharmacopoeia V* - Volume 2 (2018). Medical Publishing House - Hanoi.
2. Vo Van Chi (2004). *Dictionary of Vietnamese Medicinal Plants*, Volumes 1 and 2, Medical Publishing House, Hanoi.
3. Do Tat Loi (2006). *Vietnamese Medicinal Plants and Herbs*, Medical Publishing House, Hanoi.
4. Nguyen Tan Thanh, Le Thi My Chau, Nguyen Thi Huyen, Hoang Thi Thanh Xuan, Tran Dinh Thang (2023). *Study of ultrasonic extraction conditions of total triterpenoid saponins and polysaccharides from the roots of Millettia speciosa*. Journal of Agriculture and Rural Development, pp. 67-73. Hanoi, June.

of Health (QCVN 8-1:2025/BYT; QCVN 8-2:2011/BYT; Decision No. 46/2007/QĐ-BYT of the Ministry of Health). Microbiological indicators: *E. coli*, *Salmonella*: Not detected; Total aerobic bacteria $1,0 \times 10^2$ Cfu/g; Total number of yeast and mold spores 12 Cfu/25g. Thus, the product meets food safety requirements.

minutes); extraction using ultrasonic waves (power 500W, ultrasonic frequency 20 kHz). The product, *M. speciosa* extract, has a light yellow color and a characteristic aroma. The bioactive content of alkaloids is 4,04% dry matter and maackiain is 9,0 mg/100g dry matter. Microbiological indicators: *E. coli*, *Salmonella*: Not detected; Total aerobic bacteria $1,0 \times 10^2$ Cfu/g; Total number of yeast and mold spores 12 Cfu/25g. The product meets food safety requirements.

5. Nguyen Tan Thanh, Tran Dinh Thang, Le Thi My Chau, Doan Manh Dung, Nguyen Thi Huong, Nguyen Ba Thanh (2024). *Production process of total saponin extract powder from Millettia speciosa root*. Useful solution.

6. *Determination of alkaloid content*: NIFC.05.M.111 (Gravitational method).

7. Duan Z. (2011). "The Study on Biological Characteristics and Cultivation Management Measures of *M. speciosa* Champ.", *Tropical Forestry*, 39, 22-25.

8. Huang, B., Xu, L., & Li, Z. (2008). "The reasearch of stem tissue culture from *M. speciosa*", *Journal of Anhui Agricultural Sciences*, 32(2), 993 - 994.

9. Yao S and Bai L., (2012). "Study on Seed Germination Characteristic of *Milletia speciosa* Champ.", Seed, 31, 36 - 38.
10. Zhang Dehua, Renshu, H., & Lou, Z. (2010). "Progress on the Method of Extraction of Alkaloids", Chinese Wild Plant Resource, 29, 15 - 20.
11. Zhang, S., Yin, T., Ling, X. *et al* (2008). "Interactions between thrombin and natural products of *Milletia speciosa* Champ. using capillary zone electrophoresis", Electrophoresis, 29 (16 SPEC. ISS.), 3391 - 3397.
12. Zhao, X. N., Wang, X. F., Liao, J. Bin *et al* (2015). "Antifatigue Effect of *Milletia speciosa* Champ. (Leguminosae) Extract in Mice", Tropical Journal of Pharmaceutical Research, 14(3), 479 - 485.
13. Zhenyu Zhao *et al* (2017). Optimum extraction of Flavonoids from *Milletia speciosa* Champ. by ultrasound, microwave and Soxhlet and evaluation of in vitro antioxidant activity. Measurement and Characterization; 11(4).
14. Zhi Huang *et al* (2020). New Polysaccharide compound from *Milletia speciosa* Champ. root: Isolation, structural characteristics and immunomodulatory activity. International Journal of Biological Macromolecules, 145: 547 - 557.
15. Joong - Ho Kwon, Gee-Dong Lee. Jacqueline Belanger, J.R.Jocelyn Pare (2003). Effect of ethanol concentration on the efficiency of extraction of ginseng saponins when using a microwave-assisted extraction (MAPTM). International Journal of Food Science & Technology, 38(5), page 615-622.
16. Ministry of Health. Vietnamese Pharmacopoeia V - Volume 2 (2018). Medical Publishing House - Hanoi.
17. Nguyen Thi Hang, Nguyen Thi Ha Ly, Nguyen Thi Phuong, Phuong Thien Thuong, Nguyen Thi Bich Thu, Nguyen Dinh Quan (2019). Research on isolation and quantitative analysis of maackiain in the roots of *Milletia speciosa*. Journal of Medicinal Plants, 24(3): 220-226.
18. TCVN 6846:2007, Microorganisms in food and animal feed - Methods for the detection and quantification of presumptive *Escherichia coli* - Most probable number counting technique.
19. TCVN 4884-1:2015, Microorganisms in the food chain - Methods for the quantitative determination of microorganisms - Part 1: Colony counting at 30°C by the plate pouring technique.
20. TCVN 8275-2:2010, Microorganisms in food and animal feed - Methods for the quantitative determination of yeasts and molds - Part 2: Colony counting technique in products with water activity less than or equal to 0.95.

- Ngày gửi bài: 29/12/2025
 - Ngày phản biện: 11/05/2026
 - Ngày duyệt đăng: 02/06/2026