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A Production Process of Extract from the Root of Vietnamese Purple Morinda Officinalis How

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ABSTRACT

Highly concentrated extracts, known as extracted preparations, are collected by evaporating or drying solutions containing biologically active substances (BASs) found in herbs or animal parts through a leaching process with the assistance of appropriate solvents. Generally, highly concentrated extracts derived from the herbs are produced through two primary stages: (1) leaching BASs from the herbs using suitable solvents, and (2) evaporating or drying the prepared solution to obtain the final product. The leaching process is a crucial step that significantly influences recovery efficiency, product quality, and production costs. In this work, the operating conditions of a leaching system were investigated to extract BASs from MO's root (MO = Morinda officinalis How), which are the main components in the highly concentrated extract of MO. The results indicate that the maximum yield of BASs dissolved in an ethanol solution reaches 57.3% from MO powder (dimensions approximately 0.56 to 0.58 mm), corresponding to an ethanol solution of 60% (v/v), a temperature of 60 °C, and a leaching time of 6 hours. Based on these findings, a production process for the highly concentrated extracts from Vietnamese purple MO has been established, aiming to create a facilely used product chain, thereby enhancing the value of this rare medicinal plant.

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1. Introduction

Morinda officinalis How (MO) is a medicinal plant Ba Kich which was previously harvested from wild sources in various low mountainous regions across several countries such as China, India, North Korea, and Laos. In Vietnam, it is found in numerous provinces, including Quang Ninh, Lang Son, Bac Giang, Ha Giang, Cao Bang, Tuyen Quang, Thai Nguyen, Bac Can, Lao Cai, Yen Bai, Phu Tho, Lai Chau, Son La, Hoa Binh, Ninh Binh, Thanh Hoa, Nghe An, and Quang Nam.

In recent years, with the adoption of tissue culture methods, MO cultivation has expanded to thousands of hectares in some regions, necessitating post-harvest processing technology to establish a product chain and increase the value of this rare medicinal plant.

According to modern medicine, MO enhances health, strengthens tendons, boosts the body's resistance, and treats various conditions such as male infertility, osteoporosis, and hypertension. In traditional medicine, the root of MO is often soaked in alcohol. However, this method may not be suitable for individuals who cannot consume alcohol.

Currently, with the surplus production of Morinda officinalis from thousands of hectares of cultivation, traditional methods of utilization are insufficient. Hence, there is a pressing need to extract the active compounds from MO's root for convenient preservation and to further enhance the value of this valuable medicinal plant.

Previous and ongoing research has identified over 100 bioactive compounds from MO, with polysaccharides, oligosaccharides, anthraquinones, and iridoid glycosides being the primary components. Pharmacokinetic studies have proven that the main components of MO are non-toxic, and the results indicate that MO has emerged as a good source of traditional medicine. However, their

effectiveness is low because their concentrations in MO are not high. The extraction of natural bioactive compounds (BASs) from MO's roots will increase their value, and this has attracted the attention of scientists both domestically and internationally for a long time. In terms of the technology for exploiting these compounds on an industrial scale, the current state is still weak and lacking.

MO's root contains components such as glycosides, anthraquinones, polysaccharides, mono- and oligosaccharides, carboxylic acids and their derivatives, volatile oils, pigments, and minerals such as K, Na, Mg, Al, Fe, P, Na, Ba, Zn, Cu, Sr, Pb, Ti, Sn, Ni, V, Co, W, Li, Mo, and Be [1]–[5]. Therefore, the extraction from MO's roots will contain these aforementioned substances and minerals [4]. Le Thi Tham et al. [6] disclosed that the leaching process was carried out one to two times using an ethanol solution of 50% (v/v) at 45 °C in a range of leaching time from 60 to 90 hours, while the ratio of raw material (g) and solvent (ml) changed from 1/10 to 1/4 under gentle stirring, resulting in 58.78% of soluble matter in the obtained extraction. The obtained extract was concentrated under 0.8 atm pressure to collect the concentrated extract and dried to obtain the MO's extract powder. Similarly, the reference [7] performed the extraction of MO's powder with a particle size of less than 1 mm, which was ground from the sliced, cored, and dried root of MO in conditions like a ratio of solid to liquid of 1/9 and a solution containing 60% ethanol for 60 hours to extract 99.9% of the anthraquinone from the initial raw material. It should be noted that the leaching time was prolonged from 60 to 90 hours, making it challenging to apply these methods to make products from the MO [6], [7]. In contrast, Duduku and co-workers [8] leached powder of MO's root using ethanol solvent of 70% (v/v) for only 6 hours to produce a dry extract. However, the ratio of solid and liquid is 1/30, which increases the solvent cost or the cost of solvent regeneration, which is a problem that needs consideration.

According to diffusion theory, the mechanism of the leaching process of BASs from the MO's root occurs in three successive stages [9], including 1) The solvent penetrates the porous structure of the MO's root; 2) BASs dissolve into the solvent within the porous capillaries; 3) The dissolved BASs diffuses out of the pore of the MO's root. Therefore, the slowest stage will determine the overall rate and efficiency of the extraction process. Among the three stages, the first and the second stages have a relatively high rate compared to the third one. In the third stage, the diffusion of BASs from inside the pore system to the outside one occurs in a stagnant liquid, which is extremely slow. To reduce the time of this stage and increase the speed of the leaching process, it should be conducted as options below:

a) Reducing the diffusion distance, or particle size, by grinding the MO's root into smaller particles while also increasing the solid-liquid interfacial area can improve the efficiency of mass transfer, according to equation (1) as below:

$$m = K(x_1^* - x_2)F \quad (1)$$

Herein, m (kg·s−1) represents the obtained BASs amount, determined according to the material balance equation (2):

$$m = G_1(x_{10} - x_1) \quad (2)$$

G_1(kg·s−1) is the amount of material introduced for extraction; x_i is the BASs's concentration calculated in weight percentage in which x_1^*; x_{10}; and x_1 stand for the solid phase at balance with the liquid phase; at the initial time; and final time, respectively, while x_2 stands for the liquid phase.

F (kg·m−1·s−1) is the surface area of the solid-liquid interface and can be estimated from the equation: $F = a \cdot G_1$ (in which a (m2·m−3) stands for a specific surface area that can be estimated from the following equation $a = \frac{1}{I}$ with I (m−1) is the fineness of the extracted material).

K (m·s−1) stands for the mass transfer coefficient and is determined according to equation (3):

$$K = \frac{1}{\frac{\delta}{D_1} + \frac{1}{\beta_2}} \approx \frac{D_1}{\delta} \quad (3)$$

D_1(m2·s−1) is a diffusion coefficient in the solvent within the pores; δ (m) is the particle size of the material ; β_2 (m·s−1) is the mass transfer coefficient in the liquid phase outside the solid particles.

A diffusion coefficient is defined as

$$D_1 = \frac{7.4 \cdot 10^{-12}\sqrt{\varphi M_2}T}{\mu_2 V_{BAS}^{0.6}} \quad (4)$$

Where, φ is a coefficient stating the influence of the solvent; M2 (g·mol−1) is the molecular weight of the solvent; T(K) is the temperature of the solvent; μ2 (cP) is the solvent viscosity and V_{BAS}(cm3·mol−1) is the molar volume of BASs. Accordingly, it shows that D_1 depends on the nature of the solvent and the characteristics of BASs. Besides, D_1 is also proportional to the temperature, implying that a higher temperature leads to an increase in D_1, resulting in reduced extraction time.

Equation (4) indicates that increasing the temperature of the solvent will increase the diffusion coefficient in the extraction solvent. However, since the temperature rises to the boiling point of the solution, the formation of vapor bubbles can interrupt the solid-liquid interfacial contact, halting the process [10]. Additionally, heating the solvent to increase the temperature leads to increased energy consumption and higher production costs. Furthermore, increasing the temperature too much can alter the BASs from the root of MO, specifically, and herbal substances in general.

From the above analysis, it is clear that the extraction of BASs from MO's roots is the key factor that determines the efficiency, quality, and cost of MO's extract production line. The extraction process of BASs from MO's root depends on the nature of the extraction solvent; for BASs, a suitable concentration of ethanol-water solution is often used, depending on the raw material and the desired product. Temperature not only affects efficiency but also quality, as BASs are susceptible to heat denaturation. The duration of the extraction process influences both productivity and cost, as well as the final product's value. This article will examine the effects of these factors on the extraction efficiency of BASs from purple MO, a valuable medicinal herb found in Vietnam. The article also investigates the impact of the solid-to-liquid ratio on the recovery efficiency of BASs.

2. Materials and Methods

2.1. Materials and laboratory equipment

Roots of purple MO (around 2.0 kg) originating from Quang Ninh are provided by Rung Vang Co. Ltd. (31 Tran Trieu Luat, Ward 7, Go Vap District, Ho Chi Minh City, Vietnam). Ethanol of 96 v/v and Whatman filter paper (GF/C, 1.2 μm pore size, density 1200 kg/m3) are purchased from Tan Hung Phat Co. Ltd. (Vietnam).

Equipment and tools used for conducting experiments are an analytical balance (Ohaus PX2202, USA), a drying oven (Memmert UN160, Germany), a crusher, a water-bath (Memmert WNB22, 220 V-50 Hz, 2000 W, Germany), a particle size analyzer, a digital thermometer, glassware beakers, bottles, and pipettes.

2.2. Experimental methods

MO's root washing, cutting into pieces, coring, and then being placed in the oven for drying until reaching a constant weight. After removal from the oven, they are allowed to cool for moisture content measurement. Subsequently, they are finely ground into powder, and a sample of the crushed powder is taken for particle size distribution measurement. The results indicate an average size of 51.10794 μm and a nominal size of 80.43832 μm (refer to Figure 2).

The extraction process of biologically active compounds from the MO's root is depicted in Figure1.

Further research focuses on the effects of ethanol concentration in the solvent, temperature, and extraction time, following the same method (refer to Figure 1). The experimental design follows an alternating variable plan while maintaining particle size and a solid-to-liquid ratio (S/L ratio)) of 1:10. The arrangement of research experiments is outlined in Figures 1, 2, and Table 1.

The total amount of substances extracted from MO's root is calculated using the equation (5):

$$m = (m_{01} + m_{02} + m_{03}) - (m_{03} + m_2) \quad (5)$$

Recover efficiency of substances extracted from MO's root is calculated using the equation (6):

$$E = \frac{m}{m_{01}}.100\% \quad (6)$$

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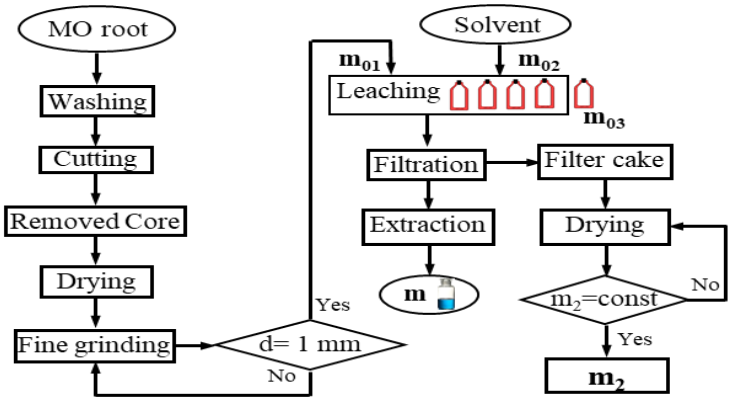

Figure 1. *Experimental diagram for extracting biologically active substances from MO's root.*

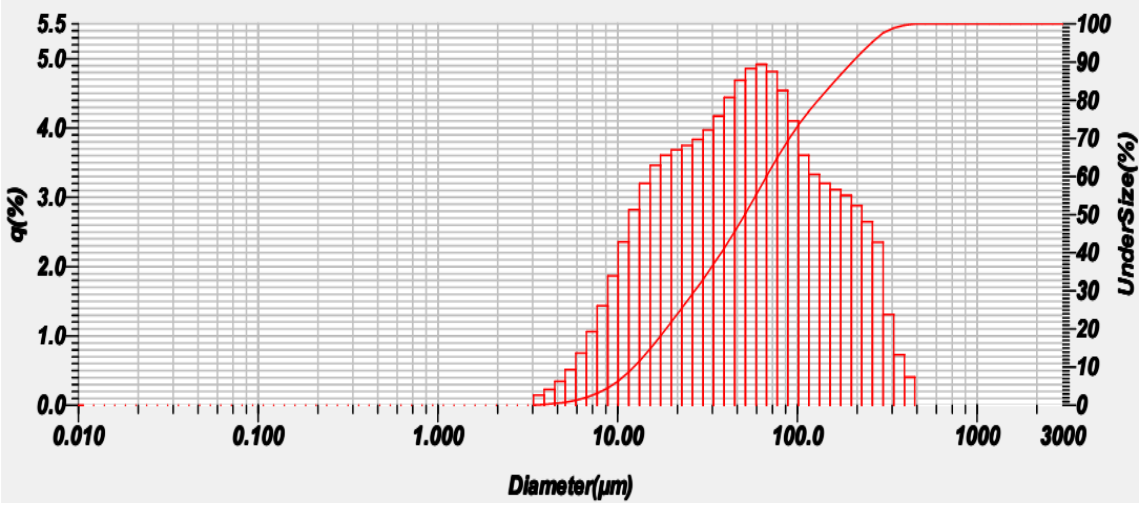

Figure 2. *Particle size distribution*

Table 1. *Experimental Design Matrix for Studying the Effects of Ethanol Concentration, Temperature, and Extraction Time on the recovery efficiency (E) based on the ratio of solid to liquid (S/L ratio)*

Parameters	**Experimental domain**			
Extraction time, h	2	4	6	8
Ethanol concentration, % (v/v)	50	65	80	
Temperature, °C	40	60	80	
S/L ratio, g/mL	1/5	1/10	1/20	

The experiment proceeded as follows: the temperature was fixed according to the values specified in Table 1. Following the steps outlined in the diagram in Figure 1, the recovery efficiency (E) of each sample was determined based on the ethanol concentration at the designated time points in Table 1. Each measurement was performed three times to obtain an average value. The experimental results are presented in Table 2, Table 3, and Figure 3.

3. Results and discussion

3.1. The impact of temperature, concentration, and extraction time on separation efficiency

Experimental results illustrating the influence of temperature, ethanol concentration in the solution, and extraction time on recovery efficiency, with a weight ratio of solid and liquid of 1/10, are shown in Table 2 and Figure 3.

The extraction efficiency varies with temperature, ethanol concentration in the solvent, and time, as depicted in Figure 3.

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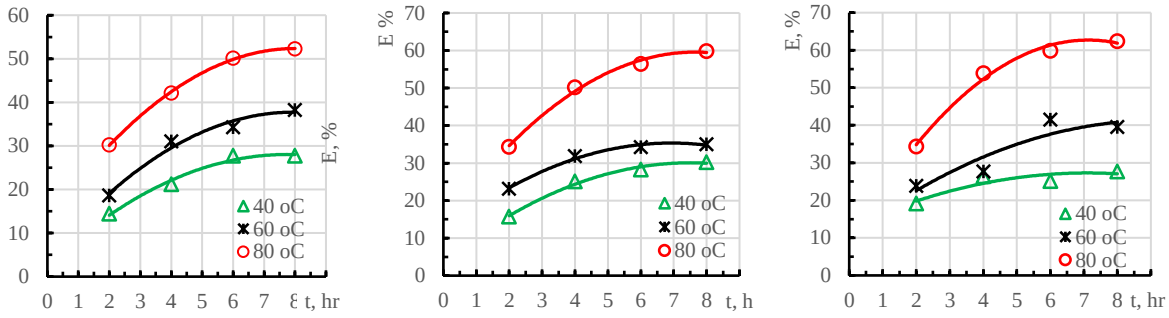

Figure 3. *Changes in recovery efficiency of active substances from Morinda officinalis How's root according to temperature [a) – 40°C, b) – 60°C, c) – 80°C], concentration, and time.*

The experimental findings, as presented in Table 2 and Figure 3, reveal several key insights. At a solid-to-liquid ratio of 1:10, increasing both temperature and ethanol concentration in the solvent results in a rapid enhancement of recovery efficiency. However, beyond 6 hours of extraction time, the rate of increase in recovery efficiency diminishes. Therefore, for the powder of MO's root, which has an average size of approximately 0.5 mm, it is optimal to limit the extraction duration to around 6 hours. This timeframe contrasts significantly with the prolonged extraction process for lychees [6], which can extend up to 60-90 hours due to their lack of grinding.

Interestingly, this 6-hour extraction duration aligns with the time frame observed from the reference [8], albeit with a solid-to-liquid ratio of 1/30, leading to higher solvent consumption.

Moreover, when escalating the ethanol concentration from 50% (v/v) to 65% (v/v) and subsequently to 80% (v/v), the recovery efficiency exhibits a progressive increase over time. However, the rate of increase from 50% (v/v) to 65% (v/v) exceeds that from 65% (v/v) to 80% (v/v). This phenomenon can be attributed to the polar effect of ethanol on non-polar biologically active substances of various sizes. Consequently, to mitigate solvent expenses, it is advisable to employ alcohol solvents containing 50-60% (v/v), consistent with the findings in the previous report [11]. Such a strategy would effectively reduce the overall cost associated with extraction solvents.

Table 2. *The impact of temperature, concentration, and extraction time on extraction efficiency*

No.	Extraction time (h)	Ethanol concentration (v/v)	Temperature (°C)	Efficiency (%)
1	2	50	40	14.39
2	2	50	60	18.61
3	2	50	80	30.20
4	2	65	40	15.71
5	2	65	60	23.17
6	2	65	80	34.30
7	2	80	40	19.17
8	2	80	60	28.30
9	2	80	80	38.25
10	4	50	40	21.16
11	4	50	60	31.82
12	4	50	80	46.22
13	4	65	40	23.86
14	4	65	60	31.04
15	4	65	80	53.82
16	4	80	40	26.39
17	4	80	60	33.91

18	4	80	80	42.13
19	6	50	40	39.50
20	6	50	60	41.78
21	6	50	80	57.33
22	6	65	40	27.68
23	6	65	60	41.47
24	6	65	80	56.44
25	6	80	40	25.08
26	6	80	60	34.22
27	6	80	80	50.15
28	8	50	40	39.63
29	8	50	60	36.46
30	8	50	80	59.82
31	8	65	40	30.28
32	8	65	60	39.55
33	8	65	80	62.41
34	8	80	40	27.70
35	8	80	60	35.01
36	8	80	80	52.26

3.2. The effect of the solid-to-liquid ratio on recovery

The experimental findings of the effect of the solid-to-liquid ratio on recovery efficiency relate to different temperatures and ethanol concentrations after 6 hours, as presented in Table 3

Table 3. *Effect of ratio of solid to liquid at different temperatures and Ethanol Concentrations after 6 hours on recovery*

No.	The ratio of solid to Liquid g/mL	Ethanol concentration (v/v)	Temperature (°C)	Efficiency (%)
1	1/5	50	40	24.20
2	1/5	50	80	39.70
3	1/5	80	40	16.20
4	1/5	80	80	41.00
5	1/10	50	40	39.50
6	1/10	50	80	57.30
7	1/10	80	40	25.10
8	1/10	80	80	50.20
9	1/20	50	40	29.60
10	1/20	50	80	56.10
11	1/20	80	40	29.80
12	1/20	80	80	57.60

From the obtained data, it is evident that when extracted with the ratio of solid to liquid is 1/5, the recovery efficiency remains relatively low, reaching a maximum of only 41.0%, depending on the temperature and ethanol concentration. However, with the ratio of solid to liquid being 1/10, the recovery efficiency notably improves, reaching a maximum of 57.3%, akin to the findings in [6], but with a significantly shorter processing time of only 6 hours.

On the other hand, with the ratio of solid to liquid being 1/20, although there is a slight increase in recovery efficiency, reaching a maximum of only 57.6%, the heightened solvent cost outweighs the benefits, especially in actual production scenarios.

Furthermore, the experimental data indicates that at higher temperatures, the recovery graph exhibits a more significant increase. This phenomenon can be attributed to the decrease in solvent viscosity, which reduces diffusion resistance (as per formula 4), consequently enhancing the diffusion coefficient and, subsequently, the mass transfer coefficient (as per formula 3), thereby increasing extraction yield (as per formula 1).

3.3. Building process diagram for producing the purple Morinda officinalis How's extract powder

After harvesting, Morinda officinalis How's root undergoes a series of advanced processes for extraction. Firstly, they are thoroughly cleaned in Device 1, then drained and cut into pieces measuring (1-2) cm, and finally cored using Device 2. Next, they are dried in Dryer 3 with heated air at a temperature of 60-65 °C from Calorifier 4. The dried product from Dryer 3 is finely crushed to a size of less than 1 mm in Mill machine 5 and then quantified, along with a 60% alcohol solution from Tank 6, in a ratio of solid to liquid of 1/10, before entering Extraction Device 7. It should be noted that BASs are compounds that are easily denatured by temperature. Therefore, the leaching process is oriented to be carried out within a low-temperature domain for the maintenance of the BASs' natural characteristics. Herein, the leaching process was conducted within their allowed temperature range of 40-80 °C with the optimal temperature at 60 °C. The jacketed-system Extraction Devices 7 is equipped with steam at 2.0 atm pressure to maintain the desired temperature. After 6 hours of extraction, the extract is separated and stored in Container 14. It is then concentrated in Concentrator 8 at a pressure of 0.5 atm (the boiling temperature of the mixture is about 60-70 °C) to an 80% concentration and stored in Container 13.

The residue separated from Extractor 7 was repurposed as fertilizer. The Base Condenser System 9, Non-condensing Air Separator 10, Vacuum Pump 11, and Water Tank 12 worked together to maintain the stability of Evaporation System 8. This ensured that overheating and loss of biologically active substances in the product were avoided.

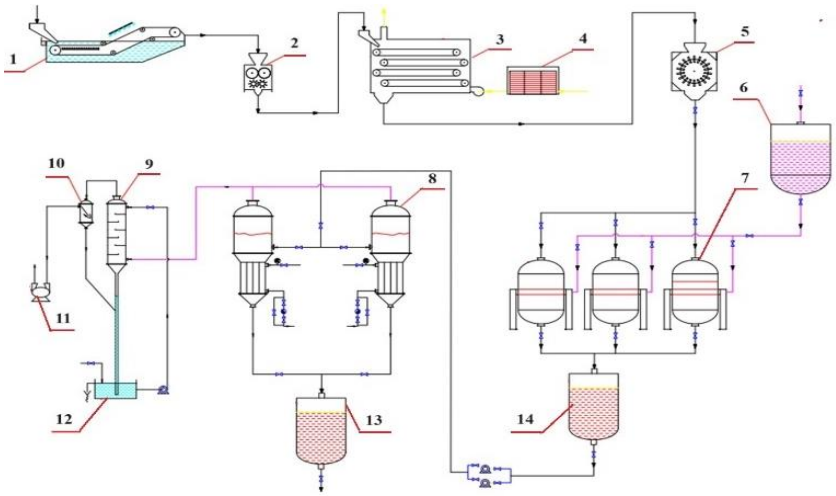

Figure 4. *Process Diagram for Producing the purple Morinda officinalis How's extract powder.*

4. Conclusions

Based on the research findings, several key conclusions emerge:

To shorten the extraction time, it's essential to grind the roots of MO to a size smaller than 1 mm before extraction. However, determining the optimal particle size requires a closer examination based on the overall cost, encompassing the grinding process, residue filtration, and extraction.

Utilizing a solid-to-liquid ratio of 1:10 and the powder of MO's roots with a size less than 1 mm allows for extraction with a recovery rate exceeding 57.3% at a temperature of 60°C and an ethanol concentration of 60% in the solvent. Subsequently, the extraction solution is concentrated under the pressure of 0.5 atm, corresponding to the boiling temperature of the mixture around 60-70 °C.

These insights lay the foundation for advancing production technology for the purple MO's extract powder. This progress opens avenues for expanding the product line and enhancing the value of this esteemed medicinal plant in Vietnam, as delineated in Figure 4.

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Conflict of Interest

The authors declare that they have no conflict of interest.

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