EFFECT OF EXTRACTION METHOD ON THE RECOVERY EFFICIENCY OF POLYPHENOL COMPOUNDS FROM *Muntingia calabura* L. LEAVES

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ABSTRACT

This research presents the results of recovering polyphenolic compounds from the waste and by-product material of *Muntingia calabura* L. leaves using extraction methods like maceration, ultrasound-assisted extraction, and ultrasound-assisted enzymatic extraction. In the maceration method, several factors were studied: solvent type (ethanol, methanol, water); solid-liquid ratios (1:5, 1:10, 1:20, 1:30, 1:40); and extraction times (3; 5; 7; 9; 11 hours). This method achieved a total polyphenol content of 32.24 mgGAE/g. Using ultrasound-assisted extraction, the following factors were examined: ultrasound power (100; 200; 300 W); solid-liquid ratios (1:5, 1:10, 1:20, 1:30, 1:40); and times (3; 5; 10; 30; 50 minutes). This method yielded a total polyphenol content of 38.265 mgGAE/g. Additionally, response surface methodology with a central composite design (RSM-BBD) was applied to optimize the polyphenol extraction process from *M. calabura* leaves. The model was designed with 15 experiments, including 3 central points, using ultrasound-assisted enzymatic extraction with 3 factors: enzyme concentration (0.5; 1; 2; 2.5; 3%), pH activation (4, 5, 6), and temperature activation (30, 40, 50, 60, 70°C). The ANOVA analysis results showed a high value of R^2 and $p < 0,0001$ indicating the model's statistical significance, leading to a total polyphenol content of 74,0125 mgGAE/g. The FTIR spectrum of the *M. calabura* leaf extracts indicated the presence of functional groups characteristic of polyphenolic compounds, including aromatic alcohols (-OH), alkenes (-CH), esters (-C=O), amino acids (C=C), and alcohols and ethers (C-C).

Keywords: Muntingia calabura L. leaves, polyphenols, maceration method, ultrasound, enzyme, cellulase.

1. INTRODUCTION

One of the potential plant species that can be tested for its antioxidant capacity is *M. calabura* L. leaves. The content of bioactive compounds contained in *M. calabura* leaves includes phenolics, flavonoids, tannins, triterpenes and saponins. This plant is native to Central America, western South Asia, it is grown wild, found along roadsides, and commonly used as a shade tree [1]. The *M. calabura* tree has low economic value, and public knowledge about the benefits of *M. calabura* as a medicinal and food source is quite limited. However, the leaves of the *M. calabura* can be utilized to produce personal care and cosmetic products [2–4]. However, *M. calabura* leaves are often wasted and become trash [5, 6].

Over the past several decades, scientists around the world have been striving to explore and study the various pharmacological effects that the *M. calabura* tree can offer to humans. According to information gathered from domestic and international journals published over the past 10 years (2010-2020), the *M. calabura* tree has shown significant potential for new activities in the future [5, 6]. The presence of several bioactive compounds in *M. calabura* leaves, particularly flavonoids and polyphenols, makes the leaf extract possess antioxidant activity [7], anti-infection, anti-inflammatory, anti-cancer, and antibacterial activities [4, 8]. The potential antibacterial and antifungal applications of crude extracts prepared in different solvents from *M. calabura* leaves against Gram-positive and Gram-negative bacteria, such as *Escherichia coli*, *Salmonella typhimurium*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Bacillus subtilis*, *Bacillus megaterium*, *Klebseilla pnemoniae* [9].

Extraction methods applied to extract polyphenols from various natural materials have been extensively studied. Traditional extraction methods have evolved over more than a century to extract phenolic compounds using solid-liquid systems, where the plant material base involves processes like maceration, infusion, or Soxhlet extraction to release different phenolics depending on their solubility. Typically, dry samples are pre-treated mechanically to reduce particle size, maximizing the contact between the material and the solvent. Traditional extraction methods have the advantage of not requiring complex equipment, are ease of use, efficiency, and wide-ranging applicability [10], [11]. However, there are some drawbacks, such as long processing times, solvent toxicity, and residuals that may remain in the final product, necessitating additional time-consuming purification steps, which can increase the overall cost of the process [10].

Due to the issues related to the limitations of traditional extraction methods, current research is developing and applying more efficient and safer alternative extraction methods, including ultrasound-assisted extraction, microwave-assisted extraction, ultrasound-assisted extraction combined with enzymes, and supercritical fluid extraction, … [13].

Polyphenol extraction processes supported by technology often operate at high pressure or temperature, or with the help of enzymes, reducing both extraction time and resource consumption (especially strong and toxic solvents) while generating minimal waste. Technology-assisted extraction enhances quality standards in three strategic directions: reducing solvent volume, shortening extraction time, and saving energy. These fundamental achievements lead to significant improvements in recovery rates, selectivity, and extraction yields [14].

Therefore, selecting suitable extraction methods is a top priority for many industries to achieve high economic efficiency by reducing energy costs and complying with green chemistry requirements. This study surveys and optimizes influencing factors such as solvent type, solid-to-solvent ratio, ultrasound power, enzyme concentration, etc., for extraction methods like maceration, ultrasound-assisted and ultrasound-assisted enzymatic extraction, aiming to identify the extraction method that achieves the highest recovery efficiency of polyphenols from the waste and by-product source of *M. calabura* leaves.

2. MATERIALS AND METHODS

2.1. Materials

- *Materials*: *M. calabura* leaves, collected from roadside trees in Tan Phu District, Ho Chi Minh City, washed, processed, and removed impurities. They are dried and ground to a particle size of 0.5 – 1.0 mm, then stored in 50 (g) polyamide bags using vacuum sealing.

- *Chemicals*: Gallic acid (P ≥99.0%, China); Folin–Ciocalteu reagent by Merck (2N, Germany); Na_2CO_3 (P ≥99.0%, China); HCl (C = 37.0 – 38.0%, China); Cellulase enzyme (Sigma, USA); Ethanol solvent (P ≥98.0%, China); Methanol solvent (P ≥99.0%, China).

2.2. Methods

2.2.1. Extraction process of polyphenols using maceration method

M. calabura leaf powder are soaked in solvent (ethanol, methanol, water) with solid-liquid ratios (1:5, 1:10, 1:20, 1:30, 1:40 w/v) and extraction times (3; 5; 7; 9; 11 hours). Polyphenol extraction was carried out by using the maceration method. The extract was centrifuged at 4000rpm for 10 min. The solvent is removed by rotary evaporation, and the polyphenol-rich extracts from *M. calabura* leaves is dried to obtain the concentrated extract.

2.2.2. Ultrasound-assisted extraction process of polyphenols

M. calabura leaf powder is added to ethanol with solid-liquid ratios (1:5; 1:10; 1:20; 1:30; 1:40 w/v). Polyphenol extraction is performed using ultrasound-assisted extraction at different ultrasound power (100W, 200W, 300W) and times (3; 5; 10; 30; 50 minutes). The extract was centrifuged at 4000rpm for 10 min. The solvent is removed by rotary evaporation, and the polyphenol-rich extracts from *M. calabura* leaves is dried to obtain the concentrated extract.

2.2.3. Ultrasound-assisted enzyme extraction process of polyphenols

The process of surveying and selecting fixed conditions for polyphenol extraction using ultrasound-assisted extraction has led to improvements when combining with enzymes. The optimization of polyphenol extraction using ultrasound-assisted enzymatic extraction involves key influencing factors such as X_1 (enzyme concentration), X_2 (pH activation), and X_3 (temperature activation), which are presented using the Box-Behnken Design (BBD) with JMP.

Table 1. Coded Values for Independent Variables

No	Independent Variables	Unit	Code	Patterns		
				-1	0	+1
1	Enzyme concentration	%	X_1	0.5	2	3
2	pH activation	-	X_2	4	5	6
3	Temperature activation	°C	X_3	30	50	70

2.2.4. Determination of total polyphenols content

The quantity of total polyphenols content in the extracts was determined using the Folin-Ciocalteu reagent of the Akkol et al. (2008) method. The reaction mixture consisted of 0.1 mL of extract, then added 7.5 mL of Folin–Ciocalteu and allowed to stand for 3 min followed by the addition of 5.0 mL of 7.5% Na_2CO_3.The solution mixture was incubated for 1 hours in the dark, then the absorbance was measured at a wavelength of 765 nm by using UV-Visible spectrophotometry. Phenolic content is expressed in mg by weight of gallic acid equivalent/g extract (mg GAE/g dry fraction) [10].

The total polyphenols (TPP) in a sample can be calculated using the following formula:

$$X \text{ (mg GAE/g dry fraction)} = \frac{(x.V.f)}{(1000.m)}$$

Where:

X: the total polyphenols calculated in milligrams of gallic acid per gram of dry fraction (mgGAE/g dry fraction)

x: the amount of gallic acid in milligrams, determined from the calibration curve (ppm)

V: is the specimen solution's volume; f: the dilution factor

1000: the conversion factor to grams; m: the mass of leaves powder (g)

2.2.5. Determination FTIR spectrum

The FTIR spectrum was obtained using an FTI IMPACT Nicolet 410 in the range of 4000-400 cm^{-1}. The polyphenol-rich extracts from *M. calabura* leaves were dried for 2 days in a vacuum oven at 60°C and then pelletized with KBr for FTIR analysis.

2.2.6. Statistic analyis

Data were reported as mean ± SD. The results were analyzed with one-way analysis of variance (ANOVA) using JMP 10.0. Significant differences were analyzed using the least significant difference (LSD) test. The significance was established at $P < 0.05$.

3. RESULTS AND DISCUSSION

3.1. Extraction of polyphenols using the maceration method

The extraction of *M. calabura* leaves involves a soaking process with a solvent to draw out and release phenolic compounds based on their solubility. To enhance the efficiency of extraction methods, dried *M. calabura* leaves are mechanically pre-treated to reduce particle size, maximizing the contact between the material and the solvent. The solvent volume should be enough to cover the material layer by at least 1 cm. It's crucial to choose a solvent with a polarity similar to that of polyphenolic compounds and to ensure a soaking duration sufficient to maximize the extraction of these compounds [10].

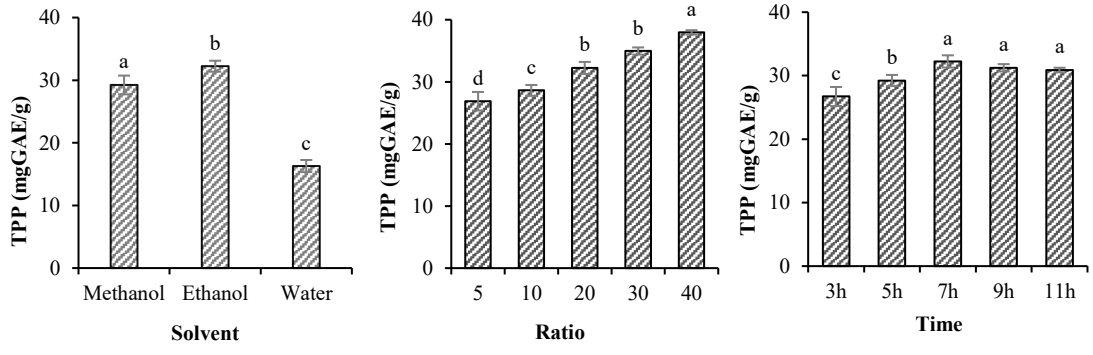


Figure 1. TPP extracted from *M. calabura* leaves using the maceration method

Legend: a, b, c, d ($p \leq 0.05$): indicate statistically significant differences; the data are the averages from three replicates

As shown in Figure 1, when using ethanol as extraction solvent, phenolic content was 32.24 mgGAE/g dry fraction, which is higher than the other two solvents. This is because water and methanol have high polarity, while polyphenols have lowest polarity similar to

ethanol, making them better soluble in ethanol, thus enhancing extraction efficiency [15]. Additionally, the polyphenol content increases with the solid-liquid ratio and time, which is because the longer time and which can be ascribed to the increase in surface contact between the plant matrix and the solvent, leading to better extraction of polyphenols [10], [11]. However, soaking for extended periods (beyond 9 hours) can lead to the degradation of natural compounds, reducing their bioactivity [16]. Also, use of large amount of solvents (beyond a 1:40 ratio) can be wasteful and leave residues in the final product, necessitating additional purification steps, which can be time-consuming and impact the overall cost of the process [10]. Thus, for polyphenol extraction using the maceration method, the optimal conditions are: ethanol as the solvent , solid-liquid ratio 1:40 (w/v), and 7 hours extraction time, yielding a total polyphenol content of 32.24 mgGAE/g.

3.2. Ultrasound-assisted extraction process of polyphenols

In the polyphenol extraction process using technology such as ultrasound, the cell structure is disrupted, allowing the polyphenol compounds within the cells to be released [11]. This process reduces extraction time and solvent consumption (especially strong and toxic solvents) while producing minimal waste, leading to improved recovery, selectivity, and extraction yield.

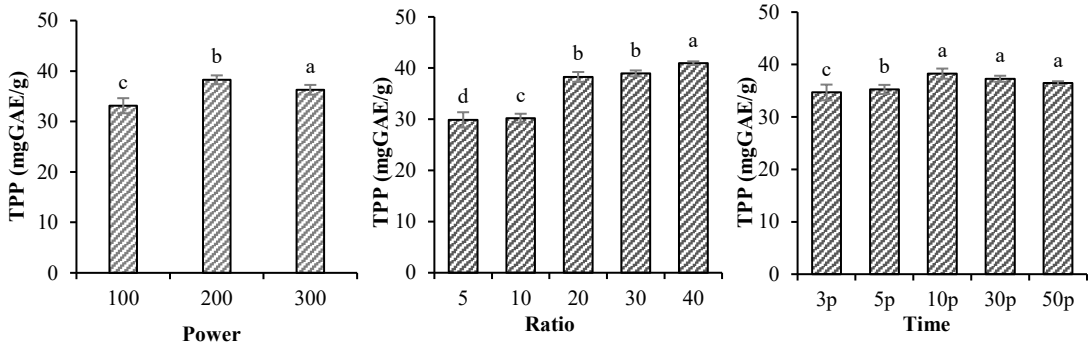


Figure 2. TPP extracted from *M. calabura* leaves using ultrasound-assisted extraction
Legend: a, b, c, d ($p \leq 0.05$): indicate statistically significant differences; the data are the averages from three replicates

The results in Figure 2 indicate that as ultrasound power increased (from 100 to 200 W), the capacity to break down cell walls increases, leading to a higher extraction efficiency of polyphenols. However, when high-power ultrasound (300W) will produce free hydroxyl groups to degrade polyphenols, which will hurt phenolic substances and their antioxidant activity in the extracts [18], [19]. When the solid-liquid ratio is increased (from 1:20 to 1:40), the extraction did not result in a significant change in the TPC; and using ultrasound energy for longer time (30 to 50 minutes) raising the temperature, the degradation of polyphenolic substances, the total polyphenols content of the extract showed a downward trend. Meanwhile, although there are variations in TPP at different solid-liquid ratios and times, these differences are not mathematically significant when extracting at a ratio of 1:20 (w/v) and extraction time of 10 minutes. Thus, with the assistance of ultrasound at a power of 200 W, compared to the maceration method, the TPP efficiency lowers (38.265 mgGAE/g) while reducing the time by a factor of 42 (10 minutes), and the amount of solvent used is halved (at a ratio of 1:20).

3.3. Optimization of ultrasound-assisted enzymatic extraction of polyphenols

Ultrasound-assisted enzymatic extraction technology is a safe and sustainable approach. Combined with the stimulation of ultrasound waves, it enhances the contact between the

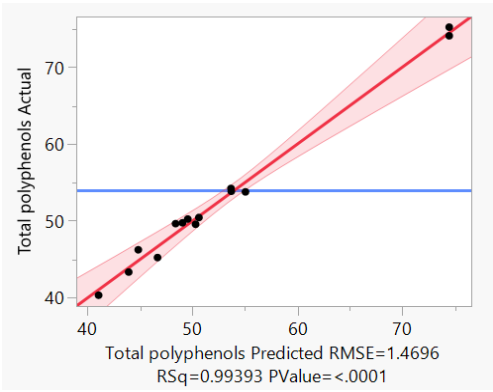
material's surface and the enzyme, effectively breaking down cell walls, thereby increasing the extraction efficiency and yielding a higher recovery of polyphenols [14].

The optimization of phenolic extraction from *M. calabura* leaves was enhanced using the Response Surface Methodology (RSM) technique, shown in Table 1. This technique is used to create a matrix for 15 experiments. The total polyphenols results (Y) and the model-predicted results (Y') of the 15 experiments generated by JMP 10.0 software are presented in Table 2.

Table 2. Table of experimental values and model-predicted results

Experiments	**X_1**	**X_2**	**X_3**	**Total polyphenols (mgGAE/g)**	
				Y	**Y'**
1	1	4	50	40.3452 ± 0,1412	40.6573
2	3	4	50	50.4536 ± 0,1911	50.5394
3	1	6	50	53.8632 ± 0,1706	53.2064
4	3	6	50	50.2568 ± 0,1733	50.1316
5	1	5	30	49.6574 ± 0,1307	49.3162
6	3	5	30	49.7675 ± 0,1807	49.2637
7	1	5	70	49.5754 ± 0,2513	49.6853
8	3	8	70	53.7896 ± 0,2029	53.8642
9	2	4	30	43.3578 ± 0,1403	43.0403
10	2	6	30	45.2453 ± 0,1395	45.5378
11	2	4	70	46.2563 ± 0,1905	46.1951
12	2	6	70	54.2368 ± 0,1933	54.0976
13	2	5	50	75.2355 ± 0,1802	75.2219
14	2	5	50	74.7479 ± 0,1333	74.1393
15	2	5	50	73.9683 ± 0,1932	74.0942

Using JMP 10.0 software, after removing factors that had an insignificant impact on the objective variable of total polyphenols, the analysis of variance and model validation results presented in Figure 3 show a correlation coefficient $R^2 = 0.9939$. This indicates that the experimental process was carefully and tightly controlled. With a p-value of <.0001* (<0.05), it can be concluded that the variables X (enzyme concentration, pH activation, temperature activation) have a statistically significant impact on the response values (TPP), validating the use of this order of correlation. This suggests that the model is appropriate and can accurately predict the study's objective.



Summary of Fit	
RSquare	0.99393
RSquare Adj	0.983005
Root Mean Square Error	1.469608
Mean of Response	54.01735
Observations (or Sum Wgts)	15

Figure 3. Variance for the quadratic polynomial model

This result presented in Figure 3, indicates a good fit of the regression equation to the experimental data, with a p-value of <.0001* indicates that only < 0.01% of the change in the Fit value is due to noise that the model couldn't account for. This result demonstrates that the regression equation has a good fit with the experimental data, leading to high statistical reliability. The correlation coefficient $R^2 = 0.9939$ indicates that 99.39% of the variation in total polyphenols is due to the impact of the independent variables: enzyme concentration, pH activation, and temperature activation. Lack of fit testing was employed to assess the adequacy of the fit. The ANOVA results for the lack of fit test indicated no inadequacy of the model concerning total polyphenol ($p > 0.05$), suggesting that the model adequately represented the experimental data.

Table 3. ANOVA variance for the regression model

Source	DF	Estimate	F-value	P-value
Model	9	74.485133	87.79	<.0001*
Enzyme concentration	1	1.3532875	2.60	0.0480*
pH activation	1	2.89865	5.58	0.0026*
Temperature activation	1	1.9787625	3.81	0.0125*
Enzyme concentration* pH activation	1	– 3.4287	– 4.67	0.0055*
Enzyme concentration* Temperature activation	1	1.026025	1.40	0.2215
pH activation* Temperature activation	1	1.52325	2.07	0.0929
Enzyme concentration* Enzyme concentration	1	–11.24872	– 14.60	<.0001*
pH activation* pH activation	1	– 14.67215	– 19.18	<.0001*
Temperature activation* Temperature activation	1	– 12.70437	– 16.61	<.0001*

(*): *significance level at $p < 0.05$*

ANOVA analysis is used to evaluate the model, as shown in Table 3, and the regression equation that represents the relationship between the factors of enzyme concentration, pH activation, and temperature activation with total polyphenols, with the coded variables, is as follows:

$$Y = 87.79 + 2.60X_1 + 5.58X_2 + 3.81X_3 - 4.67X_1X_2 - 14.60X_1^2 - 19.18X_2^2 - 16.61X_3^2 \quad (*)$$

The regression equation indicates that total polyphenols can be accurately predicted under the influence of all three first- and second-order factors X_1, X_2, and X_3; and is simultaneously influenced by the interaction between factors ($X_1 \times X_2$), as the model's inadequacy is statistically significant ($p<0.05$, $R^2=0.99$).

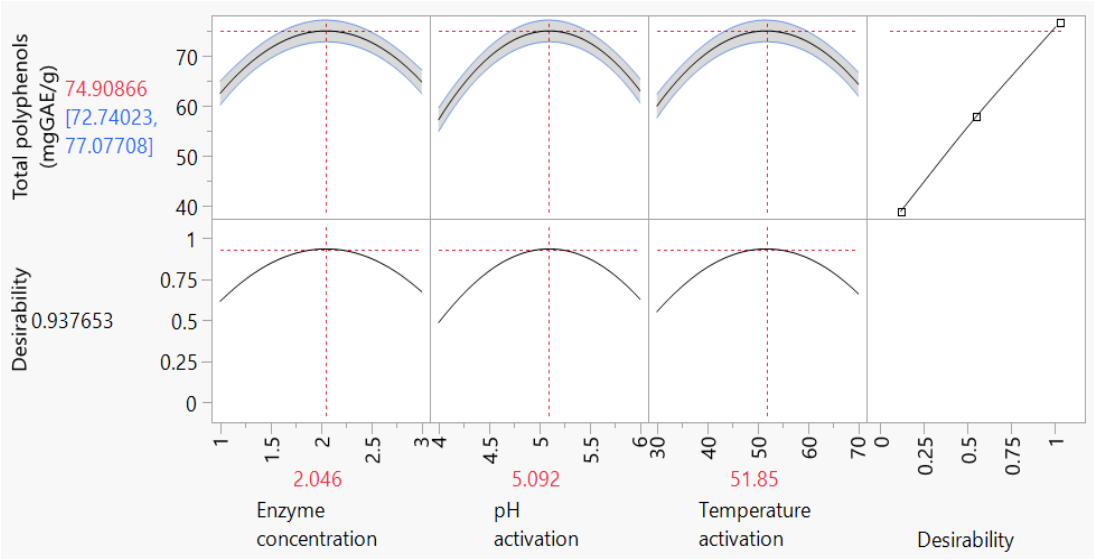


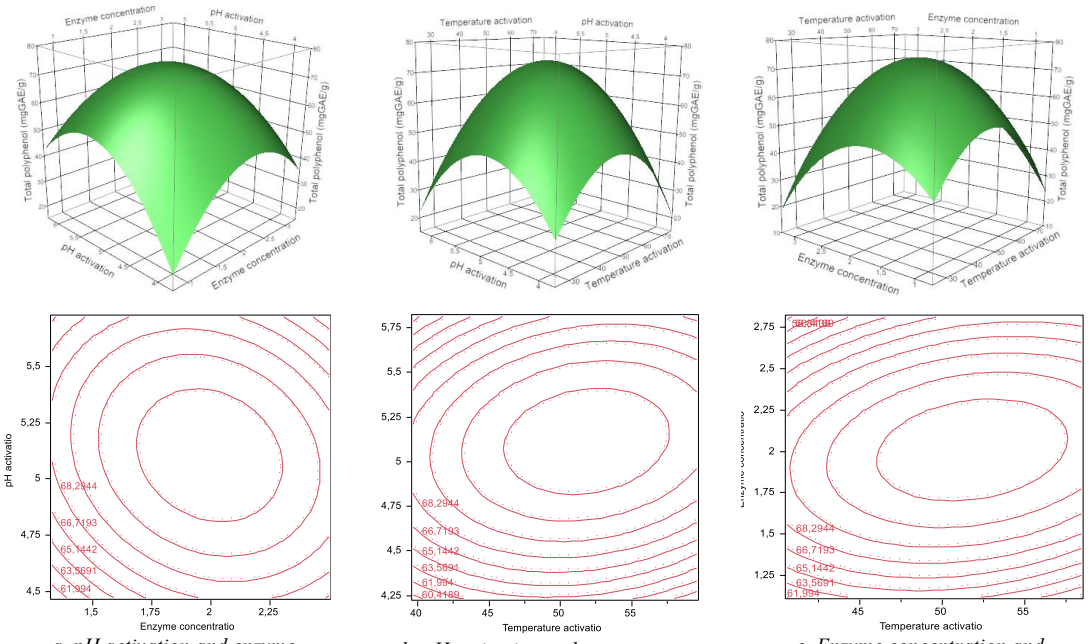
Figure 4. Graph predicting the desired values of total polyphenols for responses

The experimental prediction model gives the optimal value for the study of total polyphenols using temperature as a variable at 51.85°C, active enzyme concentration at 2.046%, and pH activation at 5.092, yielding a total polyphenol content of 74.9086 mgGAE/g (Figure 4). The actual experimental total polyphenol content is 74.0125 mgGAE/g. The predicted results and experimental outcomes show no statistically significant difference, demonstrating that the optimized model is completely appropriate and meaningful for the study of total polyphenols.

The response surface methodology (RSM) and the contour plots shown in Figure 5 can better illustrate the influence of the independent variables on the total polyphenols (TPP) when one factor is fixed.

Figures 5a and 5b indicate that the process achieves maximum TPP when the pH activation is at 5.092. This is because polyphenols, being strong antioxidants, are prone to oxidation at high pH levels [10], [12]; at lower pH, polyphenols are less likely to be oxidized, but the activation of enzymes becomes less efficient.

Figures 5b and 5c show that as the activation temperature increases, the TPP also increases, reaching an optimal point at 51.85°C, which is suitable for the active range of cellulase enzyme. This result aligns with the information provided by the manufacturer, indicating that cellulase enzyme operates best within a temperature range of 50-55°C. If the temperature exceeds the optimal range, stronger oxidation reactions occur, reducing the polyphenol content [13]. Cellulase is an enzyme capable of degrading cellulose by breaking down the β-1,4-glycosidic bonds in the polymer, or it can directly break the ether and ester bonds between polyphenols and the polymer structure of plant cell walls [14]. Plant cells contain various types of biopolymers such as cellulose, hemicellulose, pectin, etc., arranged in complex structures. The cellulase enzyme enhances the hydrolysis of plant cell wall polymers, breaking down cellulose, collapsing the cell walls, and releasing intracellular polyphenols [15–18].



a. pH activation and enzyme concentration

b. pH activation and temperature activation

c. Enzyme concentration and temperature activation

Figure 5. Response surface models and contour plots illustrating the effects of pairs of factors

The results shown in Figures 5a and 5c show that TPP increases with cellulase enzyme concentrations between 1.5% and 2.3%, but if the cellulase enzyme ratio is increased to 2.5%, the polyphenol content increases only slightly. This could be because when the enzyme ratio is too high, the rate of polyphenol extraction begins to slow down due to saturation [19].

3.3. FTIR spectrum

The FTIR spectrum of the *M. calabura* leaf extract with various extraction methods is shown in Figure 6. The polyphenol extracts are characterized by rich aromatic hydroxyl structures and their ability to form complexes with proteins [20].

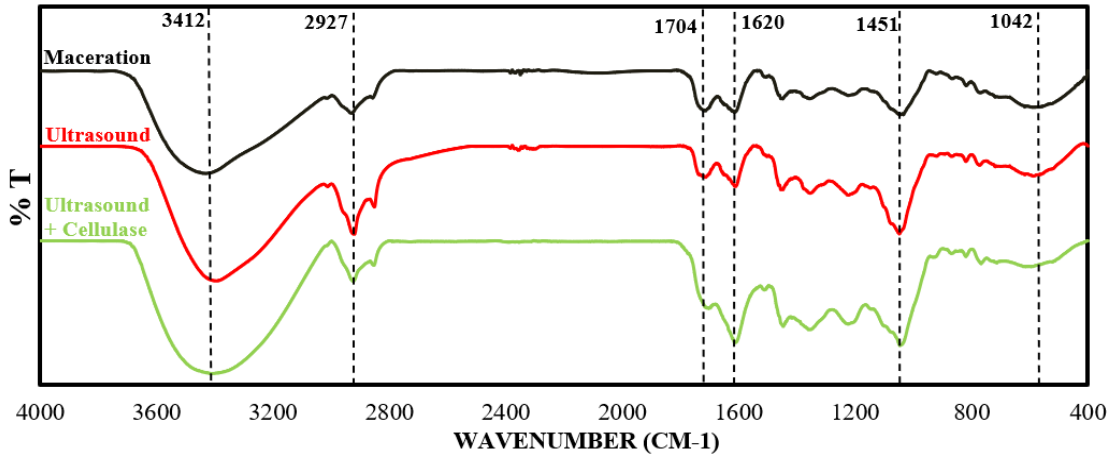


Figure 6. FTIR spectrum of *M. calabura* leaf extract compared across different extraction methods

The polyphenolic compounds in the *M. calabura* leaf extract contain the following functional groups: aromatic alcohols (-OH), alkenes (-CH), esters (-C=O), amino acids (C=C), and alcohols and ethers (C-C) [21]. The FTIR spectra of the *M. calabura* leaf extracts obtained through soaking, ultrasound-assisted, and ultrasound-assisted enzymatic extraction all exhibit the characteristic spectral bands of polyphenolic compounds. However, in the FTIR spectrum of polyphenol extracts from the ultrasound-assisted enzymatic extraction, the intensity and breadth of the bands are more significant compared to those in the spectra from the soaking and ultrasound-assisted extraction.

The spectral band observed in the range of 3500-3100 cm^{-1} is characteristic of the stretching vibrations of the -OH group, which belongs to the aromatic alcohol functional group- an important chemical component in *M. calabura* polyphenol extracts that reflects its antioxidant mechanism [22–24]. The peak in the range of 2932-2925 cm^{-1} is characteristic of the stretching vibrations of the C-H group, encompassing -CH, $-CH_2$, and $-CH_3$, which originate from carbohydrates and sugars [23], [25]. The absorption value of 1704 cm^{-1} is characteristic of stretching vibrations, while the band at 1732 cm^{-1} is indicative of the stretching vibrations of the C=O group in esters, mainly stemming from gallic acid derivatives in polyphenols [26, 27]. The peaks in the range of 1655-1603 cm^{-1} are characteristic of stretching vibrations and tensile vibrations of the C=C group [28], in the wavelength range of 1611-1444 cm^{-1}, characteristic of the vibrations of the C-C group [29] and some absorption bands in the range of 1045-879 cm^{-1} most clearly show the tensile vibrations of the C-OH group [30].

4. CONCLUSIONS

The study investigated the impact of different extraction methods: maceration, ultrasound-assisted extraction, and ultrasound-assisted enzymatic extraction on the efficiency of polyphenol recovery from *M. calabura* L. leaves. The maceration method was carried out under the following extraction conditions: ethanol solvent with ratio 1:40 (w/v), and 7 hours for extraction time, phenolic content was 32.24 mgGAE/g. Improvement through the use of ultrasound-assisted extraction at an ultrasound power of 200W increased the total polyphenol content (TPP) to 38.265 mgGAE/g with extraction time of 10 minutes and 1:20 for solid-liquid ratio. The most effective method was ultrasound-assisted enzymatic extraction, with the highest TPP reaching 74.0125 mgGAE/g. This was achieved using response surface methodology with a central composite design to optimize the polyphenol extraction process from *M. calabura* leaves. The predicted results from the model and the actual experimental results showed no statistically significant difference, indicating that the optimized model is entirely appropriate and meaningful. The optimal conditions for ultrasound-assisted enzymatic extraction were a pH activation of 5.092 with an activation time of 36.01 minutes at a temperature of 51.85°C. The FTIR spectrum of the *M. calabura* leaf extracts showed the presence of aromatic alcohol groups (-OH), alkenes (-CH), esters (-C=O), amino acids (C=C), and alcohols and ethers (C-C), characteristic of polyphenolic compounds.

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TÓM TẮT

NGHIÊN CỨU ẢNH HƯỞNG CỦA PHƯƠNG PHÁP TRÍCH LY ĐẾN HIỆU QUẢ THU HỒI CÁC HỢP CHẤT POLYPHENOL TỪ LÁ TRỨNG CÁ (*Muntingia calabura* L.)

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Nghiên cứu này trình bày kết quả thu hồi các hợp chất polyphenol từ nguồn nguyên liệu phế phụ phẩm lá trứng cá (*Muntingia calabura* L.) với các phương pháp trích ly bằng ngâm chiết, có hỗ trợ siêu âm, enzyme kết hợp hỗ trợ siêu âm. Phương pháp trích ly bằng ngâm chiết khảo sát các yếu tố: loại dung môi (ethanol, methanol, nước); tỉ lệ nguyên liệu:dung môi (1:5, 1:10, 1:20, 1:30, 1:40) và thời gian ngâm chiết (3; 5; 7; 9; 11 giờ) đạt hiệu quả tổng polyphenols 32.24 mgGAE/g. Sử dụng phương pháp trích ly có hỗ trợ siêu âm khảo sát các yếu tố: công suất siêu âm (100; 200; 300 W); tỉ lệ nguyên liệu:dung môi (1:5, 1:10, 1:20, 1:30, 1:40) và thời gian siêu âm (3; 5; 10; 30; 50 phút) có kết quả tổng polyphenols 38.265 mgGAE/g. Đồng thời ứng dụng phương pháp bề mặt đáp ứng với thiết kế mô hình lặp tâm (RSM-BBD) để tối ưu quy trình trích ly các hợp chất polyphenol từ lá trứng cá. Mô hình được thiết kế 15 thí nghiệm với 3 thí nghiệm tại tâm để xây dựng cho ba yếu tố khảo sát bằng phương pháp bằng phương pháp enzyme có hỗ trợ siêu âm với nồng độ enzyme (0.5; 1; 2; 2.5; 3%), pH hoạt hóa (4; 5; 6), nhiệt độ hoạt hóa (30; 40; 50; 60; 70°C). Kết quả phân tích ANOVA cho hệ số R^2 cao và $p < 0.0001$ chứng tỏ mô hình có ý nghĩa thống kê, được kiểm chứng cho kết quả tổng polyphenols cao nhất đạt 74.0125 mgGAE/g. Phổ FTIR của các cao chiết lá trứng cá cho thấy sự tồn tại của các nhóm chức rượu thơm (-OH), anken (-CH), ester (-C=O), amino acid (C=C) và rượu, ete (C-C) đặc trưng của các hợp chất polyphenol.

Từ khóa: Muntingia calabura L., lá trứng cá, polyphenols, ngâm chiết, siêu âm, enzyme, cellulase.