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Research article

Enhanced Enzyme-assisted Aqueous Extraction of Polyphenols from *Ficus auriculata* Fruits: Optimization and Assessment of Bioactive Properties

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Abstract

The *Ficus auriculata* fruit is not only a nutritionally valuable food but also a rich source of polyphenols, which contribute to cardiovascular health, cancer prevention, blood sugar regulation, and cholesterol reduction. This study aimed to optimize the extraction conditions for polyphenol-rich extracts from fig fruit using response surface methodology (RSM). The effects of enzyme concentration, solvent-to-solid ratio, and extraction time on total polyphenol content (TPC) were evaluated. The optimized conditions were determined to be an enzyme concentration of 0.74%, a solvent-to-solid ratio of 23:1 (mL/g), and an extraction time of 65 min. Under these conditions, the TPC in the extract reached 2.65 g GAE/100 g dry matter, closely aligning with the predicted value of 2.70 g GAE/100 g dry matter. Scanning electron microscopy (SEM) analysis confirmed significant structural modifications in enzyme-treated samples, indicating enhanced extraction efficiency. Furthermore, GC-MS profiling identified 13 bioactive compounds in the optimized extract, suggesting their potential role in antioxidant activity. These findings highlight the potential of *F. auriculata* fruit as a valuable source of natural polyphenols, supporting its application in functional foods and pharmaceutical formulations.

Keywords: *Ficus auriculata*; antioxidant; extraction; optimization; polyphenol

1. Introduction

Ficus auriculata, commonly known as the Roxburgh fig or elephant ear fig, belongs to the Moraceae family, which includes over 700 species distributed across tropical and subtropical regions worldwide (Zhang et al., 2022; Bhatt et al., 2024). This species is widely found in South and Southeast Asia, particularly in countries such as India, China, Nepal, Bhutan, Pakistan, Myanmar, Thailand, Vietnam, and Malaysia (Bhatt et al., 2024). In Vietnam, *F. auriculata* is mainly cultivated in mountainous provinces including Tuyen

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Quang, Phu Tho, Yen Bai, Hoa Binh, and Thai Nguyen, with the highest density recorded in Thua Thien Hue province (Morris & Van Bay, 2002; Dai et al., 2020).

The species is characterized by its large, broad leaves and distinctive fruit, which changes color from green to red or purple upon ripening. The ripe fruit is highly valued for its sweet taste and nutritional content, being rich in dietary fiber, natural sugars, and essential minerals. It is consumed fresh and also processed into various products such as juices, jams, and salads (Paramanandam et al., 2021; Rasool et al., 2023; Bhatt et al., 2024). In addition to its nutritional value, *F. auriculata* is recognized in traditional medicine for its health-promoting properties, including diuretic, laxative, and digestive effects. It has also been traditionally used in the treatment of diabetes and constipation (Rasool et al., 2023). These therapeutic potentials are largely attributed to its diverse phytochemical composition, which includes polyphenols, anthocyanins, flavonoids, tannins, and antioxidant compounds (Gaire et al., 2011; Rasool et al., 2023; Bhatt et al., 2024). Various parts of the plant—such as roots, leaves, stems, bark, and latex—have been used in traditional remedies due to their pharmacological properties (Gaire et al., 2011; Bhatt et al., 2024).

The extraction of bioactive compounds, particularly phenolic compounds, is essential for unlocking the medicinal potential of *F. auriculata*. The efficiency, yield, and quality of these compounds depend significantly on the extraction method employed (Ince et al., 2014; Zhong et al., 2019; Shahinuzzaman et al., 2021). Conventional solvent-based extraction methods, despite their widespread use, present several drawbacks, including the degradation of thermolabile compounds and the reliance on toxic organic solvents. To address these limitations, a range of green extraction technologies—such as ultrasound-assisted extraction (UAE), microwave-assisted extraction (MAE), and enzyme-assisted extraction (EAE)—have garnered increasing attention for their potential to enhance both yield and sustainability (Ince et al., 2014; Zhong et al., 2019; Tran et al., 2023). Among these, EAE is emerging as a promising eco-friendly approach for recovering polyphenols. This method utilizes specific enzymes such as cellulase, pectinase, and protease to degrade plant cell walls, thereby facilitating the release of intracellular bioactive compounds while maintaining their structural and biological integrity (Yazdi et al., 2019; Gligor et al., 2019; Stanek-Wandzel et al., 2024). Compared to traditional techniques, EAE offers several advantages, including higher extraction efficiency, reduced solvent usage, and better preservation of heat-sensitive compounds (Pinelo & Meyer, 2008; Stanek-Wandzel et al., 2024). These advantages make EAE highly suitable for sustainable applications in the food and pharmaceutical industries.

Although enzyme-assisted extraction (EAE) holds significant promise as a green and efficient technique, its application in extracting polyphenols from *Ficus auriculata* fruit remains limited. In particular, the use of individual enzymes such as cellulase and pectinase, as well as the systematic identification and characterization of key bioactive compounds in the extract, have not been adequately studied. To address these gaps, the present study aimed to optimize the EAE process for extracting total polyphenols from *F. auriculata* using response surface methodology (RSM). The efficiency of enzyme-based extraction was compared with that of conventional solvent-based methods. In addition, major antioxidant compounds was identified and quantified using gas chromatography–mass spectrometry (GC-MS). The antioxidant and antimicrobial activities of the extracts was evaluated through DPPH and ABTS radical scavenging assays, along with antibacterial inhibition tests. This research contributes to the scientific understanding of *F. auriculata* as a functional food source and supports the development of green extraction strategies for potential applications in both food and pharmaceutical sectors.

2. Materials and Methods

2.1 Materials

Fresh fig fruits (*Ficus auriculata*) were obtained from Tan Binh District, Ho Chi Minh City, and brought to the laboratory. The fruits were cleaned thoroughly to eliminate impurities, damaged, or pest-infested pieces. Cleaned figs were sliced thinly (2-3 mm thickness) and evenly placed on stainless steel trays for drying at 50°C until the moisture content dropped below 10%. The dried fig slices were finely ground and sifted through a 0.3 mm stainless steel mesh for consistency. The resulting fig powder was stored in airtight PET bags and used for all subsequent experiments.

For chemicals and reagents, analytical-grade chemicals and reagents were utilized. The antioxidant compounds 1,1-diphenyl-2-picrylhydrazyl (DPPH) and 2,2'-azinobis-(3 ethylbenzothiazoline-6-sulfonic acid) ($ABTS^{\bullet+}$) were purchased from Sigma-Aldrich (St. Louis, MO, USA). The Folin-Ciocalteu reagent was acquired from Merck (Germany). Additional chemicals such as potassium persulfate, ethanol (99.7%), methanol (98%), gallic acid monohydrate, vitamin C, and anhydrous sodium carbonate were supplied by Alpha Chemika (India).

For the enzymatic assays, pectinase Ultra SP-L (3300 polygalacturonase units/mL) and cellulase 1.5 (700 endoglucanase units/mL) were utilized. These enzymes, produced by Novozymes (Denmark), were provided by Brenntag Vietnam.

2.2 Methods

2.2.1 Enzyme assisted extraction method

The enzymatic extraction process was based on Fernández et al. (2015) with some adjustments. Optimal extraction conditions were determined by referring to the enzyme manufacturers' specifications in Table 1 for temperature and pH ranges. To start the extraction, 2.00 g of dried *Ficus auriculata* powder was weighed and mixed with distilled water at a ratio of 20 mL/g. A 0.8% concentration of the enzyme solution was added to the mixture. The extraction was carried out at specific temperature and pH levels tailored for each enzyme.

Table 1. Characteristics of enzymes

Enzyme	Optimum Conditions			Source
	Activity	pH	T (°C)	
Cellulase	700 EGU/mL	4.5 - 5.0	50 - 55°C	*Trichoderma reesei*
Pectinase	3300 U/mL	4.0 - 4.5	45 - 50°C	*Aspergillus niger*

Single-factor experiment

The reaction occurred in a temperature-controlled orbital shaker (New Brunswick Scientific G-24, USA) at 150 rpm for 60 min in the absence of light to reduce oxidation. After completion, the mixture was centrifuged at 4°C for 10 min at 2500 g. The supernatant was then filtered using Whatman No. 41 filter paper. The total phenolic content (TPC) was measured, and the enzyme with the highest TPC was chosen for further experiments.

The study aimed to analyze the main factors affecting the extraction of polyphenols from *Ficus auriculata* powder by examining the impact of enzyme concentration (ranging from 0.4% to 1.2%), solvent-to-solid ratio (varying from 10 to 35 mL/g), and hydrolysis time (ranging from 30 to 90 min). Each variable was tested systematically under controlled conditions to enhance extraction efficiency and maximize polyphenol yield. Initially, the effect of enzyme concentration was investigated by dispersing 2.00 g of *Ficus auriculata* powder in water at a constant solvent-to-solid ratio of 20:1 mL/g and conducting the extraction for 60 min using enzyme concentrations of 0.4% to 1.2%. Subsequently, the impact of solvent-to-solid ratio was assessed by suspending 2.00 g of the powder in different ratios (10-35 mL/g), stirring the mixture continuously, adding 0.8% enzyme, and incubating for 60 min under optimized enzymatic conditions. The influence of extraction time was then studied by conducting enzyme-assisted extractions for various durations (30, 45, 60, 75, and 90 min) with the previously optimized solvent ratio and enzyme concentration. Comparisons were also made with a traditional extraction method following a modified protocol based on Cádiz-Gurrea et al. (2019) for a thorough assessment of polyphenol extraction efficiency.

2.2.2 Optimization of the extraction methodology

The extraction was carried out with enzyme-assisted hydrolysis, utilizing pectinase and cellulase as the primary enzymes. Enzyme concentrations were systematically varied (0.6%, 0.8%, and 1.0%), alongside different solvent to solid ratios (15, 20, and 25 mL/g) and extraction times (45, 60, and 75 min). Optimization was conducted using response surface methodology (RSM) following a Box-Behnken design, with three center-point replications, and analyzed using Statgraphics Centurion XV software. The levels of different variables are given in Table 2. The present experimental design comprised of 15 combinations using 3 central points to assess the effect on total phenolic content (TPC). The data was fitted to a second order regression equation.

2.3 Analytical methods

2.3.1 Determination of total polyphenol content (TPC)

Total polyphenol content (TPC) was determined using a modified Folin-Ciocalteu method according to Yazdi et al. (2019). In this procedure, 0.5 mL of the extract was mixed with 2.5 mL of 10% Folin-Ciocalteu reagent and vortexed thoroughly. After allowing the mixture to react at room temperature for 4 min, 2.5 mL of a 7.5% sodium carbonate (Na_2CO_3) solution was added, and the reaction proceeded for 60 min. The absorbance was measured at 765 nm, and TPC was expressed as gallic acid equivalents per 100 grams of dry matter (g GAE/100 g dry matter). This assay was repeated three times for each extract, and the mean±standard deviation was calculated.

2.3.2 DPPH and ABTS radical scavenging activity

The antioxidant properties of the extracts were assessed using the DPPH and ABTS radical scavenging assays, following the method of Tran et al. (2023). In the DPPH assay, different concentrations of extract were mixed with the DPPH solution, vortexed, and kept in the dark at room temperature for 30 min. Absorbance was then measured at 517 nm,

Table 2. Box-Behnken design with independent variables and its experimental response values

Run	Factors			Y
	A	B	C	TPC (g GAE/100g dry matter)
	Enzyme Concentration(%)	Solvent to Solid Ratio (mL/g)	Extraction Time (min)	
1	0.6	15	60	1.96 ± 0.01
2	1	15	60	2.04 ± 0.01
3	0.6	25	60	2.49 ± 0.02
4	1	25	60	2.01 ± 0.01
5	0.6	20	45	1.84 ± 0.01
6	1	20	45	2.00 ± 0.01
7	0.6	20	75	2.39 ± 0.02
8	1	20	75	2.23 ± 0.02
9	0.8	15	45	1.95 ± 0.01
10	0.8	25	45	2.14 ± 0.02
11	0.8	15	75	2.05 ± 0.01
12	0.8	25	75	2.35 ± 0.01
13	0.8	20	60	2.54 ± 0.01
14	0.8	20	60	2.65 ± 0.003
15	0.8	20	60	2.65 ± 0.01

A, B, and C, respectively, denote the enzyme concentration (%), solvent to solid ratio (mL/g), and extraction time (min). The values are mean ±standard deviation of three replications.

with vitamin C (5-25 µg/mL) as the reference standard. For the ABTS assay, radicals were produced by combining 7 mM ABTS with 2.45 mM potassium persulfate (1:1) in the dark for 12-16 h. The solution was then diluted to achieve an absorbance of 0.70±0.05 at 734 nm before analysis. A mixture of 0.1 mL extract and 0.9 mL ABTS solution was incubated for 6 min at room temperature, and absorbance was measured at 734 nm. The radical scavenging activity was calculated as the percentage of inhibition, as shown in equation 1:

$$\textit{Scavenging activity (\%)} = \left[\mathbf{1} - \frac{\boldsymbol{Abs_{sample}}}{\boldsymbol{Abs_{control}}}\right] \times \mathbf{100} \quad (1)$$

where Abs_{sample} represents the absorbance of the extract-treated solution, and $Abs_{control}$ refers to the absorbance of the radical solution without the extract. The IC_{50} value, indicating the extract concentration required to inhibit 50% of DPPH radicals, was determined. All experiments were performed in triplicate to ensure accuracy and reproducibility of the results.

2.3.3 GC-MS analysis

The *Ficus auriculata* powder extracts were analyzed using a PerkinElmer Clarus 600 GC-MS system, equipped with a capillary column (30 m × 0.25 mm i.d., 0.25 µm film thickness). High-purity helium gas (99.99%) was used as the carrier at a constant flow rate of 1.0 mL/min. The injector, transfer line, and ion source were all maintained at 290°C, and ionization was conducted by electron impact (EI) at 70 eV. The oven temperature was

initially set at 60°C (held for 2 min), then ramped at 3°C/min to 280°C, according to the procedure described by Al-Owaisi et al. (2014). Before analysis, the extracts were diluted 1:100 (v/v), filtered through a 0.22 µm membrane, and 1 µL of each sample was injected in split mode (30:1). The system operated in full-scan mode (mass range 40-550 amu), and volatile compounds were identified by comparing their mass spectra and retention times with those available in the **NIST Mass Spectral Library (NIST 11)** and **Wiley Registry of Mass Spectral Data.** The relative abundance of each compound was expressed as a percentage of the total ion chromatogram area. All analyses were performed in triplicate to ensure reproducibility.

2.3.4 Bacteriostatic assay

A bacteriostatic assay was conducted to evaluate the antibacterial and antifungal properties of the extracts against *Staphylococcus aureus*, *Salmonella typhimurium*, and *Candida albicans* using the coated plate method. Individual colonies of the microorganisms were cultured on solidified Mueller-Hinton agar, and wells were created into which the extract solution was added (Stochmal et al., 2021). After 24 h of incubation at 37°C, the inhibition zones around the wells were examined to determine antimicrobial effectiveness. The diameter of the clear zones indicating microbial suppression was measured precisely with an automatic colony counter.

2.4 Statistical analysis

All experiments were conducted three times, and the results were presented as the mean±standard deviation. For each assay, the number of repetitions was indicated to ensure the reliability and statistical significance of the results. Statistical analysis was carried out using ANOVA and LSD tests at a significant level of 5%. Data were processed using Microsoft Excel 2019 and Statgraphics Centurion XV (Version XV), which was employed for response surface methodology (RSM) to enhance experimental conditions.

3. Results and Discussion

3.1 The effect of enzyme type on total polyphenol content (TPC)

The impact of enzyme-assisted extraction on the total polyphenol content (TPC) of *Ficus auriculata* fruit was investigated using pectinase and cellulase. As shown in Figure 1a, enzymatic treatments significantly enhanced TPC compared to conventional solid–liquid extraction without enzyme addition. The control sample, extracted without enzymes, exhibited a TPC of only 1.19 g GAE/100 g dry matter. In contrast, pectinase-assisted extraction yielded the highest TPC of 2.42 g GAE/100 g dry matter, representing a 2.04-fold increase over the control and a 1.10-fold increase compared to cellulase-assisted extraction (2.19 g GAE/100 g dry matter). The inferior yield of the conventional method is attributed to the rigid structure of the plant cell wall, which impedes the release of bound polyphenols (Gligor et al., 2019; Yazdi et al., 2019). Polyphenols are often trapped within intracellular compartments or bound to cell wall polysaccharides via hydrophobic interactions and hydrogen bonds (Pinelo & Meyer, 2008; Fernández et al., 2015). Enzymes facilitate the release of these compounds by degrading cell wall components and hydrolyzing ester linkages, thereby enhancing cell permeability and improving extraction of polyphenol compounds (Gligor et al., 2019; Stanek-Wandzel et al., 2024).

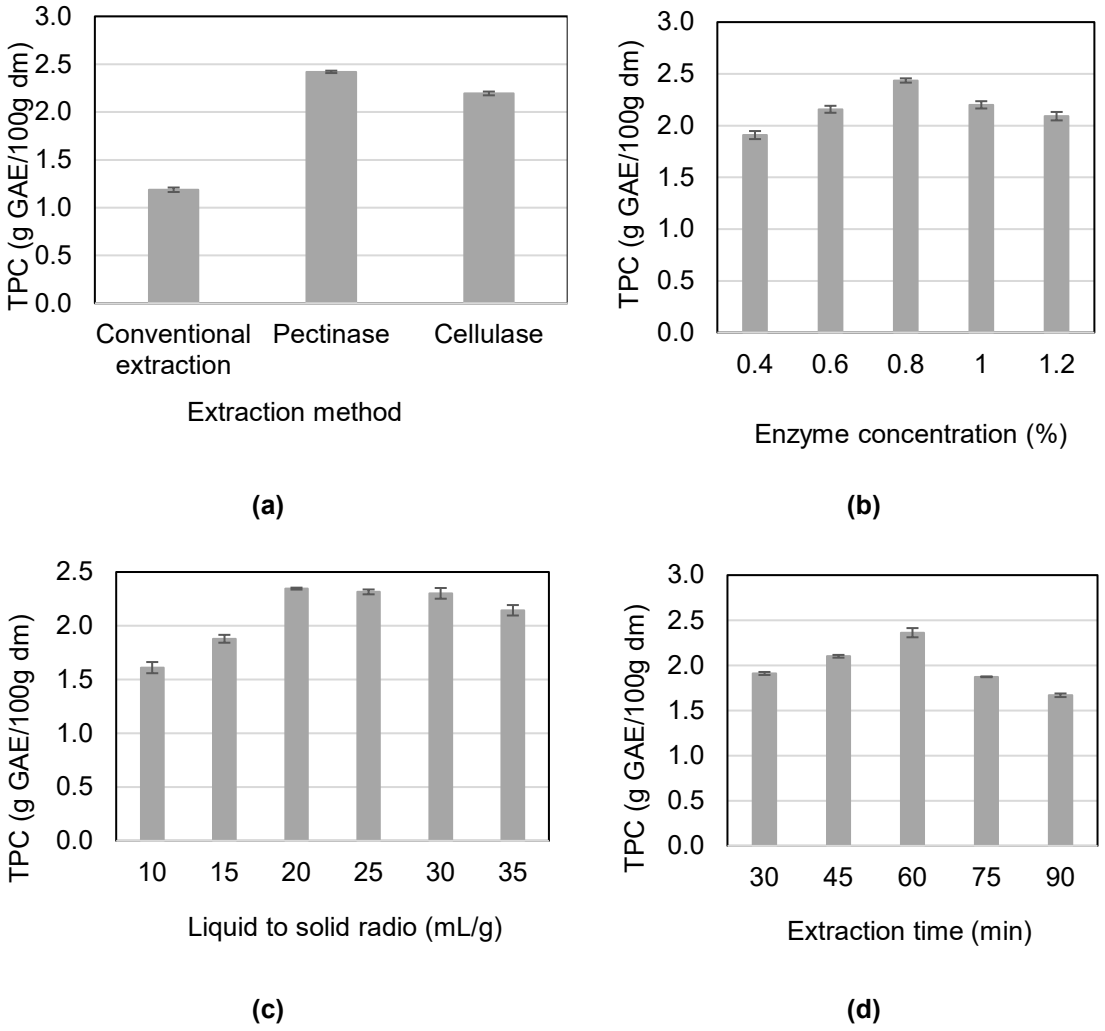


Figure 1. Effects of (a) different enzymes; (b) enzyme concentration (%); (c) solvent to solid ratio (mL/g); (d) extraction time (min) on the yield of phenolic extracts from *Ficus auriculata* fruits

Among the enzymes tested, pectinase proved more effective than cellulase. This is likely due to the specific role of pectin in the cell wall architecture of *Ficus auriculata*, where it acts as a major structural barrier to polyphenol release. Pectinase hydrolyzes this component more effectively than cellulase, which mainly targets cellulose (Fernández et al., 2015). These findings are consistent with previous studies, which reported that pectinase yielded greater phenolics than cellulase (Fu et al., 2008; Fernández et al., 2015). Based on its superior performance, pectinase was selected for further optimization.

3.2 Effect of enzyme concentration on total polyphenol content (TPC)

The influence of different pectinase concentrations (0.4%-1.2%) on TPC was evaluated under constant extraction conditions: 20 mL/g solvent-to-solid ratio and 60 min extraction time. As depicted in Figure 1b, increasing pectinase concentration led to a significant rise in TPC, from 1.91 g GAE/100 g dry matter at 0.4% to a peak of 2.44 g GAE/100 g dry matter at 0.8%. The enhanced extraction is attributed to the enzymatic hydrolysis of ester and ether bonds between polyphenols and the cell wall matrix, facilitating polyphenol release (Pinelo & Meyer, 2008; Al-Owaisi et al., 2014; Fernández et al., 2015; Stanek-Wandzel et al., 2024). Beyond 0.8%, however, TPC declined, reaching 2.20 g GAE/100 g dry matter at 1.0% and 2.09 g GAE/100 g dry matter at 1.2%. This reduction may be due to enzyme saturation or self-inhibition, where excessive enzyme molecules reduce overall activity. Moreover, higher enzyme concentrations can promote oxidation, leading to the formation of quinones and polymerized products that decrease extractable polyphenol levels (Stanek-Wandzel et al., 2024). Similar trends were observed in previous studies (Miron et al., 2013; Boulila et al., 2015; Ahmed et al., 2016; Islam et al., 2023), indicating that optimal enzyme concentration depends on the substrate and extraction conditions. Therefore, 0.8% pectinase was identified as optimal for subsequent experiments.

3.3 Effect of solvent to solid ratio on the total polyphenols content (TPC)

The ratio of solvent to solid plays a vital role in the extraction of polyphenols from *Ficus auriculata* fruit. In this study, various ratios ranging from 10:1 to 35:1 (mL/g) were examined, as shown in Figure 1c. The findings indicate that increasing the solvent to solid ratio from 10 to 20 mL/g resulted in a significant increase in total polyphenol content (TPC) from 1.61 g GAE/100g dry matter to 2.35 g GAE/100g dry matter. However, beyond this point, further increases in the solvent volume did not lead to substantial improvements, and TPC slightly decreased to 2.14 g GAE/100g dry matter at a 1:35 ratio. This trend can be explained by two conflicting factors. Initially, a higher solvent ratio improves the contact surface area between the substrate and solvent, aiding in mass transfer and enhancing extraction efficiency. Nevertheless, excessive solvent dilutes enzyme concentration, reducing catalytic efficiency. Moreover, elevated solvent levels increase dissolved oxygen content, potentially accelerating the oxidative degradation of polyphenols, thereby lowering TPC recovery. Similar observations were made by Islam et al. (2023) in banana peel polyphenol extraction, where TPC peaked at 25 mL/g and declined at 30 mL/g due to dilution effects. Furthermore, studies by Casazza et al. (2011) and Yazdi et al. (2019) demonstrated that surpassing the optimal solvent to solid ratio level leads to excessive resource consumption without enhancing extraction efficiency, consistent with the results of this study. Based on these findings, a solvent to solid ratio of 20 mL/g was identified as the most effective condition for maximizing TPC extraction, maintaining enzyme efficiency, and minimizing solvent wastage in subsequent experiments.

3.4 Effect of extraction time on polyphenols content

The duration of extraction time is a crucial factor affecting the efficiency of extracting polyphenols. Research indicates that prolonging the extraction time can improve the retrieval of phenolic compounds (Casazza et al., 2011; Yazdi et al., 2019; Islam et al., 2023). However, excessive extraction time may cause structural degradation and decrease bioactivity due to hydrolysis or oxidation. To investigate the influence of extraction time on

total polyphenol content (TPC), experiments were carried out with durations ranging from 30 to 90 min while keeping other extraction conditions constant - pectinase concentration at 0.8% and a solvent-to-solid ratio of 20 mL/g. The results, as shown in Figure 1d, indicate that extraction time significantly affects TPC yield. The TPC was lowest at 30 min of extraction as shorter timeframes do not allow for complete polyphenol release, resulting in lower efficiency (Casazza et al., 2011; Yazdi et al., 2019; Islam et al., 2023). When the extraction time was extended to 60 min, the TPC peaked at 2.36 g GAE/100g dry matter, which is 1.24 times higher than the 30-min extraction and 1.12 times higher than the 45-min extraction. However, beyond 60 min, at 75 and 90 min, the TPC started to decline to 1.87g GAE/100g dry matter and 1.67 g GAE/100g dry matter, respectively. This decline is due to prolonged extraction exposing the extract to higher temperatures, light, and oxygen, leading to oxidation that degrades phenolic compounds and decreases TPC (Pinelo & Meyer, 2008; Yazdi et al., 2019). Based on these findings, 60 min was identified as the optimal extraction time, balancing efficiency and compound stability. Extending the extraction time further not only decreases TPC yield but also raises enzyme consumption, thereby increasing production costs without significant benefits.

3.5 Optimization of TPC from *Ficus auriculata*

To maximize the extraction efficiency of total polyphenol content (TPC) from *Ficus auriculata* fruit, a response surface methodology (RSM) based on the Box-Behnken design was applied. This model allowed simultaneous evaluation of three critical factors: enzyme concentration (%), solvent-to-solid ratio (mL/g), and extraction time (min). A total of 15 experimental runs, including three center points, were conducted to build the regression model, and the experimental matrix is detailed in Table 2.

Analysis of variance (ANOVA) results (Table 3) revealed that the quadratic regression model was highly significant, with an F-value of 33.11 and a p-value < 0.001, indicating strong statistical reliability. Furthermore, the lack-of-fit test yielded a p-value of 0.47 (>0.05), confirming the model's adequacy. The high coefficient of determination (R^2 = 0.99, adj-R^2 = 0.97) also suggests excellent agreement between experimental and predicted values, and that the model could be reliably used for prediction and optimization under similar extraction conditions. These findings were consistent with previous optimization studies, which reported that a reliable experimental model should have a lack-of-fit P-value greater than 0.05 and R^2, adj-R^2 values greater than 0.8 (Yazdi et al., 2019; Islam et al., 2023; Tran et al., 2023). The second-order polynomial regression equation derived was:

$$Y=-13.85+16.90A+0.40B+0.18C-7.75A^2-0.01A\times B-0.05A\times C-0.008B^2-0.001C^2 \quad (2)$$

where A, B, and C represent enzyme concentration, solvent-to-solid ratio, and extraction time, respectively. The coefficients of the model reveal that enzyme concentration had the most substantial linear and quadratic effects on TPC, followed by solvent ratio and time. The interaction terms AB and AC also reached statistical significance, implying that enzyme concentration modulates the influence of both solvent volume and extraction duration. This underscores the necessity of considering variable interactions rather than optimizing each factor independently.

Table 3. Analysis of variables for regression model of response in extraction conditions

Source	Sum of Squares	Df	Mean Square	F-Ratio	P-Value
A: Enzyme concentration	0.05	1	0.05	23.82	0.04
B: Solvent to solid ratio	0.23	1	0.23	109.31	0.009
C: Extraction time	0.09	1	0.09	41.98	0.02
A×A	0.35	1	0.35	172.32	0.006
A×B	0.04	1	0.04	19.26	0.05
A×C	0.09	1	0.09	41.18	0.02
B×B	0.13	1	0.13	63.83	0.02
B×C	0.003	1	0.003	1.44	0.35
C×C	0.26	1	0.26	126.61	0.008
Lack-of-fit	0.008	3	0.003	1.27	0.47
Pure error	0.004	2	0.002		
Total (corr.)	1.15	14			
			R^2	0.99	
			Adj-R^2	0.97	

The response surface plots (Figure 2) further illustrate these effects. When extraction time was fixed at 60 min, TPC increased with both enzyme concentration and solvent ratio, peaking around 0.8% enzyme and 23-25 mL/g solvent, beyond which a decrease occurred—likely due to saturation or polyphenol degradation. At a constant solvent-to-solid ratio of 20 mL/g, the maximum TPC was observed near 0.75-0.8% enzyme and 60-65 min, with declines at higher values attributed to oxidative or enzymatic degradation processes (Pinelo & Meyer, 2008; Stanek-Wandzel et al., 2024). When enzyme concentration was fixed at 0.8%, TPC increased with both time and solvent ratio up to 65 min and 25 mL/g, respectively, followed by a similar decline.

Among the variables, enzyme concentration was the most influential factor. This finding is consistent with literature indicating that enzyme-assisted cell wall disruption significantly enhances polyphenol release (Boulila et al., 2015; Ahmed et al., 2016). However, excessive enzyme concentrations may lead to unfavorable conditions such as feedback inhibition or increased oxidation. Likewise, while increasing the solvent ratio can facilitate solute diffusion and enzyme accessibility, excessive volumes may dilute enzyme activity or promote oxidative degradation. The interdependence of these parameters highlights the necessity of a holistic optimization strategy rather than a univariate approach. Under the optimized conditions predicted by the model—0.74% enzyme concentration, 23 mL/g solvent-to-solid ratio, and 65 min of extraction—the theoretical maximum TPC was 2.70 g GAE/100 g dry matter. Experimental validation under these conditions yielded an actual TPC of 2.65 g GAE/100 g dry matter (mean of triplicate runs), with a relative deviation of only 1.7%, well within the acceptable margin of error (<5%). This close match between predicted and experimental values validates the model's reliability and further reinforces the effectiveness of the combined parameter optimization. Compared to the best single-variable condition (2.36 g GAE/100 g dry matter) and the conventional extraction method (1.19 g GAE/100 g dry matter), the optimized process improved TPC yield by 1.13 and 2.2 times, respectively, highlighting the substantial benefits of this systematic approach.

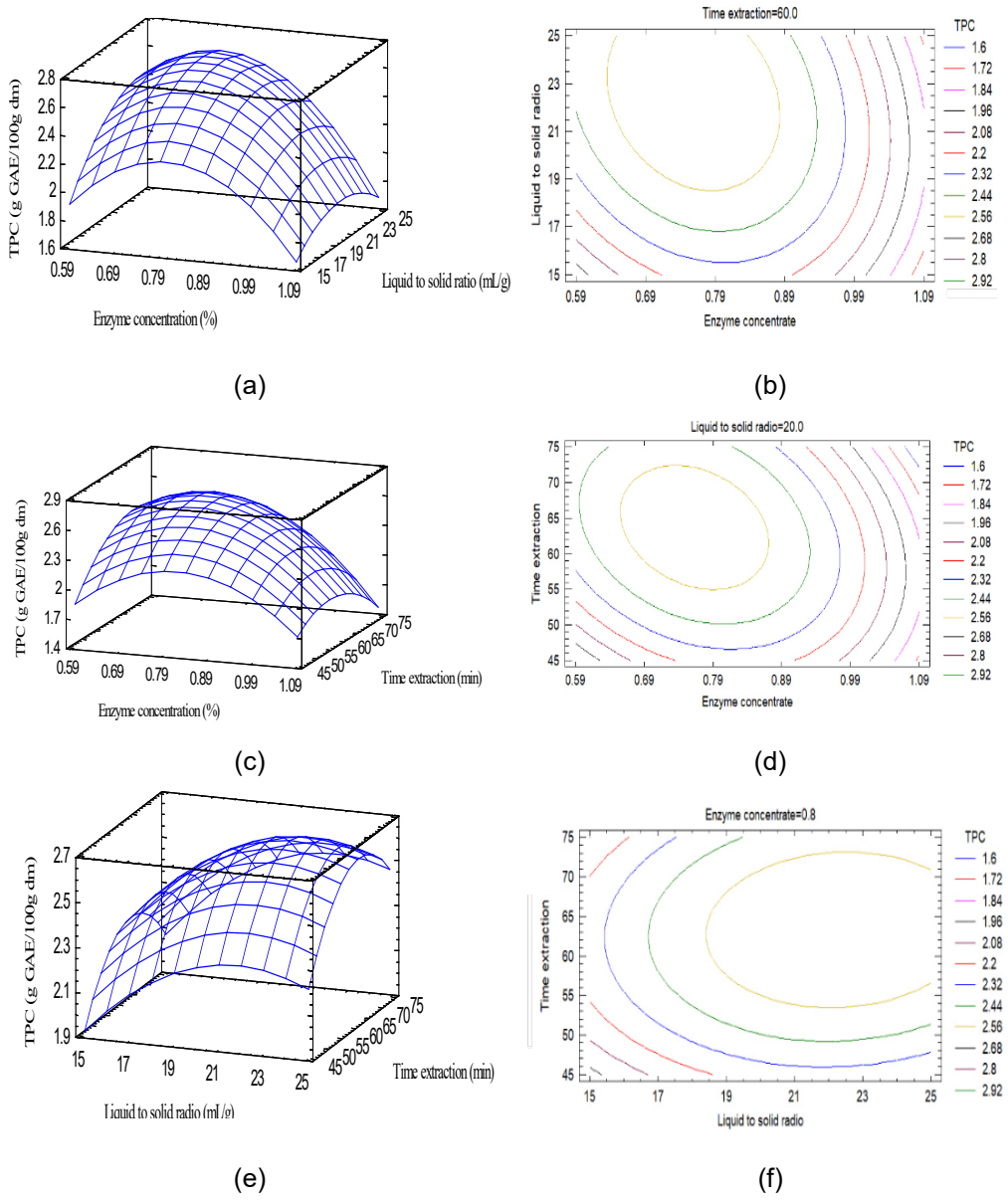
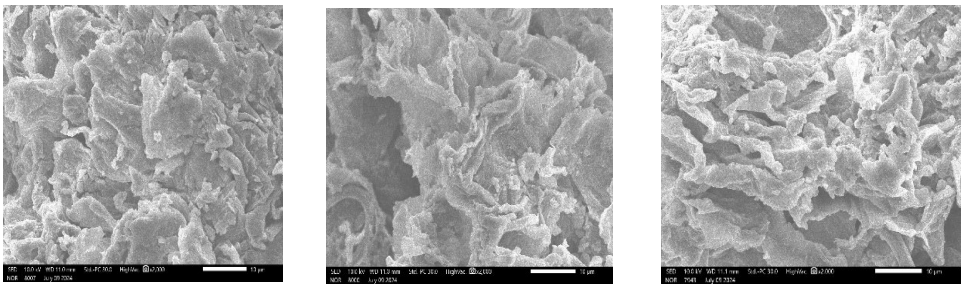


Figure 2. Response surface (a,b,c) and contour (b,d,f) for interactions between three independent extraction parameters on the extraction yields of phenolic extracts from *Ficus auriculata*: (a-b) enzyme concentration × liquid to solid ratio; (c-d) enzyme concentration × extraction time; (e-f) liquid to solid ratio× extraction time

3.6 The antioxidant activity and antimicrobial activity of extract under optimized conditions

To highlight the superiority of enzyme-assisted extraction over conventional solvent extraction, a comparative analysis was performed. The total polyphenol content (TPC) obtained through enzyme-assisted extraction reached 2.65 g GAE/100 g, representing a 1.6-fold increase compared to traditional solvent extraction. This enhancement can be attributed to the enzymatic hydrolysis of cell wall polysaccharides, which improves mass transfer and promotes the release of intracellular bioactive compounds (Yazdi et al., 2019; Islam et al., 2023). Further evidence is provided by scanning electron microscopy (SEM) images (Figure 3)—whereas solvent-extracted samples (Figure 3b) maintained an intact cellular structure, enzyme-treated samples (Figure 3c) displayed significant cell wall disruption, facilitating greater polyphenol recovery.



(a) **(b)** **(c)**

Figure 3. SEM images of: (a) *Ficus auriculata* powder (b) *Ficus auriculata* powder extracted by conventional extraction method, and (c) at optimal enzyme assisted extraction (0.74%, 23 mL/g, 65 min)

A comparative assessment of the antioxidant and antibacterial activities of *Ficus auriculata* extracts obtained via enzyme-assisted extraction (EAE) and conventional extraction (CE), with vitamin C as a reference standard, highlights notable differences in bioactivity. Antioxidant capacity, evaluated through ABTS and DPPH radical scavenging assays, demonstrated that the extract derived from enzyme-assisted extraction exhibited significantly lower IC_{50} values (112.48 µg/mL for ABTS and 170.23 µg/mL for DPPH) in comparison to the conventional extract (172.61 µg/mL and 230.15 µg/mL, respectively). These results suggest that EAE enhances the release of bioactive polyphenols, contributing to greater free radical scavenging activity (Yazdi et al., 2019; Tran et al., 2023). However, vitamin C, a well-known antioxidant, displayed the most potent activity, with the lowest IC_{50} values (12.30 µg/mL for ABTS and 15.11 µg/mL for DPPH), reaffirming its superior free radical neutralization potential (Kim et al., 2002).

In terms of antibacterial activity, the EAE extract demonstrates significantly higher inhibition against *Staphylococcus aureus* (ATCC 25923) and *Salmonella Typhimurium* (ATCC 14028) compared to the conventional extract (Figure 4). The inhibition index for the EAE extract (19.91 mm for *S. aureus* and 14.10 mm for *S. Typhimurium*) are substantially larger than those of the conventional extract (12.45 mm and 10.04 mm, respectively) (Table 4). This enhanced antimicrobial effect is likely due to a higher concentration of bioactive

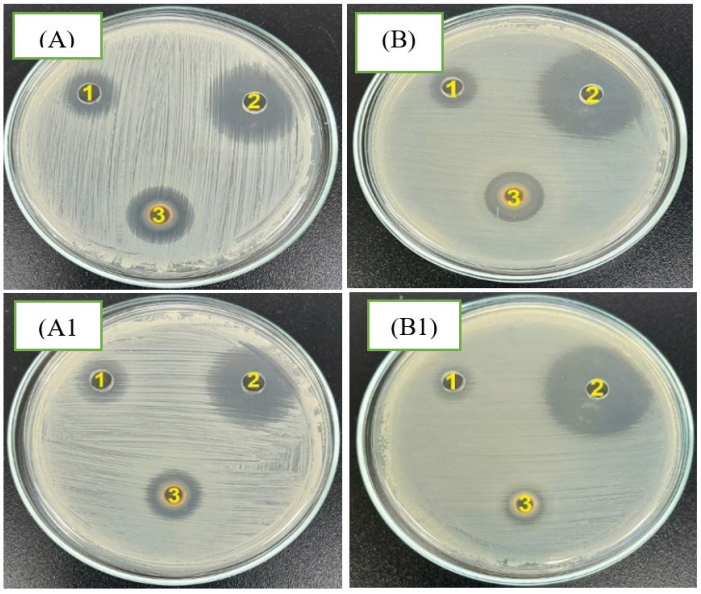


Figure 4. Antibacterial activity of *Ficus auriculata* extracts: enzyme-assisted extraction against *Staphylococcus aureus* (A) and *Salmonella Typhimurium* (A1), and conventional extraction against *S. aureus* (B) and *S. Typhimurium* (B1)

Table 4. Comparison of antioxidant activity and antibacterial activity of *Ficus auriculata* extract

Samples	$ABTS^{\bullet+}$ (IC_{50})	DPPH (IC_{50})	Inhibition Index (mm)	
			***Staphylococcus aureus* ATCC 25923**	***Salmonella Typhimurium* ATCC 14028**
Enzyme assisted extraction extract	112.48^{b}±4.33	170.23^{b}±2.13	19.91^{b}±1.02	14.10^{b}±0.22
Conventional extract	172.61^{a}±3.75	230.15^{a}±2.16	12.45^{c}±0.89	10.04^{c}±0.42
Vitamin C	12.30^{c}±0.66	15.11^{c}±0.78	23.91^{a}±1.02	29.10^{a}±0.22

Different letters in the same column indicate statistically significant differences between treatments ($p < 0.05$). The values are mean±SD of duplicate runs.

compounds released through enzymatic hydrolysis, which improves the extract's efficacy against pathogenic bacteria (Daglia, 2012; Al-Owaisi et al., 2014). Furthermore, these results suggest that variations in phenolic composition and extraction methods significantly influence antioxidant potency among *Ficus* species. Nevertheless, vitamin C shows the most pronounced antibacterial activity, with inhibition zones of 23.91±1.02 mm for *S. aureus* and 29.10±0.22 mm for *S. Typhimurium*, aligning with its recognized antimicrobial properties (Kallio et al., 2012).

The findings highlight the advantages of enzyme-assisted extraction over conventional methods, demonstrating its potential for improving the functional properties of *Ficus auriculata* extracts. By enhancing the bioavailability of polyphenols and antimicrobial compounds, EAE presents a promising alternative for developing natural antioxidants and antimicrobial agents in food and pharmaceutical applications.

3.7 GC-MS analysis of bioactive compounds

Gas chromatography–mass spectrometry (GC-MS) analysis of the enzyme-assisted extract from *Ficus auriculata* fruit powder showed a diverse profile of 13 bioactive compounds, including polyphenols, fatty acids, phytosterols, and triterpenoids (Table 5, Figure 5). Notably, gallic acid was identified as the most abundant constituent, with a relative abundance of 70.78%, significantly surpassing levels typically reported in other antioxidant-rich fruits such as pomegranates and grapes, which generally contain gallic acid in the range of 10-40% (Stochmal et al., 2021; Stanek-Wandzel et al., 2024). This high concentration indicates that *F. auriculata* fruit has strong free radical-scavenging capability, making it a promising functional food ingredient with potent antioxidant properties.

Table 5. Chemical composition of various extracts of *Ficus auriculata fruits*

No.	Retention time RT (min)	Identified compounds	Relative abundance (%)
1	2.28	2,4-Dihydroxy-2,5-dimethyl-3(2H)-furan-3-one	0.27
2	5.29	6-Azabicyclo[3.2.1]]octane	0.28
3	6.4	ND (Not determined)	0.61
4	7.5	Pyrogallol	0.42
5	8.85	Ferulic acid	5.58
6	9.91	Gallic acid	70.78
7	10.20	1,3-Benzodioxole, 4-methoxy-6-(2-propenyl)-	0.49
8	12.94	3,4-Dimethyl-Caffeic acid	15.12
9	13.40	Hexadecanoic acid	0.64
10	14.17	Phytol	0.65
11	14.30	Linolenic acid	0.79
12	16.19	ND (Not determined)	0.56
13	22.19	ND (Not determined)	0.27
14	25.75	β-Sitosterol	0.88
15	27.68	Olean-12-en-3β-ol, acetate	0.55
16	28.45	Lup-20(29)-en-3-ol, acetate, (3β)	2.11

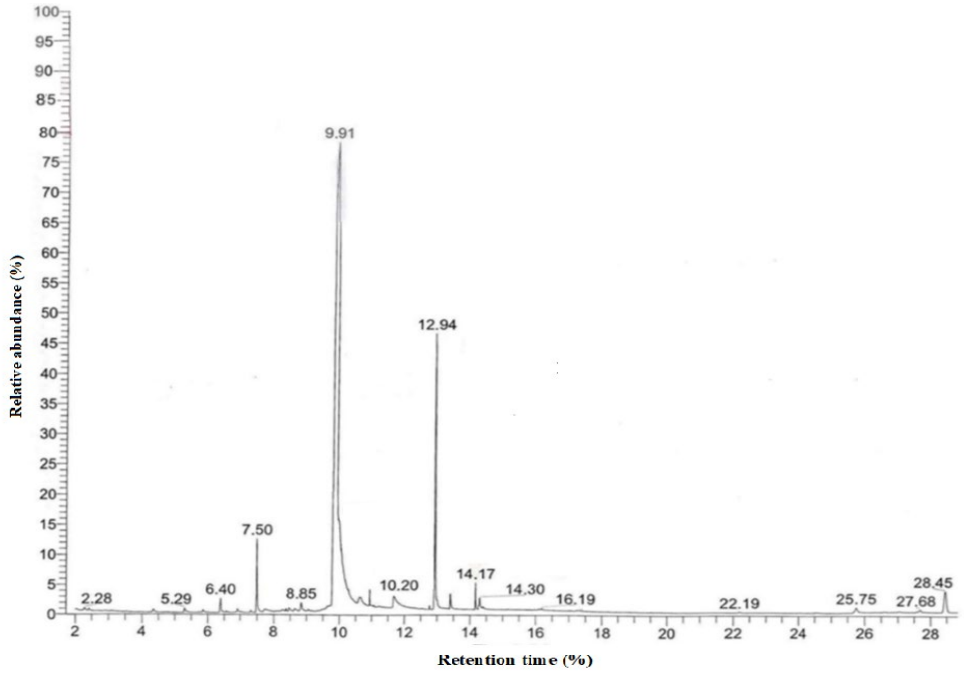


Figure 5. GC-MS chromatogram of enzyme assisted extract of *Ficus auriculata fruits*

The extract also contained 3,4-dimethyl-caffeic acid (15.12%) and ferulic acid (5.58%), both hydroxycinnamic acid derivatives known for their anti-inflammatory and antimicrobial properties. Ferulic acid, in particular, has been extensively studied for its health benefits and is commonly found in plant cell walls, contributing to oxidative stability and cellular protection (Stochmal et al., 2021; Stanek-Wandzel et al., 2024). In comparison, earlier studies on *Ficus carica* and other fig species predominantly reported chlorogenic acid and rutin as major phenolics (Veberic et al., 2008; Kamiloglu & Capanoglu, 2015). This variation in phenolic profile across *Ficus* species may be attributed to species-specific metabolic pathways**,** environmental factors, or differences in extraction methodology, highlighting the unique phytochemical signature of *F. auriculata*. Beyond polyphenols, several lipophilic bioactives were also detected. Linolenic acid (0.79%) and hexadecanoic acid (0.64%) are essential fatty acids associated with cardiovascular benefits and anti-inflammatory action. Additionally, β-sitosterol (0.88%), a plant sterol structurally similar to cholesterol, is recognized for its cholesterol-lowering effects and protective roles in heart health (Genser et al., 2012; Durrani et al., 2024).

The presence of pyrogallol (0.42%) and phytol (0.65%)**,** both exhibiting antimicrobial and antioxidant activity, adds further therapeutic relevance. Most notably, two triterpenoid derivatives, olean-12-en-3β-ol acetate (0.55%) and lup-20(29)-en-3-ol acetate, (3β) (2.11%), were identified. These compounds are widely reported in medicinal plants and are known for their anti-inflammatory, antimicrobial, and hepatoprotective effects (Kurç et al., 2023). Their presence underscores the multifunctional pharmacological potential of the extract. This study not only confirms the rich bioactive composition of *F. auriculata* fruit extract but also highlights its potential as a functional food and nutraceutical ingredient.

4. Conclusions

The study successfully optimized the enzyme-assisted extraction (EAE) of polyphenols from *Ficus auriculata* fruit using pectinase and comprehensively evaluated the bioactive potential of the extract. Pectinase showed the highest efficiency among the enzymes tested, resulting in a total polyphenol content of 2.42 g GAE/100 g dry matter, a 2.04-fold improvement compared to conventional extraction methods. By applying response surface methodology (RSM) based on the Box-Behnken design, the study identified the optimal extraction conditions: 0.74% pectinase concentration, 23:1 mL/g solvent-to-solid ratio, and 65 min of extraction time. Under these conditions, the predicted total polyphenol content (2.70 g GAE/100 g dry matter) closely matched the experimentally validated value (2.65 g GAE/100 g dry matter), confirming the effectiveness of the optimization approach. Phytochemical analysis using GC-MS identified gallic acid as the primary phenolic compound in the extract, along with 3,4-dimethyl-caffeic acid and ferulic acid, known for their antioxidant and antimicrobial properties. The extract demonstrated strong radical scavenging capacity and moderate antibacterial activity against *Staphylococcus aureus* and *Salmonella Typhimurium*, highlighting the potential of *Ficus auriculata* fruit as a sustainable source of natural bioactive compounds for various applications. Future research should focus on upscaling the extraction process, enhancing polyphenol bioavailability, exploring synergistic interactions with other bioactive compounds, conducting in vivo studies, and formulation trials to validate the efficacy and safety of products derived from *Ficus auriculata* for practical use.

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6. Authors' Contributions

Conceptualization; methodology, validation; formal analysis and investigation (Pham Thi My Tien, Nguyen Thi Thanh Lam, Nguyen Minh Tam); Data curation (Tran Chi Hai, Nguyen Ngoc Yen Nhi, Pham Thi Phuong Linh, Le Ngoc Nhu); Supervision (Pham Thi My Tien and Tran Chi Hai); Visualization, writing—review and editing (Tran Chi Hai and Phan Van Man);

7. Conflicts of Interest

The authors state that they do not have any conflicts of interest related to this article.

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References

Ahmed, I., Zia, M. A., Hussain, M. A., Akram, Z., Naveed, M. T., & Nowrouzi, A. (2016). Bioprocessing of citrus waste peel for induced pectinase production by *Aspergillus niger*; its purification and characterization. *Journal of Radiation Research and Applied Sciences*, 9(2), 148-154. https://doi.org/10.1016/j.jrras.2015.11.003

Al-Owaisi, M., Al-Hadiwi, N., & Khan, S. A. (2014). GC-MS analysis, determination of total phenolics, flavonoid content and free radical scavenging activities of various crude extracts of *Moringa peregrina* (Forssk.) Fiori leaves. *Asian Pacific Journal of Tropical Biomedicine*, 4(12), 964-970. https://doi.org/10.12980/APJTB.4.201414B295

Bhatt, S. C., Kumar, V., Naik, B., Gupta, A. K., Saris, P. E. J., Kumar, V., Rajput, V., & Rustagi, S. (2024). *Ficus auriculata* Lour., an underutilized nonconventional alternative fruit to *Ficus carica* with nutraceutical potential. *Discover Sustainability*, 5(1), Article 254. https://doi.org/10.1007/s43621-024-00480-3

Boulila, A., Hassen, I., Haouari, L., Mejri, F., Amor, I. B., Casabianca, H., & Hosni, K. (2015). Enzyme-assisted extraction of bioactive compounds from bay leaves (*Laurus nobilis* L.). *Industrial Crops and Products*, 74, 485-493. https://doi.org/10.1016/j.indcrop.2015.05.050

Cádiz-Gurrea, M. D. L. L., Zengin, G., Kayacık, O., Lobine, D., Mahomoodally, M. F., Leyva-Jiménez, F. J., & Segura-Carretero, A. (2019). Innovative perspectives on *Pulicaria dysenterica* extracts: phyto-pharmaceutical properties, chemical characterization and multivariate analysis. *Journal of the Science of Food and Agriculture*, 99(13), 6001-6010. https://doi.org/10.1002/jsfa.9875

Casazza, A. A., Aliakbarian, B., & Perego, P. (2011). Recovery of phenolic compounds from grape seeds: Effect of extraction time and solid–liquid ratio. *Natural Product Research*, 25(18), 1751-1761. https://doi.org/10.1080/14786419.2010.524889

Daglia, M. (2012). Polyphenols as antimicrobial agents. *Current Opinion in Biotechnology*, 23(2), 174-181. https://doi.org/10.1016/j.copbio.2011.08.007

Dai, T. D., Tam, N. T., Huong, T. T. G., Tuyet, N. T., & Van, D. T. (2020). Triterpenoids and phenolics from the leaves of *Ficus hirta* Vahl. in Tuyen Quang Province, Vietnam. *International Journal of the Science of Food and Agriculture*, 4(2), 138-142.

Durrani, A. K., Khalid, M., Raza, A., Rasool, I. F. U., Khalid, W., Akhtar, M. N., Khan, A. A., Abdullah, Z., & Khadijah, B. (2024). Clinical improvement, toxicity and future prospects of β-sitosterol: a review. *CyTA-Journal of Food*, 22(1), Article 2337886. https://doi.org/10.1080/19476337.2024.2337886

Fernández, K., Vega, M., & Aspé, E. (2015). An enzymatic extraction of proanthocyanidins from País grape seeds and skins. *Food Chemistry*, 168, 7-13. https://doi.org/10.1016/j.foodchem.2014.07.021

Fu, Y.-J., Liu, W., Zu, Y.-G., Tong, M.-H., Li, S.-M., Yan, M.-M., Efferth, T., & Luo, H. (2008). Enzyme assisted extraction of luteolin and apigenin from pigeonpea [Cajanuscajan (L.) Millsp.] leaves. *Food Chemistry*, 111(2), 508-512. https://doi.org/10.1016/j.foodchem.2008.04.003

Gaire, B. P., Lamichhane, R., Sunar, C. B., Shilpakar, A., Neupane, S., & Panta, S. (2011).

Phytochemical screening and analysis of antibacterial and antioxidant activity of *Ficus auriculata* (Lour.) stem bark. *Pharmacognosy Journal*, 3(21), 49-55. https://doi.org/10.5530/pj.2011.21.8

Genser, B., Silbernagel, G., De Backer, G., Bruckert, E., Carmena, R., Chapman, M. J., Deanfield, J., Descamps, O. S., Rietzschel, E. R., Dias, K. C., & März, W. (2012). Plant sterols and cardiovascular disease: a systematic review and meta-analysis. *European Heart Journal*, 33(4), 444-451. https://doi.org/10.1093/eurheartj/ehr441

Gligor, O., Mocan, A., Moldovan, C., Locatelli, M., Crișan, G., & Ferreira, I. C. F. R. (2019). Enzyme-assisted extractions of polyphenols – a comprehensive review. *Trends in Food Science and Technology*, 88, 302-315. https://doi.org/10.1016/j.tifs.2019.03.029

Ince, A. E., Sahin, S., & Sumnu, G. (2014). Comparison of microwave and ultrasound-assisted extraction techniques for leaching of phenolic compounds from nettle. *Journal of Food Science and Technology*, 51, 2776-2782. https://doi.org/10.1007/s13197-012-0828-3

Islam, M. R., Kamal, M. M., Kabir, M. R., Hasan, M. M., Haque, A. R., & Hasan, S. M. K. (2023). Phenolic compounds and antioxidants activity of banana peel extracts: Testing and optimization of enzyme-assisted conditions. *Measurement: Food*, 10, Article 100085. https://doi.org/10.1016/j.meafoo.2023.100085

Kallio, J., Jaakkola, M., Mäki, M., Kilpeläinen, P., & Virtanen, V. (2012). Vitamin C inhibits *Staphylococcus aureus* growth and enhances the inhibitory effect of quercetin on growth of *Escherichia coli* in vitro. *Planta Medica*, 78(17), 1824-1830. https://doi.org/10.1055/s-0032-1315388

Kamiloglu, S., & Capanoglu, E. (2015). Polyphenol content in figs (*Ficus carica* L.): Effect of sun-drying. *International Journal of Food Properties*, 18(3), 521-535. https://doi.org/10.1080/10942912.2013.833522

Kim, D.-O., Lee, K. W., Lee, H. J., & Lee, C. Y. (2002). Vitamin C equivalent antioxidant capacity (VCEAC) of phenolic phytochemicals. *Journal of Agricultural and Food Chemistry*, 50(13), 3713-3717. https://doi.org/10.1021/jf020071c

Kurç, M. A., Orak, H. H., Gülen, D., Caliskan, H., Argon, M., & Sabudak, T. (2023). Antimicrobial and antioxidant efficacy of the lipophilic extract of *Cirsium vulgare*. *Molecules*, 28(20), Article 7177. https://doi.org/10.3390/molecules28207177

Miron, T. L., Herrero, M., & Ibáñez, E. (2013). Enrichment of antioxidant compounds from lemon balm (*Melissa officinalis*) by pressurized liquid extraction and enzyme-assisted extraction. *Journal of Chromatography A*, 1288, 1-9. https://doi.org/10.1016/j.chroma.2013.02.075

Morris, J., & Van Bay, A. (2002). *An overview of the NTFP sub-sector in Vietnam*. Non-Timber Forest Product Research Centre (NTFP-RC), Hanoi.

Paramanandam, V., Jagadeesan, G., Muniyandi, K., Manoharan, A. L., Nataraj, G., Sathyanarayanan, S., & Thangaraj, P. (2021). Comparative and variability analysis of different drying methods on phytochemical, antioxidant and phenolic contents of *Ficus auriculata* Lour. fruit. *Phytomedicine Plus*, 1(3), Article 100075. https://doi.org/10.1016/j.phyplu.2021.100075

Pinelo, M., & Meyer, A. S. (2008). Enzyme-assisted extraction of antioxidants: Release of phenols from vegetal matrixes. *Electronic Journal of Environmental, Agricultural and Food Chemistry*, 7(8), 3217-3220.

Rasool, I. F. U, Aziz, A., Khalid, W., Koraqi, H., Siddiqui, S. A., Al-Farga, A., Lai, W.-F., & Ali, A. (2023). Industrial application and health prospective of fig (*Ficus carica*) by-products. *Molecules*, 28(3), Article 960. https://doi.org/10.3390/molecules28030960

Shahinuzzaman, M., Akhtar, P., Amin, N., Ahmed, Y., Anuar, F. H., Misran, H., & Akhtaruzzaman, M. (2021). New insights of phenolic compounds from optimized fruit extract of *Ficus auriculata*. *Scientific Reports*, 11(1), Article 12503. https://doi.org/10.1038/s41598-021-91913-w

Stanek-Wandzel, N., Krzyszowska, A., Zarębska, M., Gębura, K., Wasilewski, T., Hordyjewicz-Baran, Z., & Tomaka, M. (2024). Evaluation of cellulase, pectinase, and hemicellulase effectiveness in extraction of phenolic compounds from grape pomace. *International Journal*

of Molecular Sciences, 25(24), Article 13538. https://doi.org/10.3390/ijms252413538

Stochmal, A., Skalski, B., Pietukhov, R., Sadowska, B., Rywaniak, J., Wójcik-Bojek, U., Grabarczyk, Ł., Żuchowski, J., & Olas, B. (2021). Evaluation of antimicrobial, antioxidant, and cytotoxic activity of phenolic preparations of diverse composition, obtained from *Elaeagnus rhamnoides* (L.) A. Nelson leaf and twig extracts. *Molecules*, 26(10), Article 2835. https://doi.org/10.3390/molecules26102835

Tran, C. H., Nghiem, M. T., Dinh, A. M. T., Dang, T. T. N., Van Do, T. T., Chu, T. N., Mai, T. H., & Phan, V. M. (2023). Optimization conditions of ultrasound-assisted extraction for phenolic compounds and antioxidant activity from *Rubus alceifolius* Poir leaves. *International Journal of Food Science*, 2023(1), Article 7576179. https://doi.org/10.1155/2023/7576179

Veberic, R., Colaric, M., & Stampar, F. (2008). Phenolic acids and flavonoids of fig fruit (*Ficus carica* L.) in the northern Mediterranean region. *Food Chemistry*, 106(1), 153-157. https://doi.org/10.1016/j.foodchem.2007.05.061

Yazdi, A. P. G., Barzegar, M., Sahari, M. A., & Gavlighi, H. A. (2019). Optimization of the enzyme-assisted aqueous extraction of phenolic compounds from pistachio green hull. *Food Science and Nutrition*, 7(1), 356-366. https://doi.org/10.1002/fsn3.900

Zhang, Z.-R., Yang, X., Li, W.-Y., Peng, Y.-Q., & Gao, J. (2022). Comparative chloroplast genome analysis of Ficus (Moraceae): Insight into adaptive evolution and mutational hotspot regions. *Frontiers in Plant Science*, 13, Article 965335. https://doi.org/10.3389/fpls.2022.965335

Zhong, L., Yuan, Z., Rong, L., Zhang, Y., Xiong, G., Liu, Y., & Li, C. (2019). An optimized method for extraction and characterization of phenolic compounds in *Dendranthema indicum* var. *aromaticum* flower. *Scientific Reports*, 9(1), Article 7745. https://doi.org/10.1038/s41598-019-44102-9