AN INVESTIGATION INTO THE TOTAL PHENOLIC COMPOUNDS EXTRACTED FROM *PERILLA FRUTESCENS* (*L.*) BRITT LEAVES AND THEIR APPLICATION ON BLOOD SUGAR REGULATION

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Abstract - This study investigated the extraction of total phenolic compounds from *Perilla frutescens (L.)* Britt leaves collected in Tuy Loan, Danang City, Vietnam. The extraction process was optimized based on ethanol concentration (%), material-to-solvent ratio (w/v), extraction time (h), and temperature (°C). The highest yield (0.27 ± 0.015 ‰) was achieved at 80% ethanol, a 1:40 (w/v) ratio, 3 hours, and 60°C. The extract exhibited potential anti-diabetic activity with an IC50 value of 5120 ± 0.45 µg/mL, compared to acarbose (119.73 ± 8.26 µg/mL). The phenolic compound powder met the safety standards for food ingredients set by the Ministry of Health of Vietnam, including microbial and heavy metal limits. This research contributes to the scientific understanding of plant-derived bioactive compounds, supports the use of P. frutescens as a functional food ingredient, and promotes the economic and medicinal value of local Vietnamese herbs.

Key words – Anti-diabetic activity; extraction processing; IC_{50}; major factors; perilla leaves; *Perilla frutescens* (*L.*) Britt

1. Introduction

Perilla frutescens (L.) Britt, clarified in the *Lamiaceae* family, is one of the annual aromatic herbaceous plants that is commonly cultivated in the tropical zones of Asian countries, *e.g.*, *viz.* "sisu" in China, "shiso" in Japan, and "tía tô" in Vietnam. *Perilla frutescens* (L.) Britt leaves are commonly used as an edible vegetable food ingredient for their pleasant taste (salads, sushi, garnish, and soup) and as a medicinal herb for their many health benefits in treating depression-related diseases and asthma in a long medical history [1-3]. The most popular *Perilla frutescens* (L.) Britt with red leaves is growing in Vietnam and is recognised as a valuable herb due to its fundamental content of bioactive compounds [4]. The bioactive compounds, *viz.* total phenolic compounds, isolated from *Perilla frutescens* (L.) Britt red leaves, *i.e.*, phenolics, flavonoids, anthocyanins, and tannins, exhibit a variety of activities, including anti-oxidant, anti-allergy, anti-inflammation, anti-tumour, anti-bacterial, and anti-diabetic activities [5-7].

Diabetes, one of the serious chronic diseases, occurs when the human body does not produce enough insulin, a regulated blood glucose hormone, and/or when the internally produced insulin cannot be effectively secreted. The most common effect of uncontrolled diabetes is hyperglycemia symptoms, which lead to serious damage to many of the body's systems, especially the nerves and blood vessels. Based on the annual statistical report of WHO, adults aged 18 years old had diabetes disease with a percentage of 14% and this number increased by 7% in comparison to the case in 1990. Ca. 1.6 million deaths occurred before the age of 70 years caused by this disease, and accounted for 47% of total deaths in the world. Kidney disease resulted in 530.000 deaths was also led by this disease, and high blood glucose due to diabetes causes around 11% of cardiovascular deaths. Since 2000, mortality rates from diabetes have been noticeably increasing. By contrast, the probability of dying from the three main noncommunicable diseases (cardiovascular diseases, cancer, and chronic respiratory diseases) between the ages of 30 and 70 years decreased by 20% globally during the period from 2000 to 2019, while death by diabetes is exponentially increasing. Diabetes treatment coverage could be lowest in low- and middle-income countries [8]. Therefore, the bioactive compounds extracted from the local and natural resources to efficiently treat diabetes and resolve the economic issues of these countries have emerged as an attractive goal for the scientific community.

Total phenolic compounds (phenolics, flavonoids, anthocyanins, and tannins) are usually known as functional ingredients synthesised in the cells of plant organs [9, 10]. These compounds were isolated from *Perilla frutescens* (*L.*) Britt can help control blood sugar, benefiting people with diabetes, resulting in increased insulin secretion and improving the body's ability to control and convert glucose as a daily carbohydrate. These compounds help enhance the activity of insulin, an important hormone in regulating blood sugar levels [11-15]. In addition, total phenolic compounds would inhibit α-glucosidase, the enzyme responsible for carbohydrate conversion to be absorbed in the intestinal system of humans, therefore, these bioactive compounds could control blood sugar [16-20]. This study investigates the major factors in the total phenolic compound extraction from *Perilla frutescens* (*L.*) Britt and the ability of crude total phenolic compounds to inhibit α-glucosidase as an indicator of sugar conversion is

evaluated in the purose of enhancing the economic value of local herb, spot for the map of medical herbs, and contributing to the scientifc community which was driven by the phenolic compounds extracted from *Perilla frutescens* (*L.*) Britt is isolated from other regions (South Korea, China, Thailand, *etc.*) [21-23].

2. Materials and Methods

2.1. Material and Material Preparation

Perilla frutescens (*L.*) Britt leaves were purchased in the Tuy Loan area, Da Nang City, Vietnam, for economic enhancement of local herbs and as a reference point for the medicinal map of herbs. The perilla plants growing after 3 months would give the highest quality of leaves. The harvested leaves were stored in a plastic bag and transported to the laboratory for further processing. The fresh and high-quality leaves were selected, washed with distilled water, and dried in a normal condition at room temperature overnight. Perilla leaves were dried at 50 °C overnight in the drying oven (Memmert, Germany), and the dried perilla leaves were ground by a household grinder (Philips, Netherlands). The particle size of perilla leaf powder was selected by sieving with a pore size mesh of 0.6 mm. The selected size of perilla leaf powder was kept in vacuum plastic bags with a mass of 10 g/bag and stored at 4 °C for further investigation.

2.2. Chemicals

Absolute ethanol (99.5%), Folin & Ciocalteu′s phenol reagent, anhydrous gallic acid (≥ 98%), phosphate buffer (100 mM, pH = 6.8), 4-nitrophenyl-*α*-D-glucopyranoside (> 99%), Na_2CO_3 (≥ 99.5%), and *α*-glucosidase isolated from *Saccharomyces cerevisiae* were provided by Merk (Sigma-Aldrich, Germany).

2.3. Effect of Major Factors on Extraction Yield of Total Phenolic Compounds

The major factors affecting the total phenolic compounds extracted from perilla leaf powder selected in this research were the concentration of ethanol (%), the ratio of material to solvent volume (w/v), extraction time (h) and extraction temperature (°C). An exact mass of perilla leaf powder (2 g) was weighed on the analytical balance (Kern & Sohn, UK) and suspended in a certain volume of ethanol (40 mL) with different concentrations. The suspension was stirred on the heat magnetic stirrer (IKA, Sigma-Aldrich, Germany) at 3 h of extraction time and an extraction temperature of 50°C. Then the suspension was filtered by a Buchner filter equipped with a sintered glass frit (DURAN®, porosity 5). The transparent-filled liquor was kept in the refrigerator at 4 °C for further analysis. The specific extraction conditions for the investigation of each extraction factor were mentioned, *vide infra*. The experiments were replicated with triple times.

2.3.1. Effect of Ethanol Concentration (%)

The different ethanol concentrations selected in this work were 99.5%, 90%, 80%, 70%, and 60%. The optimal concentration of ethanol was evaluated by the significant difference (OriginLab® Software and Minitab® Software) with the Turkey method and a 95% confidence level, which was also for other extraction conditions, was used for further investigation of the effect of the ratio of material to solvent volume.

2.3.2. Effect of the Ratio of Material to Solvent Volume (w/v)

The total phenolic compound extraction was performed at the optimal concentration of ethanol (%, *vide supra*). The selected ratios for investigation were 1:10, 1:20, 1:30, 1:40, and 1:50 (g/mL), and then the optimal ratio of material to solvent volume (w/v) was used for further screening of the effect of extraction time.

2.3.3. Effect of Extraction Time (h)

Extraction times were from 1 to 5 h (1 h increment), which were studied. The optimal concentration of ethanol and the ratio of material to solvent volume identified from previous screening (*vid.* sections *2.3.1* and *2.3.2*) were set up for the extraction protocol of total phenolic compounds from *Perilla frutescens* (*L.*) Britt leaf powder. The investigation of the extraction temperature effect was based on the optimal extraction time.

2.3.4. Effect of Extraction Temperature (°C)

The extraction temperature effect on the total phenolic compound extraction from perilla leaf powder was in the range of 30 to 80 °C (10 °C increment). Extraction was performed with the optimal concentration of ethanol (%), the optimal ratio of material to solvent volume (w/v), and the optimal extraction time (h).

2.4. Analytical Methods

2.4.1. Determination of Moisture and Ash Content

The moisture and ash content of fresh material were analysed by the standard method suggested by AOAC [24].

2.4.2. Determination of Total Phenolic Compounds

The Folin-Ciocalteu method was used to measure the total phenolic content of the extracted liquor. This method was well described in the previous research by Lamuela-Raventós *et al.* [25], Kupina *et al.* [26], and Lucas *et al.* [27]. Extraction yield in terms of the total phenolic compounds was presented in $^0/_{00}$ (based on the dried mass of *Perilla frutescens* (*L.*) Britt leaf powder), see formula (1).

$$P = \frac{m_1 \times V_1 \times 1000}{V_2 \times m_2} \quad (1)$$

Where,

P: Extraction yield (total phenolic compounds, $^0/_{00}$);

m_1: Mass of total phenolic compounds calculated by the gallic acid standard curve (mg/mL);

m_2: Dried mass of *Perilla frutescens* (*L.*) Britt leaf powder (mg);

V_1: Volume of extracted liquor (mL);

V_2: Volume of measurement liquor (mL).

2.4.3. Blood Sugar Inhibition Assays and Quality Evaluation of Total Phenolic Compound Powder

Extraction of total phenolic compounds from *Perilla frutescens* (*L.*) Britt leaf powder was performed at the optimal conditions (given at 80% ethanol concentration,

1:40 (w/v) ratio of material to solvent volume, 3 h of extraction time, and 60 °C of extraction temperature). The collected liquor after filtration was vacuumed by a rotary evaporator at 30 °C and 123 mbar until a viscous solution was formed. The concentrated total phenolic compounds were then lyophilised to obtain a powder.

The ability of the total phenolic powder to inhibit blood sugar is evaluated by the suggested method of Shai *et al.* [28] and Yamaki *et al.* [29]. *α*-amylase digests polysaccharides (*e.g.* starch) into oligosaccharides, and *α*-glucosidase further converts these oligosaccharides into glucose monomers for absorption in the intestine of humans; therefore, *α*-glucosidase, isolated from *Saccharomyces cerevisiae*, was selected as an indicator and acarbose (an anti-diabetic drug) was used as a reference in this research. The transparent mixture of phosphate buffer (130 µL, 100 mM, and pH = 6.8), *α*-glucosidase (20 µL and 0.5 UI/mL), and phenolic compounds (different concentrations in 50 µL of water) was prepared. The mixture was incubated at 37 °C for 15 min. The phenolic compounds concentrations in the wells were in the range of 12500 – 2500 – 500 – 100 µg/mL, respectively. Then, *p*-nitrophenyl-*α*-D-glucopyranoside (50 µL, 5 mM) was added and the mixture was incubated at 37 °C for 60 min. The reaction was quenched by adding 80 µL of Na_2CO_3 (0.2 M). The absorbance of the released *p*-nitrophenol was quantified at 405 nm using an ELISA Plate Reader (Biotek). The value of IC_{50} was calculated by TableCurve 2D Software (Canada). In addition, the microbiology characterisation regarding *Escherichia coli* and *Salmonella spp.*, and heavy metal analysis for quality evaluation were carried out at Quality Assurance and Testing Centre 2 (QUATEST 2).

2.5. Statistiscal Analysis

The standard deviation of experiment results and the significant differences were calculated and evaluated, respectively, by OriginLab® Software (version 2023, USA) and Minitab® Software (version 21.4.2, USA) with the Turkey method and a 95% confidence level. The experiment results were divided into distinct groups for the significant difference indicated by the letter (Table 2).

3. Result and Discussion

3.1. Moisture and Ash Contents of Fresh Perilla Leaves

The fresh perilla leaves were analysed for moisture and ash content, which were the reference for the suitable method for material storage during the research period and quality evaluation of total phenolic compound powder downstream. The moisture and ash contents are presented in Table 1. The moisture of the fresh perilla leaves was very high (86.03 ± 0.53 %, based on the wet mass). Therefore, it needed to be dried to stabilise the bioactive compounds during the research period, obtain precise experimental results, and enhance the efficiency of total phenolic compound extraction. In addition, the ash concerning the mineral of perilla leaves also has a high value of 8.39 ± 0.02 %, which was a useful reference to evaluate the extraction process and quality of total phenolic compound powder.

Table 1. *The moisture and ash content of fresh perilla leaves*

Moisture (%, based on the wet mass)	86.03 ± 0.53
Ash (%, based on the wet mass)	8.39 ± 0.02

3.2. Effect of Ethanol Concentration (%) on the Total Phenolic Compounds

The wide range of ethanol concentrations (99.5%, 90%, 80%, 70%, and 60%) was investigated in this report for the efficient extraction of total phenolic compounds. The results are depicted in Table 2. The total phenolic compounds increased with the decrease of the ethanol concentration to 80%. However, the concentration of ethanol continuously went down, and the value of total phenolic compounds decreased in parallel. The different phenolic compounds would be efficiently extracted at different ethanol concentrations. This observation is in agreement with Jiménez-Moreno *et al.* [30], de Lima Marsiglia *et al.* [31], and Chew *et al.* [32]. The ethanol concentration of 80% gave the highest total phenolic compounds of 0.15 ± 0.006 ($^0/_{00}$), which are significantly different from the others. Therefore, this concentration was selected for further screening.

Table 2. *Effect of ethanol concentration (%), the ratio of material to solvent volume (w/v), extraction time (h), and extraction temperature (°C) on the total phenolic compounds ($^0/_{00}$)*

Total phenolic compounds ($^0/_{00}$)					
Ethanol concentration (%)					
99.5%	90%	80%	70%	60%	
0.08 ± 0.01^{c}	0.12 ± 0.01^{b}	0.15 ± 0.006^{a}	0.13 ± 0.01^{ab}	0.11 ± 0.015^{bc}	
Ratio of material to solvent volume (w/v)					
1:10	1:20	1:30	1:40	1:50	
0.08 ± 0.015^{c}	0.15 ± 0.01^{b}	0.16 ± 0.006^{b}	0.21 ± 0.015^{a}	0.21 ± 0.021^{a}	
Extraction time (h)					
1	2	3	4	5	
0.14 ± 0.006^{c}	0.17 ± 0.025^{bc}	0.22 ± 0.021^{a}	0.21 ± 0.01^{ab}	0.17 ± 0.015^{bc}	
Extraction temperature (°C)					
30	40	50	60	70	
0.11 ± 0.01^{c}	0.21 ± 0.015^{b}	0.22 ± 0.015^{b}	0.27 ± 0.015^{a}	0.22 ± 0.01^{b}	

3.3. Effect of the Ratio of Material to Solvent Volume (w/v) on the Total Phenolic Compounds

The solvent volume in extraction processing holds an important key due to its role in the dynamic balance of desired products between the material (solid phase in this work) and the solvent, as well as the downstream process [33]. Table 2 shows the effect of the ratio of material to solvent volume (w/v) on the total phenolic compounds ($^0/_{00}$). The total phenolic compounds went up from 0.08 ± 0.015 to 0.21 ± 0.021 ($^0/_{00}$) since the solvent volume increased from 10 mL to 50 mL (Table 2). The significant difference analysis indicated that at ratios of 1:40 and 1:50 (w/v), the total phenolic compounds reached the maximal value and were significantly different from those at other ratios. Hence, the ratio of 1:40 (w/v) was used for further investigation of the extraction time effect on the total phenolic compounds. de Lima Marsiglia *et al.* [31] concluded the optimal ratio of material to solvent volume for total phenolic compounds extracted from jaboticaba peel was 1:20 (v/w), which was different in comparison to this

study because of the difference in material, extraction method, material pretreatment, *etc*.

Table 3. *Result of α-glucosidase inhibition by the total phenolic compound powder and acarbose*

Total phenolic compound powder		**Acarbose**	
Concentration (µg/mL)	Inhibition (%, based on the mass of *α*-glucosidase)	Concentration (µg/mL)	Inhibition (%, based on the mass of *α*-glucosidase)
12500	89.52 ± 4.33	500	77.59 ± 1.36
2500	31.42 ± 2.02	100	51.85 ± 1.47
500	12.74 ± 1.22	20	17.21 ± 1.13
100	5.24 ± 0.66	4	8.43 ± 0.84
IC_{50}	**5120 ± 0.45**	**IC_{50}**	**119.73 ± 8.26**

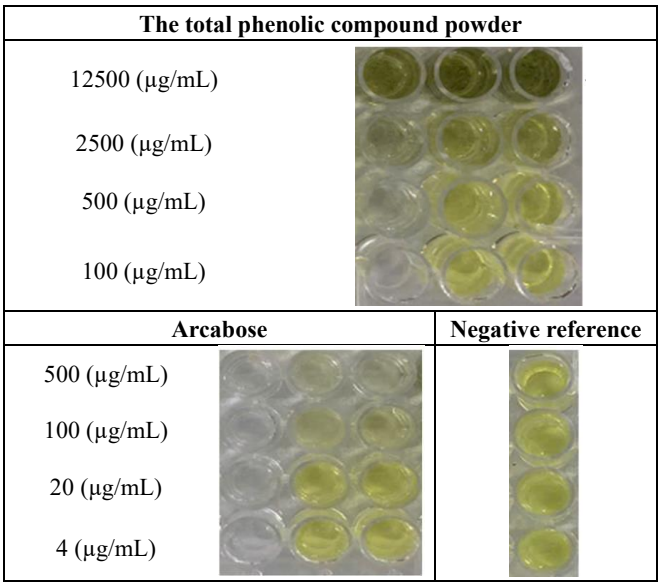

Figure 1. *Blood sugar inhibition of the total phenolic compound powder and acarbose (According to the essay of [27] and [29])*

3.4. Effect of Extraction Time (h) on the Total Phenolic Compounds

Mass transfer of desirable products from material to solvent during the extraction procedure is time-dependent, which results in the extraction time playing a crucial factor in the extraction process [33]. The total phenolic compounds at 3 h of extraction time had the highest value of 0.22 ± 0.021 ($^0/_{00}$), which was also significantly different from the others at the different extraction times (Table 2). Noticeably, the extraction time increased from 3 to 5 h, the total phenolic compounds decreased due to these compounds could be degraded under certain conditions of the extraction process (80 % ethanol concentration, ratio of material to solvent volume of 1:40 (w/v) and the extraction temperature of 50 °C). The study for the optimal extraction temperature on the total phenolic compounds was based on this value, 3 h of extraction time. The optimal time for the extraction process, given by Laloo *et al.* [34], was 72 h for the total phenol compounds extracted from *Carpobrotus edulis*. The difference in extraction solvent, material, extraction procedure*, etc.* given to this difference.

3.5. Effect of Extraction Temperature (°C) on the Total Phenolic Compounds

The thermal dynamics of soluble molecules in extraction processing are temperature-dependent [33]. Hence, screening the optimal extraction temperature would give the highest value of total phenolic compounds. The significant difference in the experimental results analyzed by both OriginLab® Software (version 2023, USA) and Minitab® Software (version 21.4.2, USA) (Table 2) showed that at 60 °C of extraction temperature the total phenolic compounds reached the highest value of 0.27 ± 0.015 ($^0/_{00}$), and it is significantly different the others. Then, the total phenolic compounds decreased with the increase of extraction temperature due to phenolic compounds degrading at high temperatures concluded by de Lima Marsiglia *et al.* [31] and Chew *et al.* [32]. Chew and his co-workers concluded the optimal extraction temperature for total phenolic compounds from *Orthosiphon stamineus* was 65 °C.

3.6. The Potential of Total Phenolic Compound Powder for Blood Sugar Inhibition and Its Quality

The extraction procedure of perilla leaf powder for the total phenolic compounds was performed at the optimal condition (80% ethanol concentration, 1:40 (w/v) ratio of material to solvent volume, 3 h of extraction time, and 60 °C of extraction temperature). The total phenolic compound powder was obtained by evaporating the solvent of the filled liquor and lyophilising the concentrated solution. The ability of phenolic compound powder to inhibit blood sugar will be indicated *via* inhibition of *α*-glucosidase. *α*-glucosidase inhibitors will delay the glucose absorption in the digestive system of humans by generating a significant reduction of postprandial hyperglycemia and postprandial insulin, which decreases HbA1c (~ 0.7%). Especially, *α*-glucosidase inhibitors for diabetic patients are used either as monotherapy or in combination with other oral hypoglycemic agents or insulin [35-37]. In this research, acarbose – an anti-diabetic drug – was utilised as a reference. The IC_{50} value of *α*-glucosidase inhibition and blood sugar inhibition essay in regards to the concentration of total phenolic compound powder and acarbose were shown in Table 3 and Figure 1, respectively.

Table 4. *Result of quality evaluation of total phenolic compound powder*

Features	**Result**
Escherichia coli (CPU/mL)	<1
Salmonella spp. (CPU/mL)	ND
Cadimi (mg/L)	ND
Lead (mg/L)	ND
Asen (mg/L)	ND
Mercury (mg/L)	ND

**ND: Not detectable*

The concentration of total phenolic compounds required to reach IC_{50} is high (5120 ± 0.45, µg/mL) in comparison to the concentration of acarbose (119.73 ± 8.26, µg/mL). The total phenolic compounds are not fractioned, isolated, and purified, which causes this

concern. However, it is one promising feature to identify that total phenolic compounds extracted from *Perilla frutescens* (*L.*) Britt is a potential anti-diabetic drug. This conclusion regarding the promising ability of total phenolic compounds for diabetic treatment in this research was well aligned with the comprehensive review of Kumar *et al.* [36], the systematic review of Yin *et al.* with 411 phenolic compounds exhibiting *α*-glucosidase inhibitor extracted from medical plants [37], and a critical review of Dirir *et al.* [38]. The critical point for fraction, isolation, purification, and characterisation of phenolic compounds for the enhancement of anti-diabetic activity was given in the intensive research of Aalim *et al.* [39], Pieczykolan *et al.* [40], and de Oliveira Raphaelli *et al.* [41]. The quality evaluation of total phenolic compound powder with these specific features in terms of microorganisms and heavy metals is depicted in Table 4. The heavy metals could not be found in the lyophilised powder product, and the microorganisms found were in the safe range based on the regulation QCVN 20-1:2024 of the Vietnamese Ministry of Health. Thus, the total phenolic compound powder obtained in this work could be used as a food ingredient.

4. Conclusion and Outlook

The total phenolic compounds extracted from *Perilla frutescens* (*L.*) Britt leaves were investigated in this research regarding the optimal extraction conditions. The moisture and ash content of fresh perilla leaves (86.03 ± 0.53% and 8.39 ± 0.02%, respectively), analysed by the AOAC method, is high, thus, they need to be dried for storage, and the quality of the final product regarding heavy metals is required to be analysed. The optimal extraction condition to reach the maximal value of total phenolic compounds (0.27 ± 0.015 $^{0}/_{00}$) was 80% ethanol concentration, 1:40 (w/v) of the ratio of material to solvent volume, 3 h of extraction time, and 60 °C of extraction temperature. The blood sugar inhibition essay with *α*-glucosidase isolated from *Saccharomyces cerevisiae* as an indicator and acarbose, an anti-diabetic drug, being a reference, indicated that total phenolic compounds are a promising anti-diabetic drug with an IC_{50} value of 5120 ± 0.45 (μg/mL). We suggest that the fractionation, isolation, and purification of crude phenolic compounds should be carried out to enhance the ability of blood sugar regulation. In addition, the quality evaluation also revealed that crude total phenolic compounds could be utilised as food ingredients based on the regulation of the Vietnamese Ministry of Health, according to QCVN 20-1:2024.

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