MALAYSIAN JOURNAL OF BIOCHEMISTRY & MOLECULAR BIOLOGY

The Official Publication of The Malaysian Society For Biochemistry & Molecular Biology (MSBMB)
http://mjbmb.org

FLAVONOIDS-ENRICHED EXTRACTION OF *Phyllanthus amarus* LEAVES AS A POTENTIAL *IN VITRO* MEDICATION FOR TREATING DIABETES

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History
Received: 17 April 2022
Accepted: 8 August 2022

Keywords:

Phyllanthus amarus, Antidiabetes, Flavonoids, Bioactivity

Abstract

This study aimed to evaluate the extraction process of *Phyllanthus amarus* for the enrichment of flavonoid content applied in treating diabetes and its complications. The investigated factors included ethanol concentration (0, 30, 70, 100%), extraction temperatures (40, 50, 60, 70 °C), extraction time (30, 60, 120 min), and solid-to-solvent ratio (1:10, 1:15, and 1:20 g:mL). The results showed that the appropriate extraction conditions for high flavonoid content were at an ethanol concentration of 70%, extracting temperature of 50 °C, extraction time of 60 min, and solid-to-solvent ratio of 1:15 g:mL. The relevance of flavonoid and α-glucosidase inhibition activity was also confirmed in this study. This enriched-flavonoid extract exhibited good α-glucosidase inhibition activity with a half-maximal inhibitory concentration (IC_{50}) of 2 µg/mL, antioxidant activity with IC_{50} of 4.5 µg/mL, and anti-inflammatory activity with IC_{20} of 400 µg/mL. Besides, the extracts also displayed activities in antimicrobial, especially gram-positive strains.

INTRODUCTION

Diabetes mellitus (DM) is a metabolic disease caused by abnormalities in insulin production, insulin action, or both. It is characterized by chronic hyperglycemia with disturbances in carbohydrate, lipid, and protein metabolism [1]. According to statistics from the International Diabetes Federation, there were around 463 million individuals with diabetes in 2019, which is expected to grow to 630 million by 2045 [2]. There are several types of DM, the most common of which are type I and type II. Type I diabetes (insulin-dependent diabetes mellitus) is a hereditary autoimmune condition that develops when the pancreas' insulin-producing cells are damaged, or the pancreas produces little or no insulin. A person with type I diabetes must take insulin regularly to achieve homeostasis in the body. Type II diabetes is also known as "insulin-independent diabetes mellitus," accounting for more than 90% of all diagnosed cases of diabetes in adulthood. Type II diabetes is caused by a condition in which the pancreas generates adequate insulin, but the body is unable to utilize it efficiently [3]. Hyperglycemia could harm body organs such as skin, nerves, eyes, kidneys, heart, and blood vessels [4,5]. According to Donath and Shoelson [6], type II diabetes has a strong connection with obesity, oxidation, and inactivity caused by a progressive failure of pancreatic islet β-cells to compensate for insulin resistance. While the cellular stresses in type II diabetes include oxidative stress, endoplasmic reticulum stress, amyloid deposition in the pancreas, ectopic lipid deposition in muscle, liver, and pancreas, and lipotoxicity and glucotoxicity may cause an inflammatory response or be linked with inflammation. For the management of DM, many medicinal therapies have been established. Type I diabetes is often managed with an external insulin delivery, whereas Type II is treated with a combination of lifestyle modifications and medication therapy [7]. However, the cost of anti-diabetic drugs is a burden for low-income people. Besides, some diabetes drugs

as metformin, thiazolidinediones, and rosiglitazone, have many adverse side effects including heart failure, gastrointestinal on the initial state (dyspepsia, nausea and diarrhea), changing weight, fluid retention, heart attack. Therefore, the use of herbal medication is a promising pathway for to control diabetes, along with lower side effects [8].

Herbal treatments are safe and successful in treating illnesses, and they could be a source for the creation of novel medications [9,10]. There are more than 800 plants utilized to treat diabetes healing; above and beyond, a variety of herbal-derived compounds such as flavonoids, phenols, triterpenoids, and alkaloids have shown prominent antidiabetic activity [11,12]. *P. amarus* Schum. & Thonn., a small herb in the family of Euphorbiaceae, is widely distributed in Nigeria, India, China, Vietnam, and Thailand [13]. *P. amarus* has been used by many countries in folk medicine for the treatment of various disease conditions related to several pharmacological activities such as antiviral, antibacterial, antiplasmodial, anti-inflammatory, antimalarial, antimicrobial, anticancer, antidiabetic, hypolipidemic, antioxidant, hepatoprotective, nephroprotective, and diuretic [14–16]. Many bioactive compounds were isolated from *P. amarus* as lignans, flavonoids, ellagitannins, alkaloids, triterpenes, sterol, and volatile oil [17]. Flavonoids are abundant in plants and have been demonstrated in several studies to have not only well-known antidiabetic and hypoglycemic properties but also activity in the treatment of diabetic complications. [18]. Gallocatechin, rutin, quercetin3-O-glucopyranoside, phyllanthusiin, quercetin, and kaempferol, which belong to flavonoids, were confirmed their presence in *P. amarus* extract [17]. These flavonoids have been shown to have a good ability to inhibit diabetes, and its complications, as quercetin is recorded to have good bioactivity in anti-diabetes, antioxidant, and treating neuropathic pain [19]. Moreover, there is little research focusing on the flavonoid extraction process from *P. amarus*.

The present study aimed to enrich the flavonoid fraction of *P. amarus* leaves extracts by investigating the effects of extraction conditions on TFE for anti-diabetes activity. The *in vitro* α-glucosidase enzyme inhibitory activity of *P. amarus* extracts in various conditions was also evaluated. Besides, the total phenols content, antioxidant, anti-inflammatory, and antimicrobial activities related to diabetes complications were determined in the obtained flavonoid-enriched extract. This information will be prime for the isolation of flavonoids in *P. amarus* and using the extraction as a novel medication for treating both diabetes and its complication.

MATERIALS AND METHODS

Materials

The *P. amarus* leaves were harvested from Binh Chanh District, Ho Chi Minh City, Vietnam, in October 2020. The identification was carried out at the Department of Ecology and Evolutionary Biology of the Faculty of Biology and Biotechnology, Ho Chi Minh City University of Science, Vietnam National University. The *P. amarus* samples were washed with tap water, dried at room temperature, crushed into a powder, and kept in sealed bags after being collected.

All the following chemicals used were in analytical grade; Folin–Ciocalteu reagent, quercetin, gallic acid (GA), para-nitrophenyl α-D-glucopyranoside (p-NPG), α-glucosidase, acarbose, bovine albumin, and DPPH were purchased Sigma-Aldrich, Singapore. Ethanol (EtOH), methanol (MeOH), sodium nitrite, sodium carbonate (Na_2CO_3), sodium hydroxide, aluminum chloride, and dimethyl sulfoxide (DMSO) was obtained from Merck with pure grade.

Preparation of Extracts

The *P. amarus* extraction was prepared by mixing 50 g of *P. amarus* powder with 500 mL solvent for 60 min at 50°C. The extraction process was replicated in the same conditions with *P. amarus* residues. Both extractions in 2 cycles were mixed and concentrated by rotary vacuum evaporation at 55 ℃ (Buchi R-215 Rotavapor). The dry weight of extracts was measured by Sartorius moisture analyzer MA37. Besides, to determine the extraction condition of flavonoid fraction of *P. amanus*, the effects of solvent concentration (0 – 100 %), temperature (40 – 70°C), time (30 – 120 min), and solid-to-liquid ratio (1:10 – 1:15 g:mL) on total flavonoid content were determined.

Phytochemicals Screening

Alkaloids, flavonoids, tannins, saponins, terpenoids, cardiac glycosides, and polyphenols were detected in the phytochemical screening of *P. amarus* [23–27]. Table 1 lists the reagents, test procedures, and results.

Determination of Total Flavonoid Excreted (TFE)

TFE of *P. amarus* extracts was determined by the aluminum chloride colorimetric method, as described by Baba et al [25]. Briefly, 10 mg of extract was dissolved in 1 mL of

methanol to obtain a stock sample. A total of 0.5 mL sample in desired concentration prepared from stock was made up of 2 mL of distilled water, and 0.15 mL of $NaNO_2$ 5%. After stabilizing in 5 min, 0.15 mL of $AlCl_3$ 10% was added to the mixture. Then, the mixture was incubated for 1 min before adding 1 mL of NaOH 1 mol/L and 1.2 mL of distilled water to stop the reaction. The mixture absorbance at 425 nm was measured by UV–Vis spectrophotometer (*Thermometer*, U.S.A.). The TFE was expressed as mg of quercetin equivalent (QUE) per g dry extraction weight.

Determination of Total Polyphenol Excreted (TPE)

The TPE in extracts was determined using Folin Ciocalteu's colorimetric method [26]. Briefly, a mixture of 40 µL of extract in DMSO and 200 µL of Folin–Ciocalteu was homogenized in a sonication bath for five min at ambient temperature before adding 600 µL of 20% Na_2CO_3 and 3,160 µL of distilled water. The mixture was incubated for 30 min at room temperature. The absorbance of the mixture at 760 nm was recorded by UV–Vis spectrophotometer (*Thermometer*, U.S.A.). The sample without Folin-Ciocaltue reagent and GA was used as a blank and standard, respectively. The TPE was expressed as mg of GA equivalent (GAE) per g dry extraction weight.

***In Vitro* α-Glucosidase Inhibitory Assay**

The anti-diabetes activity of the extracts *in vitro* was measured via the α-glucosidase inhibition activity because the α-glucosidase enzyme plays an essential catalytic role in converting polysaccharides to monosaccharides (glucose). Thus, inhibition of α-glucosidase lowers the glucose content. The investigation regarding the α-glucosidase enzyme inhibitory activity of the extract was conducted following Liu et al. [29]. The test was performed on 96 well plates. The 40 µL extracts dissolved in DMSO and 20 µL of α-glucosidase enzyme (1 U/mL) were added to the wells. Next, 100 µL of phosphate buffer (pH 6.8) was added to the mixture. The plate was incubated for five minutes at 37°C. Then, 40 µL of 0.1 mmol/L *p*-NPG was added to the reacting mixture, which was continuously incubated for 30 min at 37°C. Subsequently, ~100 µL of Na_2CO_3 0.1 M was added to terminate the reaction. The sample absorbance was measured at 405 nm using the UV–Vis spectrophotometer. Acarbose was employed as a positive control. The percent inhibition of the α-glucosidase reaction was calculated as follows:

$$I\% = \left(\frac{A-B}{A}\right) \times 100\%, \qquad (1)$$

where A is the absorbance at 405 nm of the blank (α-glucosidase and the substrate) and B is the absorbance at 405 nm of the extract (α-glucosidase, the substrate, and the sample).

The concentrations of the extracts resulting in the half-maximal inhibitory concentration (IC_{50}) of the enzyme activity were determined graphically.

DPPH Radical-Scavenging Activity

Oxidation is one of the main complications of diabetes; thus, finding an agent with antioxidant and anti-diabetes activities is necessary. The antioxidant activity of the samples was investigated via DPPH free radical scavenging assay according to Stagos' method, with slight modifications [30]. A total of 120 µL of the sample was added to 180 µL of DPPH dissolved in 80% MeOH. The mixture was incubated for 30 min at 30°C in the dark. Then, the absorbance was measured at 517 nm using the UV–Vis spectrophotometer. Here, MeOH and ascorbic acid were used as the negative control and positive control, respectively. The percentage inhibition (I%) was calculated using Eq. 2:

$$I\% = \left(\frac{A-B}{A}\right) \times 100\%, \qquad (2)$$

where A is the absorbance at 517 nm of the DPPH radical of the negative control, and B is the absorbance at 517 nm of the DPPH radical solution mixed with the sample.

The antioxidant activity was expressed by the IC_{50} value, representing the sample concentration required to inhibit 50% of the free radical scavenging activity.

***In Vitro* Anti-Inflammatory Assay**

The *in vitro* anti-inflammatory activity of the extracts was evaluated via the extracts' protective activity against albumin denaturation, as described in previous studies with slight modifications [32]. The extracts were serially diluted in DMSO, which were used as the negative control. The reaction mixture was prepared by adding 3 mL of bovine albumin dissolved in phosphate buffer with a pH of 6.4 into 2 mL of tested extract. The mixture was incubated for 5 min at 70°C. After cooling to room temperature, the sample absorbance was measured at 660 nm using a UV-Vis spectrophotometer (*Jasco*, U.S.A.). Diclofenac was used as the positive control.

The percentage inhibition of the protein denaturation was calculated using Eq. 3:

$$\%\text{Inhibition} = \frac{A_{control} - A_{sample}}{A_{control}} \times 100\%, \qquad (3)$$

where A_{control} is the absorbance of the negative control (DMSO) and A_{sample} is the absorbance of the extract.

Determination of Antimicrobial Activities

In this study, the antimicrobial activities were determined by the dish diffusion method [30] with two types of microbial

strains including: gram-positive (*Enterococcus faecalis, Staphylococcus aureus,* and *Methicillin-resistant Staphylococcus aureus)* and gram-negative (*Escherichia coli, Pseudomonas aeruginosa,* and *Salmonella enterica serovar Typhimurium).* Firstly, each strain was cultured onto a blood agar plate and incubated for 16 to 20 h at 37°C. A single colony was then cultured in 5 ml Mueller Hinton Broth for 5-6 h at 37 °C. The density of bacteria culture required for the test was adjusted to 0.5 McFarland standard, (1.0-2.0 x 10^8 CFU/mL) measured using the Turbidometer. Afterward, the antimicrobial activity of *P. amarus* extracts was assessed by the Kirby Bauer Disc Diffusion Method. Muller Hinton Agar Media was prepared and put into Petri-dishes for the test. Following the solidification of the agar medium, 0.1 ml of each microbial strain was distributed over the media. The discs were then saturated with the extracts and allowed to dry. For sample preparation, 50 µg/mL of extract dissolved DMSO 5% was deposited onto the discs. After the discs was dry completely, they were placed on the inoculated medium. The plates were incubated for 24 h at 37ºC for bacteria. The negative control was DMSO (5%). After the incubation, the plates were examined for inhibition zone, measured using calipers, and recorded. The test was repeated three times. Besides, the minimal inhibitory concentrations (MIC) of plant extract were also measured in this study.

Statistical Analysis

All experiments were performed at least in triplicate. The data were expressed as the mean value ± standard deviation for each measurement using Microsoft excel software. One-way analysis of variance (ANOVA) was used in the statistical analysis, followed by the least significant difference (LSD) test. The differences were considered significant when P-values were less than 0.05 (P-value < 0.05).

RESULTS AND DISCUSSION

Phytochemical Studies of *P. amarus* in Different Extracts

The presence of alkaloids, flavonoids, tannins, saponins, terpenoids, cardiac glycosides, and polyphenols in *P. amarus* leaves extract was confirmed by phytochemical analysis (Table 1). The presence of phytochemicals in *P. amarus* is responsible for its therapeutic effects. Both ethanolic and aqueous extracts were detected in all bio-compounds except cardiac glycosides. The alkaloids and flavonoids showed a strong presence in the ethanolic extract. The presence of alkaloids and terpenoids indicates the ability of the extract to be anti-diabetics, antioxidant, anti-malaria, and anti-hypertension activities [2]. Furthermore, the presence of two types of flavonoids (following 2 test methods) in PA extracts shows that they can reduce cholesterol *in vitro* and inhibit -glucosidase activity, which control blood glucose levels [31]. Tannins are valuable compounds in protecting wounds and preventing infections [32]. Besides, saponins are natural medicines that combat diseases and microbial invasions. They also contain hypocholesterolemia properties, which may provide some chemoprotection against heart disease [33]. The robust existence of these compounds in the *P. amarus* extract suggested the ability to enhance the current therapy options for type 2 diabetes mellitus.

Table 1. The phytochemical analysis of *P. amarus* leaves extracts in ethanol and water

Bioactive compounds	Test	*P. amarus* extraction	
		Ethanolic extraction	*Aqueous extraction*
Alkaloids	Dragendroff	++	+
	Bouchardat	++	+
Flavonoids	Lead acetate 10%	++	++
	Sulfuric acid 98%	++	+
Tannins	Gelatin 1%	++	++
Saponins	Hydrochloric acid	+	+
Terpenoids	Liebermann-Burchard	++	++
	Salkowski	+	+
Cardiac glycosides	Keller-kiliani	-	-
Polyphenols	Ferric (III) chloride	++	++

- Not detected; + Slightly positive reaction; and ++ Strong positive reaction

Effects of Extraction Conditions on TFE of *P. amarus*

Effects of Ethanol Concentration

Solvent extraction is the most widely used method for natural products, in which the selection of the solvent is crucial to this approach. In this study, ethanol is used to enrich the flavonoid fraction of extracts because of its selectivity, solubility, cost, and safety. The effects of ethanol concentration on TFE in extraction conditions of 50°C, 30 min, and 1:10 (g:mL) are shown in Fig 1a. The results illustrated that the content of flavonoids reaches a peak of 117.91 mgQUE/g with an alcohol concentration of 70%, but when the alcohol concentration increase to 100%, the flavonoid content decreases to 58.55 mg QUE/g significantly. Flavonoids exist in plants in the form of glycosides and aglycons; thus, at an ethanol concentration of 70%, both polar and non-polar compounds can be dissolved because of their polarity. According to Sathishkumar *et al.* [34], ethanol interacts with the flavonoid through noncovalent interactions and promotes quick diffusion into the solution. Aqueous ethanol can function as a good extracting agent since it can extract proteins and polysaccharides together with flavonoids and is easy to remove. Besides, the α-glucosidase enzyme inhibitory activity of *P. amarus* extracts gives the highest I% of 99.53% with 70% ethanol. As a result, the flavonoid content increased along with the inhibitory effect of alcohol α-glucosidase enzyme according to different concentrations. To obtain the maximum yield of flavonoids, 70% ethanol should be used.

Effects of Temperature

Temperature is a crucial factor affecting TFE, in which the appropriate temperature will increase the diffusion and solubility of extraction and lower the decomposition effect of components [35]. The effect of extraction temperature on TFE in the condition of 70% ethanol, 30 min, and 1:10 mg/mL is shown in Fig 1b. The TFE and α-glucosidase inhibition activity at 4 µg/mL of *P. amarus* extracts had peaks of 117.9 mg QUE/g and 99.53% at 50 °C and a tendency to decrease with increasing temperature in a range of 50 °C – 70°C. Although the high temperature could release the bonds of flavonoid compounds and protein or polysaccharides, higher than 50ºC temperatures cause degradation of some flavonoids such as flavan-3-ol, anthocyanin, flavanone, and chalcone glycoside [36]. Therefore, the temperature of 50°C is the optimal condition for flavonoid extraction from *P. amarus.*

Effects of Extraction Time

The extraction efficiency improves as the extraction duration in a given time range increases. Increasing the extraction time after the solute has reached equilibrium within and outside the solid material has no effect. The flavonoid content increased from 117.9 mg QUE/g to 186.5 mg QUE/g (Fig 2a), and subsequently declined with a longer extraction time. Flavonoids can be decomposed when heated at high temperatures over an extended period [37]. Moreover, the number of impurities may increase if the extraction time is increased [38]. The results were consistent with α-glucosidase inhibition activity, there was no significant difference in the I% value when extending the extraction time from 30 min to 60 min, but this value decreased sharply as the extraction time of 120 min due to the reduction of TFE.

Effects of Solid-to-Liquid Ratio

The greater the solvent-to-solid ratio is, the higher the extraction yield is; however, a solvent-to-solid ratio that is too high will cause excessive extraction solvent and requires a long time for concentration. Figure 2b shows the TFE at different ratios of material and solvent. For TFE, increasing the material/solvent ratio from 1:10 to 1:15 g:mL resulted in a slight increase in values from 186.5 mg QUE/g dry extraction weight to 209.24 mg QUE/g dry extraction weight. While the flavonoid content decreased with the solid-to-liquid ratio of 1:20 g:mL because when reaching a certain ratio, the flavonoid compounds were completely dissolved in the solvent, resulting in the extracted content reaching the saturation state and preventing further increase [34]. Besides, there was no significant change in the α-glucosidase inhibition activity of 1:10 and 1:15 sample, which confirm the relevance between TFE and α-glucosidase inhibition activity of *P. amarus* extract.

The Bioactivities of Enriched-Flavonoid Extraction

Based on the preliminary experiments, the extraction conditions for enriched flavonoids were at the ethanol concentration of 70%, a temperature of 50ºC, an extraction time of 60 min, and the solid to liquid ratio of 1:15 g:mL. Besides TFE, the enrichment extract contained a significant amount of TPE (227.53 mgGAE/g), thus the extract could have many bioactivities in inhibiting diabetic complications. The enriched flavonoids extract of *P. amarus* showed strong inhibitory activity against yeast α-glucosidase with IC_{50} of 2 µg/mL, compared to IC_{50} of acarbose of 6.83 µg/mL. Therefore, it could be concluded that the flavonoid extract of *P. amarus* has the potential to be used in the treatment of

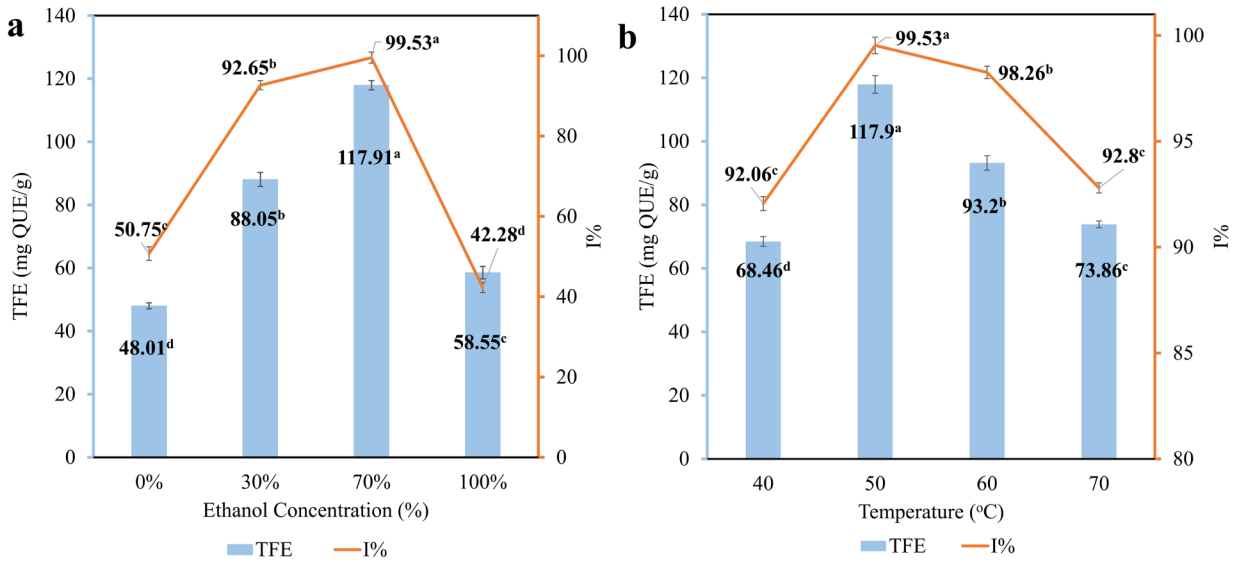

Figure 1. Effects of ethanol concentration (a) and temperature (b) on TFE and α-glucosidase inhibition activity at 4 µg/mL of *P. amarus* extracts. Data were averaged from three independent experiments and are shown as mean ± SD (n=4). The different superscript letters indicate significant differences between values, according to one-way ANOVA and LSD test ($p<0.05$)

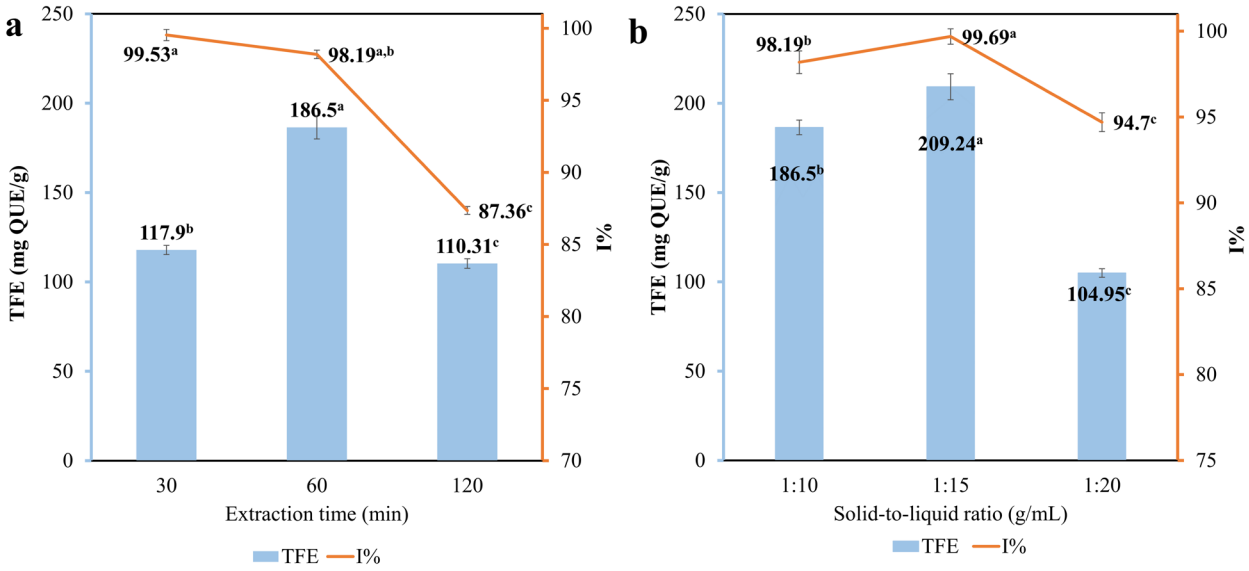

Figure 2. Effects of extraction time (a) and solid-to-liquid ratio (b) on TFE and α-glucosidase inhibition activity at 4 µg/mL of *P. amarus* extracts. The value are mean ± SD (n=4). The superscript different letters indicate significant differences between values, according to one-way ANOVA and LSD test ($p<0.05$)

type II diabetes. The antioxidant ability of the enrichment sample is quite strong, with IC_{50} of 4.5 µg/mL compared to that of Vitamin C (IC_{50} of 2.75 µg/mL). The intense antioxidant activity can be caused by the polyphenol and flavonoids in the extract, such as quercertin 3-O-glycoside, geraniin, 1,6- digalloylglucopyranoside rutin, phyllanthusiin D, and rutin [17]. Inflammation is a complicated process that involves the reaction of bodily tissues to infection, irritation, or other damages. As a result, inflammation is implicated in various illnesses, including diabetes [31]. The anti-inflammatory activity of the enrichment extract had IC_{20} is 400 µg/mL. According to William et al., extracts with inflammatory inhibition over 20% after albumin denaturation can be regarded as an anti-inflammatory drug [40]. Therefore, it is possible to rely on albumin denaturation above 20% to assess the potential anti-inflammatory activity of the enriched flavonoid extract. The anti-inflammatory activity of the extract is mainly due to the flavonoid compounds in the plant, such as quercetin, kaemferol, and chalcone. These compounds contain hydroxyl groups capable of inhibiting the biosynthesis of other mediators of the inflammatory process, such as cytokines and chemokines.

Table 2. The TFE, TPE, α-glucosidase inhibitory, antioxidation, and anti-inflammatory activities of optimal extracts

Quantity	**Sample**	
TFE (mg QUE/g)	209.24 ± 3.04	
TPE (mg GAE/g)	227.53 ± 2.46	
Bioactivities	**Sample (IC_{50})**	**Positive control (IC_{50})**
α-glucosidase inhibitory (µg/mL)	2.00 ± 0.64	6.83 ± 0.23 (Acarbose)
Antioxidation (µg/mL)	4.5 ± 0.01	2.75 ± 0.14 (Ascorbic acid)
Anti-inflammatory (µg/mL)	400 ± 2.04*	5.63 ± 1.24* (Diclofenac)

*IC_{20} value

Aside from the traditional symptoms of the disease, diabetes causes decreasing T cell response, neutrophil function, and humoral immune problems. As a result, diabetes increases susceptibility to infections, both common and those that nearly invariably afflict persons exclusively with diabetes (e.g. rhinocerebral mucormycosis) [41]. Most of the bacterial strains found in diabetic patients are *E. coli, E. faecalis, P. aeruginosa, S. aureus,* and *S. Typhimurium* [42]. The antimicrobial activities of enriched-flavonoid extract of *P. amarus* at 40 µg/mL were performed on 6 microbial strains including Gram-negative and Gram-positive by the disc diffusion method. The results are shown in Table 3. The antibacterial ability of the extract was relatively low, with the measured antibacterial diameter being quite small. The enriched-flavonoid sample was only resistant to Gram-positive strains of *E. faecalis, S. aureus*, and *MRSA* and did not affect three strains of Gram-negative due to the difference in the cell wall structure of the two groups. Gram-positive strains have a thick, reticular cell wall made of peptidoglycan. In contrast, the peptidoglycan cell wall of gram-negative ones is thinner but has a protective outer lipopolysaccharide membrane. The enriched-flavonoids extract was only effective against each group of gram-positive bacteria because it can adhere directly to the cell wall without obstructing the cell membrane like Gram-negative strains. The results of the antimicrobial activities of *P. amarus* extracts were also consistent with previous studies [43].

Table 3. The antimicrobial activities of optimal extract

Antimicrobial Agent		**Diameter of inhibition zone (mm)***	**MIC (mg/mL)**
Gram-positive bacteria	*E. faecalis*	10 ± 0.35	20 ± 0.95
	S. aureus	9.3 ± 0.23	10 ± 0.39
	MRSA	10.5± 0.12	20 ± 0.88
Gram-negative bacteria	*E. coli*	-	-
	P. aeruginosa	-	-
	S. typhimurium	-	-

- No inhibition
*at concentration of 40 µg/mL

CONCLUSION

The present study indicates the extraction conditions for the enrichment of flavonoid fraction derived from an ethanolic extract of *P. amarus* leaves. The enrichment extracts were obtained at an ethanol concentration of 70%, extracting temperature of 50°C, extraction time of 60 min, and the solid-to-liquid ratio of 1:15. The extracts were effective in the inhibition of α-glucosidase activity with IC_{50} value of 2 µg/mL, lower 3 times than the IC_{50} value of acarbose (6.83 µg/mL). In addition, the extracts had more bioactivities such as strong antioxidation capacities, anti-inflammatory, and antimicrobial. The flavonoid content is a key factor in using *P. amarus* for treating diabetes and its complications. In future work, this study can be considered primes for isolating flavonoid compounds and producing pharmaceutical preparations to treat type II diabetes.

ACKNOWLEDGEMENTS

We acknowledge the support of time and facilities from Ho Chi Minh City University of Technology (HCMUT), VNU-HCM, for this study.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest regarding the publication of this manuscript.

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