OPTIMIZING THE EXTRACT CONDITIONS FOR α-GLUCOSIDASE INHIBITORY ACTIVITIES FROM *POLYGONUM BARBATUM L.*

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ABSTRACT:

This study is to optimize the extraction conditions of *P. barbatum* for α-glucosidase inhibitory activities. Four factors were examined in this study, including the ethanol concentration (30, 50, 70, and 100%), the extraction temperature (40, 50, 60, and 70°C), the extraction time (30, 45, 60, and 90 minutes) and the solid to solvent ratio (1:5, 1:10, 1:15, and 1:20 g/mL). The study's results show that the appropriate extraction conditions for α-glucosidase inhibitory activities were the ethanol concentration of 70%, the extraction temperature of 60°C, extraction temperature of 60 minutes, and the solid to solvent ratio of 1:10 g/mL. The extract from *P. barbatum* under these conditions has good α-glucosidase inhibitiors with IC_{50} of 6.87 µg/mL, and anti-inflammatory activities with IC_{20} of 4.75 µg/mL. The extract has the potential for diabetes treatment.

Keywords: diabetes, α-glucosidase inhibitory activities, medicinal plants, *Polygonum Barbatum.*

1. Introduction

Diabetes mellitus (DM) is a metabolic disorder characterized by impaired insulin synthesis, insulin action, or both. Chronic hyperglycemia is present, as well as abnormalities in carbohydrate, lipid, and protein metabolism [1]. According to the International Diabetes Federation, there were around 463 million diabetics in 2019, with the number anticipated to rise to 630 million by 2045 [2]. Low-income persons, on the other hand, are burdened by the expense of anti-diabetic medicines. Furthermore, several diabetic medications, such as metformin, thiazolidinediones, and Rosiglitazone, have a variety of negative side effects, including heart failure, gastrointestinal symptoms (dyspepsia, nausea, and diarrhea), weight gain, fluid retention, and heart attack. As a result, using herbal medications to treat diabetes is a potential option with fewer side effects [3].

Herbal therapies are both safe and effective in the treatment of ailments, and they might be used to develop new pharmaceuticals [5]. *Polygonum barbatum,* sometimes known as "Knotweed," is a Polygonaceae herbaceous perennial plant that grows mostly in shaded, wet environments, such as along river and pond banks [6]. Polygonaceae is a family of 50 genera and 1200 species found in Asia and North America, with 19 genera and 103 species found in Pakistan [7]. *Polygonum barbatum* has

been used in traditional medicine to cure ulcers, and its roots have been utilized as an astringent by local practitioners [8]. Polygonum barbatum has cholinergic, antinociceptive, antitumor, anti-inflammatory, antivenom, and diuretic properties, according to a review of the literature [9]. However, there is little research focusing on the anti-α-glucosidase activities of *P. barbatum* extraction.

The present study aimed to optimize the extract condition of *P. barbatum* leaves for anti-diabetes activity. The *in vitro* α-glucosidase enzyme inhibitory activity of *P. barbatum* 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 extract. This information will be prime for using the extraction as a novel medication for treating both diabetes and its complication.

2. Materials and Methods

2.1. Materials and Chemicals

Plants were collected on 02/2021 in Ho Chi Minh city before washing and drying under shade until the moisture content was less than 15%. The dry leaves of 5 herbals were grounded and stored in a sealed bag for further use. The plants were authenticated by the Department of Ecology and Evolutionary Biology, Faculty of Biology and Biotechnology, Ho Chi Minh City University of Science, Vietnam National University.

Chemicals were purchased from commercial suppliers in analytical grade, as follows: ethanol (C_2H_5OH), methanol (CH_3OH), sodium nitrite ($NaNO_2$), sodium carbonate (Na_2CO_3), sodium hydroxide (NaOH), aluminum chloride ($AlCl_3$), dimethyl sulfoxide (DMSO), ferric chloride ($AlCl_3$), Folin-Ciocalteau reagent, quercetin, xanthine oxidase (4.5U/ml, from bovine milk), gallic acid.

2.2. Extraction method

A total of 20g of plant powders were soaked in 200 mL of 96% ethanol for 45 minutes at 50^{o}C. Before employing a rotary evaporator to remove the solvent, the ethanolic extract was vacuum filtered. The TPC of extracts was measured by the Folin-Ciocalteu assay, as described by Le et al. [2]. The TFC in each extract was determined by the aluminium-chloride colorimetric assay method, as described by Do et al. [10].

2.3. 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 important 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's method [29].

2.4. DPPH radical-scavenging activity

Oxidation is one of the main complications of diabetes; thus, finding an agent with antioxidant and anti-diabetes activities is a necessity. The antioxidant activity of the samples was investigated via DPPH free radical scavenging assay according to Stagos' method [30].

2.5. 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 [32].

3. Results and Discussion

3.1. Effects of extraction conditions on TFE of P. barbatum

The extraction yield of bioactive compounds from natural plants is affected by extraction variables such as solvent concentration (percent), temperature (C), duration (min), and LSR (g/g). The selection of a suitable solvent is a key step in optimizing the bio-active chemicals from the plant. By using different solvent concentrations from 30 to 100% (Fig. 2a), the anti-diabetes of plant extract peaks at 0.48 µg/mL at ethanol 70%. As can be seen from Fig. 2b, temperature time greatly influence the extraction of the bioactive compounds in the plant. When extraction temperature increases from 40 to 60^{o}C, a decrease in IC_{50} from 0.67 to 0.46 µg/mL is witnessed. According to a previous study, some thermally

susceptible compounds such as myricetin, kaempferol, rhamnetin, and quercetin might be present in the plant extract. A similar phenomenon can be seen in Fig. 2c in which IC_{50} reaches a peak of 0.41 µg/mL at 60 minutes. The α-glucosidase inhibition activity of the extract is low when the ratio is low (1:20 and 1:15) because the difference in concentration between the plant and the environment is not enough for bioactive compounds to diffuse into the solvent. The value of IC_{50} reaches a peak of 0.47 µg/mL at a ratio of 1:10 and increases with increasing solvent. (Figure)

3.2. The bioactivities of enriched-flavonoid extraction

Based on the preliminary experiments, the extraction conditions for enriched flavonoids are at the ethanol concentration of 70%, the temperature of 60°C, the extraction time of 1h, and the solid to liquid ratio of 1:10. Besides TFE, the enrichment extract contained a significant amount of TPE (351 mgGAE/g), thus the extract could be had many bioactivities in inhibiting diabetic complications. The enriched flavonoids extract of *P. barnatum* showed strong inhibitory activity against yeast α-glucosidase with IC_{50} of 0.45 µg/mL, compared to IC_{50} of acarbose of 9.81 µg/mL. Therefore, it could be concluded that the flavonoid extracts of *P. barbatum* had potential in the treatment of type II diabetes. The antioxidant ability of the enrichment sample is quite strong with IC_{50} of 6.87 µg/mL compared to that of Vitamin C (IC_{50} of 2.75 µg/mL). Inflammation is a complicated process that involves the reaction of bodily tissues to infection, irritation, or other damage. As a result, inflammation is implicated in a variety of 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 [15]. Therefore, it is possible to rely on the albumin denaturation above 20% to assess the potential anti-inflammatory activity of the enriched flavonoid extract.

Figure: Effects of ethanol concentration (a), temperature (b), extraction time (c), and solid to liquid ratio (d) on α-glucosidase inhibition activity of P. barbatum extracts.

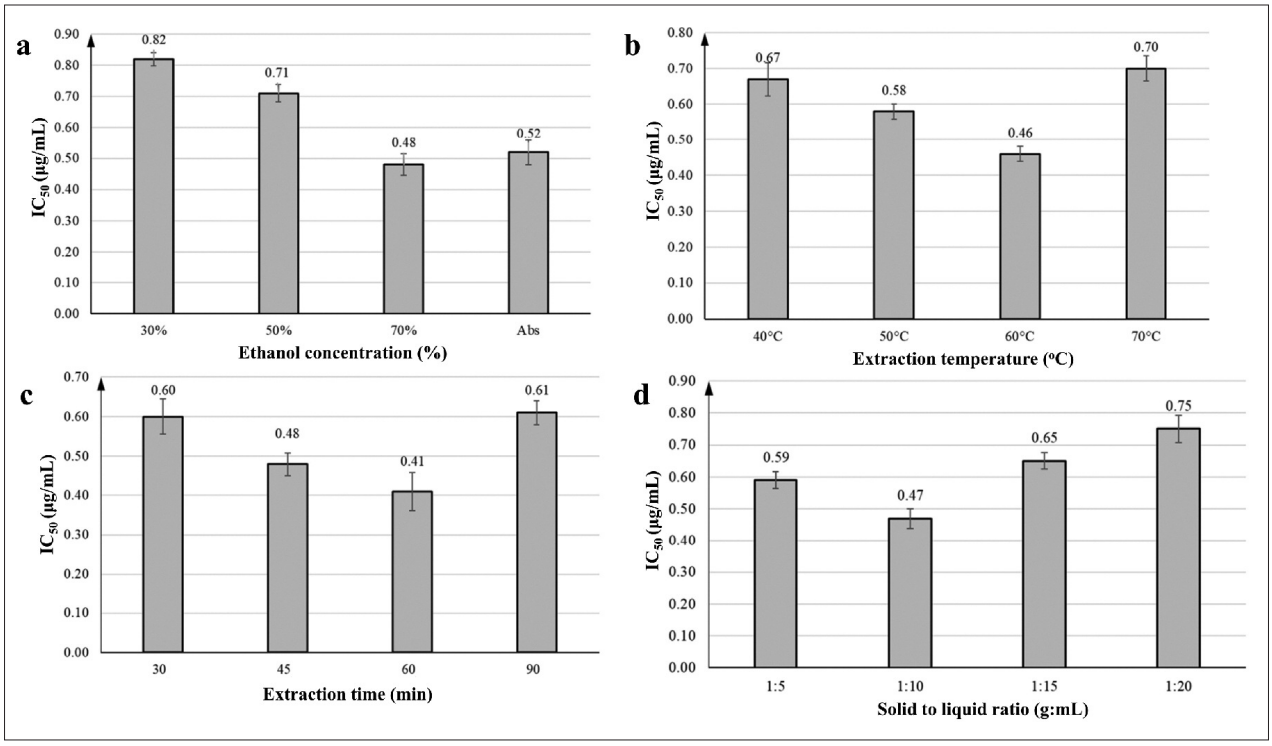

Table: The TFE, TPE, α-glucosidase inhibitory, Antioxidation, anti-inflammatory activities of enriched-flavonoids extracts of *P. barbatum*

Quantity	Sample	
TFE (mg QUE/g)	292.8 ± 4.74	
TPE (mg GAE/g)	351.08 ± 4.12	
Bioactivities	**Sample (IC_{50})**	**Positive control (IC_{50})**
α-glucosidase inhibitory (µg/mL)	0.45 ± 0.025	9.81 ± 0.16 (Acarbose)
Antioxidation (µg/mL)	6.87 ± 0.195	2.75 ± 0.16 (Ascorbic acid)
Anti-inflammatory (µg/mL)	4.75 ± 0.62 *	7.75 ± 0.53* (Diclofenac)
*IC_{20} value		

4. Conclusions

The present study indicates the extraction conditions for anti-α-glucosidase activities from an ethanolic extract of P. barbatum leaves. The optimal extracts were obtained at an ethanol concentration of 70%, extracting temperature of 60°C, the heating time of 1 hour, and solid to liquid ratio of 1:10. The extracts are effective in the inhibition of α-glucosidase activity with an IC_{50} value of 0.45 µg/mL, lower than 20 times the IC_{50} value of acarbose (9.81 µg/mL). In addition, it has more bioactivities such as strongly antioxidation capacities, and anti-inflammatory ■

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TỐI ƯU HÓA KHẢ NĂNG ỨC CHẾ ENZYME α-GLUCOSIDASE TRONG DỊCH CHIẾT CỦA *POLYGONUM BARBATUM L.*

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TÓM TẮT:

Mục tiêu của nghiên cứu này là tối ưu hóa các điều kiện chiết xuất của cây *P.barbatum* để tối đa khả năng ức chế α-glucosidase. Có 4 yếu tố được khảo sát, bao gồm: nồng độ etanol (30, 50, 70, 100%), nhiệt độ chiết (40, 50, 60, 70^{o}C), thời gian chiết (30, 45, 60, 90 phút) và tỷ lệ rắn trên dung môi (1: 5, 1:10, 1:15 và 1:20, g: mL). Kết quả cho thấy điều kiện chiết xuất thích hợp cho khả năng ức chế α-glucosidase tốt nhất ở nồng độ ethanol 70%, nhiệt độ chiết 60^{o}C, thời gian chiết 60 phút, và tỷ lệ rắn trên dung môi là 1:10 g: mL. Chiết xuất tối ưu thể hiện hoạt động ức chế α-glucosidase tốt với nồng độ ức chế IC_{50} là 0,45 µg/mL, hoạt tính kháng oxy hóa với IC_{50} là 6,87 µg/mL và hoạt tính chống viêm với IC_{20} là 4,75 µg / mL. Các kết quả cho thấy chiết xuất có tiềm năng điều trị bệnh tiểu đường.

Từ khóa: tiểu đường, α-glucosidase, dược liệu, *Polygonum Barbatum.*