DOI: https://doi.org/10.62985/j.huit_ojs.vol25.noS2_ICA.230

INVESTIGATION OF ANTIOXIDANT, α-AMYLASE, α-GLUCOSIDASE INHIBITORY AND ANTIHYPERGLYCEMIC POTENTIALS FROM *Physalis angulata* L. EXTRACT

Ngo Van Duc, Truong Ngoc Thien Kim, Bui Nguyen Ngoc Ngan, Dinh Thuy Ngoc Anh, Nguyen Ngoc Ngoan, Tran Hoang Ngau*

Ho Chi Minh City University of Industry and Trade

*Email: *ngauth@huit.edu.vn*

Received: 18 May 2023; Revised: 19 June 2023; Accepted: 5 July 2023

ABSTRACT

Diabetes mellitus is a global health issue, and the prevalence of this disease has constantly increased worldwide. The commercial medications used for diabetes in current therapy are limited by their side effects and cost. There is an urgent need to discover antidiabetic agents with minimal or no side effects derived from traditional medicinal plants. *Physalis angulata* L. is a tropical herbal plant that treats various diseases, including hepatitis, asthma, rheumatism, and malaria. This study investigated the components present in the extract using gas chromatography-mass spectrometry. It evaluated the *in vitro* antioxidant potential, the α-amylase and α-glucosidase inhibitory properties, along with the *in vivo* hypoglycemic activity of *Physalis angulata* L. The results revealed the presence of twenty-six phytochemicals in the extract, with 5-Hydroxymethylfurfural being the most abundant constituent at a percentage area of 29.74%. In the DPPH scavenging assay, the IC_{50} value of the extract was measured as 962.8 µg/mL. The α-amylase and α-glucosidase inhibitory activities were evaluated, yielding IC_{50} values of 390.82 µg/mL and 90.82 µg/mL, respectively. The extract significantly decreased blood glucose levels by 48.29% and 47.25% at 200 mg/kg and 400 mg/kg, respectively. In addition, the ethanol extract demonstrated blood glucose reduction following the oral glucose tolerance test in a diabetic mouse model. These findings suggest that the ethanol extract from *Physalis angulata* L. possesses antioxidant, α-amylase, and α-glucosidase inhibitory activities and anti-hyperglycemic characteristics, making it a consideration for managing diabetes complications and therapeutic purposes.

Keywords: Physalis angulata, antioxidant, α-amylase, antidiabetic, hypoglycemic, root extract.

1. INTRODUCTION

Diabetes mellitus is a metabolic disorder characterized by high glucose concentration, resulting from insulin deficiency and impaired insulin activity. Like other serious diseases, diabetes complications are caused by many factors such as free radical generation, oxidative stress, physical inactivity, tobacco smoking, or genetic and epigenetic factors [1, 2]. These severe symptoms of diabetes consist of weight loss, blurry vision, slow-healing sores, extreme fatigue, etc. The crucial purposes of therapy are to minimize and control blood pressure, lipid, and glycemic disorders to normal. However, synthetic medication treatment may possess many disadvantages related to ineffective drugs, toxicity, and side effects [3, 4]. The present therapy focuses on an alternative solution from natural resources, which are affordable, effective, and have fewer side effects than synthetic medications. In traditional herbal medicine, many plants

possess a variety of hypoglycemic, antioxidant constituents applied to treat diabetes [5, 6]. *Physalis angulata* L. *(P. angulata*) is widely recognized as an herbal remedy for various diseases, including hepatitis, asthma, rheumatism, and malaria, and it serves as a source of phytochemicals, particularly phenolic acids, flavonoids, alkaloids, and antioxidant agents [7, 8]. These phytochemicals impart biological properties such as antioxidant, anti-inflammatory, anti-parasitic, and antitumor activities [9-11]. Research has identified extracts from the fruits and whole plants of *P. angulata* as potent hypoglycemic agents [12, 13]. However, to date, no studies have reported on the hypoglycemic activity of this extract in a streptozotocin-induced diabetic mouse model. Therefore, the present study investigates the compositions in the ethanol root extract of *P. angulata*, its in vitro antioxidant potential, and its inhibitory activities against α-amylase and α-glucosidase, as well as its in vivo anti-hyperglycemic effects in a streptozotocin-induced diabetic mouse model.

2. MATERIALS AND METHODS

2.1. Sample collection

The roots of *P. angulata* were obtained from plants growing in their natural habitat in Hamlet 3, So Pai village, Kbang district, Gia Lai province, Vietnam. The roots were collected, and all contaminants were removed before being stored at 4 °C for analyses. Healthy male Swiss albino mice (20-35 g, 3-4 weeks old) were sourced from the Institute of Drug Quality Control, Ho Chi Minh City, Vietnam. Streptozotocin (STZ), diphenyl picryl hydrazine (DPPH), ascorbic acid, α-amylase, α-glucosidase, and 4-Nitrophenyl β-D-glucopyranoside (PNPG) were sourced from Sigma Aldrich (St. Louis, MO, USA). Glucose (20%) was acquired from Fresenius Kabi Vietnam Company, Vietnam. Other chemicals were of analytical grade.

2.2. Animals

Male Swiss albino mice were housed in cages measuring 15 x 30 x 35 cm, under a 12-hour light/12-hour dark cycle and a temperature of 28 °C ± 3 °C. The mice were fed commercial pellets from Laboratory Animals of the Institute of Tropical Biology in Thu Duc District, Ho Chi Minh City. Tap water was provided to ensure the animals remained hydrated. Each mouse in a cage had adequate access to food and water throughout the experiment. The procedures involving the animals were conducted following the Guidelines for the Care and Use of Laboratory Animals from the Institute of Tropical Biology in Ho Chi Minh City. After two weeks of acclimatization, the mice were utilized for the experiments.

2.3. Preparation of plant extract

After harvesting, *P. angulata* roots were carefully washed with distilled water for 10 minutes to remove any residual contaminant particles. They were then dried in the shade for three days at room temperature ranging from 25 to 30 °C, with a humidity level of 80-90%, before being sliced into smaller pieces. Next, they were dried at 40 °C for 2 hours in a hot air machine (UF55 Memmert, Germany) and ground into a powder using a blender (OS-1500 Osako, Vietnam). At room temperature, one kilogram of the powder was macerated in ten liters of 98% ethanol for 24 hours. The extract was filtered through filter paper (Number 102, Newstar, China). The filtrate was evaporated under reduced pressure using a rotavapor apparatus (CA-1115-CE Eyela, Japan) at 40 °C. Finally, the extract was lyophilized in a lyophilizer (MDF-C8V1 Series Panasonic, Japan) under reduced pressure and stored at -20 °C for further experiments.

2.4. 2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging method

The radical scavenging ability of *P. angulata* was assessed using the DPPH assay, as previously described with modifications [14]. A weight of 1 mg of *P. angulata* extract was mixed with 1 mL of dimethyl sulfoxide (DMSO) and vortexed for 15 minutes to prepare the working solution. A hundred microliters of two-fold dilutions (15.625, 31.25, 62.5, 125, 250, 500) µg/mL of the extract were combined with the DPPH solution (0.1 mM) in a 1:1 (v/v) ratio in each well of a 96-well microplate. After a 30-minute dark incubation at room temperature, the absorbance was measured at 540 nm using the Microtiter plate reader (HumaReader HS, Germany). Ascorbic acid served as a positive control. The percentage of DPPH radical scavenging activity was calculated as follows: DPPH radical scavenging activity (%) = [(Abs control – Abs samples) / Abs control] x 100, where Abs control is the absorbance of the control (DPPH without the extract) and Abs samples are the absorbance of the extract. The IC50 values were estimated using simple linear regression. These experiments were conducted in triplicate.

2.5. Alpha-amylase inhibition assay

The inhibitory potential of *P. angulata* extract against α-amylase was obtained as previously described [15]. A volume of 100 µL of the test samples (1000, 500, 250, 125, 62.5 µg/mL) was dissolved in 100 µL of phosphate-buffered saline (PBS) (0.1 M, pH 6.9) and added to 100 µL of α-amylase enzyme (3 U/mL) in the mixture. The samples were then incubated at 37 °C for 15 minutes, and the reaction was stopped by adding 500 µL of 1% 3,5-dinitrosalicylic acid, 0.2% phenol, 0.05% sodium sulfite, and 1% sodium hydroxide. The samples were boiled at 100 °C for 10 minutes, and then 200 µL of a 40% potassium sodium tartrate solution was added; afterward, the samples were cooled to room temperature. DMSO and acarbose served as the negative control and the standard reference drug, respectively. A blank was set with the concentration of the extract without the enzyme solution. The absorbance was measured at 540 nm. The percentage of the inhibitory effect on α-amylase was calculated using the following formula: Inhibition of α-amylase (%) = [(Abs control – Abs samples)/Abs control] x 100. The IC_{50} values were calculated from the dose-response curve. These experiments were conducted in triplicate.

2.6. Alpha-glucosidase inhibition assay

The inhibitory potential of *P. angulata* extract against α-glucosidase was obtained as previously described [16]. A volume of 40 µL of the test samples (1000, 500, 250, 125, 62.5 µg/mL) was dissolved in 10 µL of phosphate buffered saline (0.1 M, pH 6.8) and added to 20 µL of α-glucosidase enzyme (0.1 U/mL) in the mixture. Then, the samples were incubated at 37 °C for 15 minutes. After incubation, 20 µL of PNPG (1 mM) was added to the samples and incubated at 37 °C for another 15 minutes. The reaction was stopped by adding 80 µL of sodium carbonate (0.2 M). DMSO and acarbose served as the negative control and the standard reference drug, respectively. A blank was set with the concentration of the extract without the enzyme solution. The absorbance was measured at 405 nm. The percentage of the inhibitory effect of α-amylase was calculated using the following formula: Inhibition of α-glucosidase (%) = [(Abs control – Abs samples)/Abs control] x 100. The IC_{50} values were calculated from the dose-response curve. These experiments were performed in triplicate.

2.7. Induction of diabetes streptozotocin (STZ)

Diabetes mellitus in male Swiss albino mice in this study was induced according to previous research with some alterations [17]. Fifteen male Swiss albino mice were divided into two groups: the normal control group (NC) (n = 3) and the high-fat diet (HFD) group (n = 12). In the NC group, mice received a standard diet consisting of commercial pellets (21% protein, 5% cellulose, 6% mineral mixtures, 5% fat), while the HFD group was fed a high-fat diet (19.3% protein, 26.5% carbohydrate, and 45.6% fat). After six weeks, multiple low doses of STZ (40 mg/kg, dissolved in 0.05 M cold sodium citrate buffer at pH = 4.5) were injected into each mouse of the HFD group intraperitoneally for three consecutive days. Seventy-two hours later, the mice were tested for hyperglycemia using a glucometer (OGCare, Italy) with disposable test strips. Blood glucose (BG) levels and body weight in the NC and HFD groups were recorded at the end of each week in the morning during the experimental period. Blood glucose levels exceeding 200 mg/dL in the HFD group confirmed the induction of diabetes for the animals in this study.

2.8. Experimental design

The model mice were randomly divided into five groups, with each group consisting of three mice (n = 3). The first group was labeled as normal control mice (NC) and was allowed free access to a standard commercial pellet diet for 4 weeks. The second group, labeled diabetic control mice (DM), was given phosphate saline buffer. Standard acarbose drugs at a dose of 100 mg/kg (p.o.) were administered to the third group. The fourth and fifth groups received the *P. angulata* extract at doses of 200 mg/kg and 400 mg/kg (p.o.), respectively. Blood glucose levels and body weight were measured on the 0th, 7th, 14th, 21st, and 28th days of treatment.

2.9. Oral glucose tolerance test

The oral glucose tolerance test (OGTT) was conducted as described [18]. The OGTT was performed on the 1st day (before treatment) and the 28th day (after treatment) for each mouse in five experimental groups. After an 18-hour overnight fast, tail vein blood samples were collected for glucose measurement at 0, 30, 60, and 120 minutes following glucose administration (2 g/kg, p.o.).

2.10. Gas chromatography-mass spectrometry (GC-MS) analysis

Gas chromatography-mass spectrometry analyses of the root extract were conducted at the Center for Analytical Services and Experimentation in Ho Chi Minh City, Vietnam. The root extract was analyzed using the Gas Chromatography Trace 1310 system (Thermo Scientific, USA), equipped with a capillary column that is 30 m long, 250 µm in diameter, and 0.25 µm thick. Helium gas served as the carrier gas at a constant flow rate of 1,5 mL/min. An injection volume of 1 µL was utilized at 280 °C. The cycle was set at 70 °C per minute, increasing at 15 °C per minute, with the final temperature raised to 300 °C for 10 minutes. The phytochemicals present in the root extract were examined by comparing retention times, area percentages, and mass spectral patterns with the library database of identified constituents stored in the National Institute of Standards and Technology (NIST) library [19].

2.11. Statistical analysis

Statistical analyses were done by using GraphPad Prism 9 software (USA). Results were expressed as Mean ± Standard Deviation (SD). All results were analyzed using unpaired

Student's t-tests and one-way or two-way analysis of variance (ANOVA) tests. The statistically significant differences were determined at $P \leq 0.05$ (*), $P \leq 0.01$ (**), $P \leq 0.001$ (***), $P \leq 0.0001$ (****).

3. RESULTS AND DISCUSSION

3.1. Phytochemical analysis

As expected, several compounds were identified in the ethanol root extract. Twenty-six compounds were detected from the Gas chromatography-mass spectrometry analysis of *P. angulata* extract from roots (Table 1). There were nineteen compounds identified by the retention time, molecular formula, weight, and area (%), and seven unknown chemicals were present in this extract. The major constituents were 5-Hydroxymethylfurfural (29.74%), 9,12-Octadecadienoic acid (Z, Z)- (12.51%), Hexadecanoic acid (11.41%), 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl- (5.65%), Linolenic acid (5.61%), and Bis(2-ethylhexyl) phthalate (4.42%). Others were traced at low levels. All these major constituents might be responsible for the biological activities of the root extract of *P. angulata*.

Table 1. Compositional profile of *P. angulata* ethanol extract

No	Name of compounds	Molecular formula	Molecular weigtht (g/mol)	Retention time (RT)	Area (%)
1	Dihydroxyacetone	$C_3H_6O_3$	90.078	3.08	0.89
2	Unknown	-	-	4.86	3.48
3	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	$C_6H_8O_4$	144.12	5.66	5.65
4	5-Hydroxymethylfurfural	$C_6H_6O_3$	126.11	6.44	29.74
5	Unknown	-	-	6.6	2.3
6	Butyl acetoxyacetate	$C_8H_{14}O_3$	158.19	7.0	3.6
7	2-Hydroxy-2-methylsuccinic acid	$C_5H_8O_5$	148.11	7.51	0.81
8	Unknown	-	-	7.96	0.6
9	p-Anisaldehyde, 3-hydroxy-	$C_8H_8O_3$	152.15	8.06	0.35
10	Tropine	$C_8H_{15}NO$	141.21	8.3	1.3
11	10-Methyl-E-11-tridecen-1-ol propionate	$C_{17}H_{32}O_2$	268.4	8.66	1.88
12	Alpha-1-rhamnopyranose	$C_6H_{12}O_5$	164.16	10.06	0.48
13	Unknown	-	-	10.14	0.29
14	1-Isobutyl-7,7-dimethyl-octahydro-isobenzofuran-3a-ol	$C_{14}H_{26}O_2$	226.35	10.35	1.4
15	Unknown	-	-	10.51	0.92
16	Unknown	-	-	10.57	1.35
17	Tigloidine	$C_{13}H_{21}O_2$	223.31	10.62	1.86
18	Acetophenone, 4'-hydroxy-3',5'-dimethoxy-	$C_{11}H_{15}NO_4$	225.24	10.75	0.67
19	Ethyl p-methoxycinnamate	$C_{12}H_{14}O_3$	206.24	10.98	0.73
20	Hexadecanoic acid	$C_{16}H_{32}O_2$	256.42	12.34	11.41

21	9,12-Octadecadienoic acid (Z,Z)-	$C_{18}H_{32}O_2$	280.4	13.46	12.51
22	Linolenic acid	$C_{18}H_{30}O_2$	278.4	13.5	5.61
23	Stearic acid	$C_{18}H_{36}O_2$	284.5	13.63	1.57
24	Bis(2-ethylhexyl) phthalate	$C_{24}H_{38}O_4$	390.6	15.76	4.42
25	Terephthalic acid, bis(2-ethylhexyl) ester	$C_{24}H_{38}O_4$	390.6	16.86	3.0
26	Unknown	-	-	17.28	3.17

3.2. Antioxidant capacity

The antioxidant activity was estimated using a DPPH assay for various concentrations of the ethanol extract from the root of *P. angulata* and ascorbic acid in Figure 1. The percentage of scavenging activity of the ethanol extract was determined at various concentrations: 15.625 (9.93 ± 6.79%), 31.25 (11.27 ± 7.52%), 62.5 (13.17 ± 6.48%), 125 (15.67 ± 5.79%), 250 (20.61 ± 3.17%) and 500 µg/mL (30.58 ± 3.54%) (Figure 1A). The half maximal inhibitory concentration IC_{50} values of *P. angulata* extract and ascorbic acid were 962.8 µg/mL and 3.93 µg/mL, respectively (Figure 1B). Our research showed that P. angulata extract had weak antioxidant activity compared to a reference standard. In previous studies, the DPPH scavenging percentage of inhibition for solvent extracts of *P. angulata* leaves ranged from 80.9% to 98% values [20, 21]. The antioxidant potential of the extract was low compared with other results because of the different plant parts of *P. angulata* used for the isolation assays, such as leaves, fruits, and stems in this study.

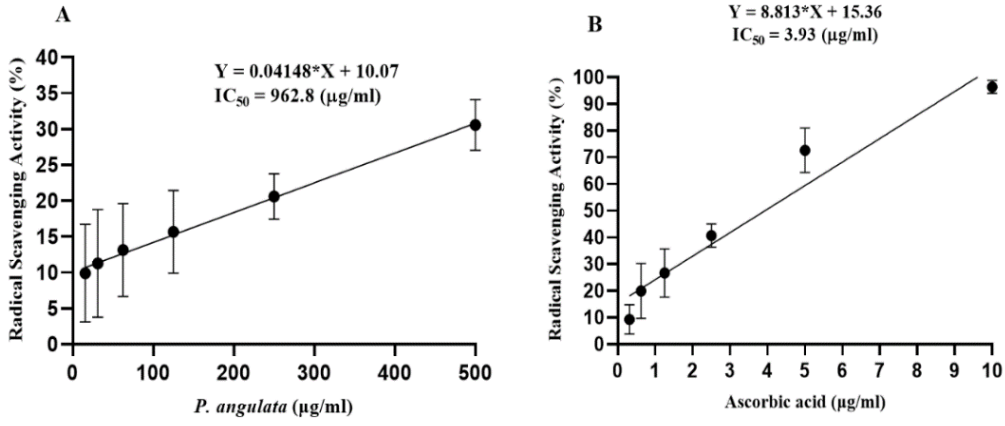

Figure 1. Antioxidant activities of *P. angulata* extract. (A). DPPH free radical scavenging activity of *P. angulata* extract. (B). Antioxidant potential of ascorbic acid. Data were expressed as mean ± SD of three independent experiments.

3.3. Inhibitory effect on α-amylase activity

The α-amylase inhibitory activity of the ethanol root extract was measured (Figure 2). As shown in Figure 2A, the percentage inhibition of pancreatic α-amylase ranged from 19.09 ± 4.25% to 59.26 ± 5.21% following the tested concentrations. The IC_{50} values for α-amylase inhibition of *P. angulata* and acarbose were 390.82 µg/mL and 30.41 µg/mL, respectively (Figure 2A-2B). These results demonstrated that the inhibitory activity of *P. angulata* extract and standard was obtained against α-amylase. The α-amylase inhibitory property exhibited in the ethanol extract was lower than that of the reference drug. It could be owing to the different interactions and affinities between the biological compounds from the extract and the active

site residues of α-amylase [22]. These findings aligned with other observations from *P. angulata* extract against α-amylase from various parts such as leaves, stems, or roots [23, 24]. According to these reports, the IC50 value of the root extract possessed a higher effect against α-amylase in the present study. Thus, it can be assumed that the *in vitro* inhibitory effect of *P. angulata* on pancreatic α-amylase could be related to secondary metabolites. According to the phytochemicals analyses, 5-Hydroxymethylfurfural and 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl- are one of the major compounds presented in the extract and might play the role as enzyme inhibitors against α-amylase [25, 26].

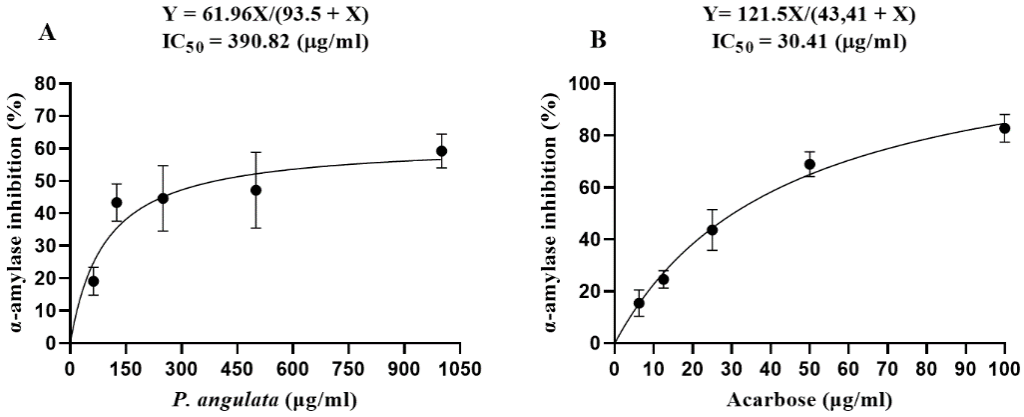

Figure 2. The inhibitory effect of *P. angulata* extract on pancreatic α-amylase. (A). Percentage inhibition of α-amylase activity of *P. angulata* extract. (B). Acarbose (positive control). Data were expressed as mean ± SD of triplicate determinations.

3.4. Inhibitory effect on α-glucosidase activity

In the current study, the α-glucosidase inhibition property of *P. angulata* extract was assessed in Figure 3. The percentage inhibition of α-glucosidase of the ethanol root extract ranged from 36.22 ± 7.19% to 88.69 ± 9.03% at the tested concentrations (Figure 3). The IC_{50} values for α-glucosidase inhibition of *P. angulata* and acarbose were 90.82 and 17.1 µg/mL, respectively (Figure 3A-3B). Inhibition of α-glucosidase activity is one of the most promising targets for treating diabetes mellitus related to starch digestion and glucose absorption. Several studies showed that the plant extracts played the role of α-glucosidase inhibitors to control hyperglycemia for the management of diabetes [27, 28]. These results were in line with other studies that showed the significant inhibition of the extract of *P. angulata* from stems [24, 29]. Nguyen reported that the IC_{50} value for α-glucosidase inhibition of the root extract was 3.674 mg/mL [24]. Our data showed a lower IC_{50} value against α-glucosidase of the ethanol extract, and as a result, the ethanol extract had a higher inhibitory potential against α-glucosidase in the current study. Hence, the root extract exhibited a remarkable inhibition against the α-glucosidase enzyme due to the presence of secondary metabolites acting as α-glucosidase inhibitors such as 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl- [26].

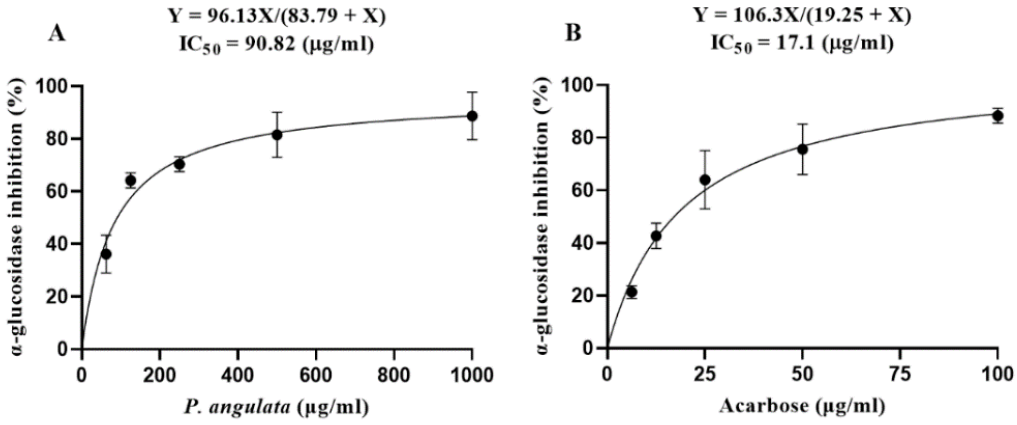

Figure 3. The inhibitory effect of *P. angulata* extract on α-glucosidase. (A). Percentage inhibition of α-glucosidase activity of *P. angulata* extract. (B). Acarbose (positive control). Data were expressed as mean ± SD of triplicate determinations.

3.5. Effect of *P. angulata* extract on blood glucose levels and body weight in STZ induced diabetic mice

Blood glucose (BG) levels and body weight were measured in Figure 4 over four weeks. These results indicated that BG levels from the different concentrations of *P. angulata* extract significantly reduced from 271.3 ± 9.07 mg/dL to 140.3 ± 7.37 mg/dL at 200 mg/kg and from 288.7 ± 56.89 mg/dL to 152.3 ± 15.89 mg/dL at 400 mg/kg after oral administration (Figure 4A). Conversely, the BG levels were significantly higher in the diabetic control group at 330.7 ± 76.27 mg/dL compared to the normal control group at 119.7 ± 11.68 mg/dL during the experimental period. The BG levels of acarbose used as the standard drug also decreased from 299.7 ± 68.16 mg/dL to 150.7 ± 34.93 mg/dL, with a reduction of 49.72%. Therefore, the BG levels of the treated diabetic groups with *P. angulata* extract decreased by 48.29% at 200 mg/kg and 47.25% at 400 mg/kg, respectively. This study indicated that twenty-eight days of oral administration of *P. angulata* extract significantly reduced BG levels in diabetic mice. Additionally, the results demonstrated that doses of 200 and 400 mg/kg remarkably decreased BG levels compared to acarbose at 100 mg/kg. Our results align with previous reports showing the anti-hyperglycemic activity of the fruit juice from *P. angulata* in streptozotocin-induced diabetic rats [12]. These data suggest that the fruit extract led to a reduction in BG levels with the oral gavage of 1 and 2 mL of fruit juice each day for two weeks. This could be attributed to the different parts of *P. angulata* that may contain similar secondary metabolites used for hypoglycemic agents in diabetes treatment.

Body weights were estimated in each group, as shown in Figure 4B. The extract treatment reduced body weights by 18.09% at 200 mg/kg and 22.29% at 400 mg/kg, respectively. The normal control group exhibited an increase of 13.15% over four weeks, while a slight reduction of 13.15% occurred in the diabetic control group without treatment. Body weight in the acarbose treatment increased by 7.9%. The mechanism behind streptozotocin-induced diabetes involves the selective inhibition of a glucose transporter, leading to DNA alkylation and resulting in pancreatic β-cell toxicity [30]. Consequently, severe body weight loss occurred due to the induction of diabetes in animals, attributed to changes in energy consumption or metabolic disorders in glucose or fat. Thus, this explains why there was decreased body weight in diabetic groups, which may require further investigation into various concentrations and treatment durations.

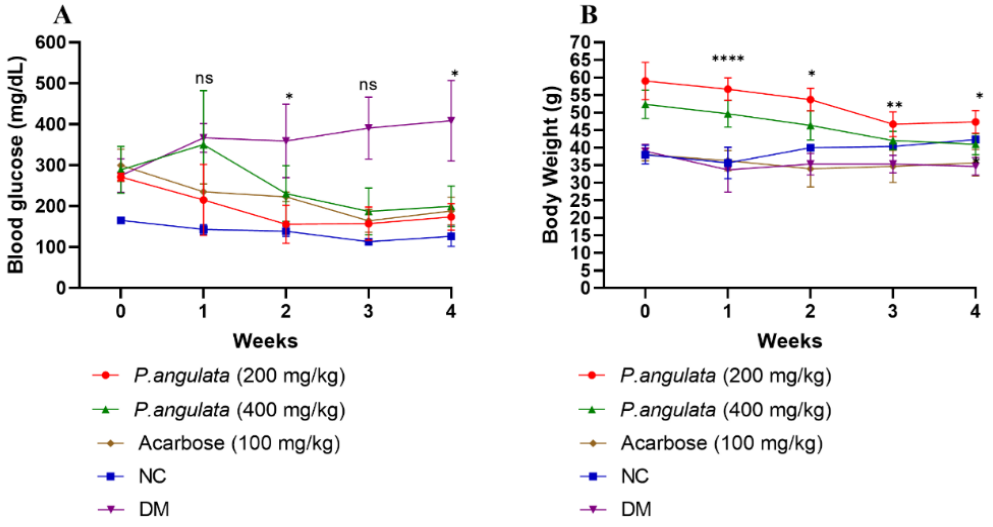

Figure 4. Effect of *P. angulata* extract on blood glucose levels and body weight. (A). Blood glucose levels. (B). Body weight. *P. angulata* (200 mg/kg): diabetic mice treated with *P. angulata* extract (200 mg/kg) (n = 3, red line), *P. angulata* (400 mg/kg): diabetic mice treated with *P. angulata* extract (400 mg/kg) (n = 3, green line), Acarbose (100 mg/kg): diabetic mice treated with acarbose (100 mg/kg) (n =3, brown line), NC: normal control mice treated with vehicle (n = 3, blue line), DM: diabetic mice treated with sodium citrate buffer (n = 3, purple line). Values were presented as mean ± SD. Statistical differences were evaluated by two-way ANOVA, $*P \leq 0.05$, $**P \leq 0.0001$, ns means not significant (compared to normal control group).

3.6. Effect of *P. angulata* extract on oral glucose tolerance test

The oral glucose tolerance tests for the experimental groups are shown in Figure 5.

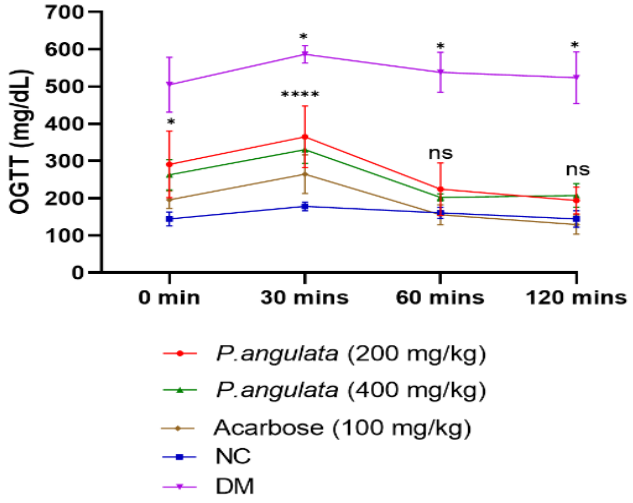

Figure 5. Effect of *P. angulata* extract on blood glucose after glucose loading. *P. angulata* (200 mg/kg): diabetic mice treated with *P. angulata* extract (200 mg/kg) (n = 3, red line), *P. angulata* (400 mg/kg): diabetic mice treated with *P. angulata* extract (400 mg/kg) (n = 3, green line), Acarbose (100 mg/kg): diabetic mice treated with acarbose (100 mg/kg) (n =3, brown line), NC: normal control mice treated with vehicle (n = 3, blue line), DM: diabetic mice treated with sodium citrate buffer (n = 3, purple line). Values were presented as mean ± SD. Statistical differences were analyzed by one- and two-way ANOVA, $*P \leq 0.05$, ns mean not significant (compared to normal control group).

In the negative control (NC) mice, the blood glucose (BG) level rose to 178 ± 11.79 (mg/dL) after 30 minutes of glucose administration and decreased to 144.3 ± 22.12 (mg/dL) after 120 minutes. In the diabetic group without treatment (DM), the values peaked at 586.3 ± 23.67 mg/dL after 30 minutes and were maintained at 538.3 ± 53.69 mg/dL and 523.7 ± 69.83 mg/dL at the 60- and 120-minute mark, respectively. Animals treated with *P. angulata* extract exhibited a significant decrease in BG levels after 120 minutes. Specifically, the BG level dropped from 365 ± 83.07 mg/dL to 194 ± 36.35 mg/dL at the concentration of 200 mg/kg, while BG levels decreased from 330.3 ± 37.17 mg/dL to 207.3 ± 31.79 mg/dL at 400 mg/kg. Thus, the reduction observed following the extract treatment highlights the effects of *P. angulata* on impaired glucose tolerance, further confirming the extract's ability to enhance insulin sensitivity in diabetic animals.

4. CONCLUSION

In conclusion, this study investigated that the ethanol extract from the root of *P. angulata* L. exhibits antioxidant activity and concentration-dependent inhibitory potentials against the α-amylase and α-glucosidase enzymes in vitro. This ethanol extract significantly reduced blood glucose levels in vivo in high-fat diet and multiple low-dose streptozotocin-induced diabetic mice. The phytochemicals from *P. angulata* L. extract also contain a diverse range of nineteen identified constituents and seven unknown compounds that may be responsible for the biochemical properties of this plant. Consequently, these results provide consistent evidence for further research into novel chemicals and their underlying mechanisms from *P. angulata* L. for application in pharmacy.

Acknowledgments: This research was supported by Ho Chi Minh City University of Industry and Trade (HUIT) (under grant number: 249/HD-DCT).

REFERENCES

1. Swanston-Flatt, S. K., Day, C., Bailey C. J. and Flatt P. R. - Traditional plant treatments for diabetes. Studies in normal and streptozotocin diabetic mice, Diabetologia **33** (8) (1990) 462-4. https://doi.org/10.1007/BF00405106
2. Mobasseri, M., Shirmohammadi, M., Amiri, T., Vahed, N., Fard, H. H. and Ghojazadeh, M. - Prevalence and incidence of type 1 diabetes in the world: a systematic review and meta-analysis, Health Promot Perspect **10** (2) (2020) 98-115. https://doi.org/10.34172/hpp.2020.18
3. Inzucchi, S.E., Bergenstal, R., M., Buse, J. B., Diamant, M., Ferrennini E., Nauck M., Peters, A., L., Tsapas, A., Wender, R., and Matthews, D., R. - Management of hyperglycemia in type 2 diabetes: a patient-centered approach: position statement of the American Diabetes Association (ADA) and the European Association for the Study of Diabetes (EASD), Diabetes Care **35** (6) (2012) 1364-79. https://doi.org/10.2337/dc12-0413
4. Pandey, A., Tripathi, P., Pandey, R., Srivatava R., and Goswami, S. - Alternative therapies useful in the management of diabetes: A systematic review, Journal of Pharmacy Bioallied Science **3** (4) (2011) 504-12. https://doi.org/10.4103/0975-7406.90103
5. Khatune, N. A., Rahman, B. M., Barman, R. K., and Wahed, M. I. I. - Antidiabetic, antihyperlipidemic and antioxidant properties of ethanol extract of *Grewia asiatica Linn.* bark in alloxan-induced diabetic rats, BMC Complementary and Alternative Medicine **16** (1) (2016) 295. https://doi.org/10.1186/s12906-016-1276-9

6. Nain, P., Saini, V., Sharma, S., and Nain, J. - Antidiabetic and antioxidant potential of Emblica officinalis Gaertn. leaves extract in streptozotocin-inducad type-2 diabetec mellitus (T2DM) rats, Journal of Ethnopharmacology **142** (1) (2012) 65-71. https://doi.org/10.1016/j.jep.2012.04.014
7. Moreira, G. C., dos Anjos, G. L., Carneiro, C. N., Ribas, R. F., and Dias F. d. S.- Phenolic compounds and photosynthetic activity in *Physalis angulata* L. (Solanaceae) in response to application of abscisic acid exogenous, Phytochemistry Letters **40** (2020) 96-100. http://dx.doi.org/10.1016/j.phytol.2020.09.018
8. Hsieh, W. T., Huang, K. Y., Lin, H. Y. and Chung, J. G. - *Physalis angulata* induced G2/M phase arrest in human breast cancer cells, Food and Chemical Toxicology **44** (2006) 974-983. https://doi.org/10.1016/j.fct.2005.11.013
9. Wang, L., Lu, S., Wang, L., Xin, M., Xu, Y., Wang, G., Chen, D., Chen, L., Liu, S., and Zhao, F. - Anti-inflammatory effects of three withanolides isolated from *Physalis angulata* L. in LPS-activated RAW 264.7 cells through blocking NF-κB signaling pathway, Journal of Ethnopharmacology **276** (2021) 114186. https://doi.org/10.1016/j.jep.2021.114186
10. da Silva, R. R., da Silva, B. J., Rodrigues A. P., Farias, L. H., da Silva M. N., Alves, T., Bastos G. N., do Nascimento, J. L., and Silva, E. O. - In vitro biological action of aqueous extract from roots of *Physalis angulata* against Leishmania (Leishmania) amazonensis, BMC Complement Alternaltive Medicine **15** (2015) 249. https://doi.org/10.1186/s12906-015-0717-1
11. Hsu, C. C., Wu, Y. C., Du, Y. C., Tseng, W. K., Wu, C. C., and Chang, F. R. - Physalin B from *Physalis angulata* triggers the NOXA-related apoptosis pathway of human melanoma A375 cells, Food and Chemical Toxicology **50** (3) (2012) 619-624. https://doi.org/10.1016/j.fct.2011.12.017
12. Iwansyah, A.C., Luthfiyanti, R., Ardiansyal, R. C. E., Rahman, N., Andriana, Y., and Hamid, H. A. - Antidiabetic activity of *Physalis angulata* L. fruit juice on streptozotocin-induced diabetic rats, South African Journal of Botany **145** (2022) 313-319. https://doi.org/10.1016/j.sajb.2021.08.045
13. Abo, K. and Lawal, I. J. o. A. S. R. - Antidiabetic activity of *Physalis angulata* extracts and fractions in alloxan-induced diabetic rats, Journal of Advanced Scientific Research **4** (4) (2013) 32-36.
14. Mariadoss, A. V. A., Park, S., Saravanakumar, K., Sathiyaseelan, A., and Wang, M. H. - Phytochemical profiling, in vitro antioxidants, and antidiabetic efficacy of ethyl acetate fraction of *Lespedeza cuneata* on streptozotocin-induced diabetic rats, Environment Science Pollution Research Intitutes **30** (21) (2023) 60976-60993. https://doi.org/10.1007/s11356-023-26412-8
15. Yang, J. Y., Park, J. H., Chung, N., and Lee, H. S. - Inhibitory Potential of Constituents from *Osmanthus fragrans* and Structural Analogues Against Advanced Glycation End Products, α-Amylase, α-Glucosidase, and Oxidative Stress, Scientific Reports **7** (1) (2017) 45746. https://doi.org/10.1038/srep45746
16. Mettupalayam Kaliyannan Sundaramoorthy, P. and Kilavan Packiam, K. - In vitro enzyme inhibitory and cytotoxic studies with *Evolvulus alsinoides* (Linn.) Linn. Leaf extract: a plant from Ayurveda recognized as Dasapushpam for the management of Alzheimer's disease and diabetes mellitus, BMC Complementary Medicine and Therapies **20** (1) (2020) 129. https://doi.org/10.1186/s12906-020-02922-7

17. Matboli, M., Eissa, S., Ibrahim, D., Hegazy, M. G. A., Imam, S. S., and Hanin, E. K. - Caffeic acid attenuates diabetic kidney disease via modulation of autophagy in a high-fat diet/streptozotocin- induced diabetic rat, Scientific Reports **7** (1) (2017) 2263.
18. Zhang, X., Jin, Y., Wu, Y., Zhang, C., Jin, D., Zheng, Q., and Li, Y. - Anti-hyperglycemic and anti-hyperlipidemia effects of the alkaloid-rich extract from barks of *Litsea glutinosa* in ob/ob mice, Scientific Reports **8** (1) (2018) 12646. https://doi.org/10.1038/s41598-017-02320-z
19. Ralte, L., Khiangte, L., Thangjam, N. M., Kumar, A., and Singh, Y. T. - GC–MS and molecular docking analyses of phytochemicals from the underutilized plant, *Parkia timoriana* revealed candidate anti-cancerous and anti-inflammatory agents, Scientific Reports **12** (1) (2022) 3395. https://doi.org/10.1038/s41598-022-07320-2
20. Ramakrishna Pillai, J., Wali, A. F., Menezes, G. A., Rehman, M. U., Wani, T. A., Arafah, A., Zargar, S., and Mir, T. M. - Chemical Composition Analysis, Cytotoxic, Antimicrobial and Antioxidant Activities of *Physalis angulata* L.: A Comparative Study of Leaves and Fruit, Molecules **27** (5) (2022). https://doi.org/10.3390/molecules27051480
21. Lima, L. G. B., Mantenegro, J., Abreu, J. P., Santos, M. C. B., Nascimento, T. P. D., Santos, M. D. S., Ferreira, A. G., Cameron, L. C., Ferreira, M. S. L. - Metabolite profiling by UPLC-MS(E), NMR, and antioxidant properties of amazonian fruits: mamey apple *(Mammea americana),* camapu *(Physalis angulata),* and uxi *(Endopleura uchi),* Molecules **25** (2) (2020). https://doi.org/10.3390/molecules25020342
22. Lim, J., Ferruzzi, M. G., and Hamaker, B. R. - Structural requirements of flavonoids for the selective inhibition of α-amylase versus α-glucosidase, Food Chemistry **370** (2022) 130981. https://doi.org/10.1016/j.foodchem.2021.130981
23. Olusola, B. O. and Adeseye, R. E. I. - In Vitro Antioxidant Alpha-amylase and Alpha Glucosidase Inhibitory Properties of Ethanol Extract of *Physalis angulata L.,* Conference Academic Union of Polytechnic zone **2020** (2020).
24. Nguyen, M. C., and Duong, D. D. - Inhibitory effects of ground cherry extracts (*Physalis angulata* L.) on α-amylase and α- glucosidase activities, CTU Journal of Science **55** (2019) 126-132. https://doi.org/10.22144/ctu.jsi.2019.053
25. Shapla U. M., Solayman Md., Alam N., Khalil M. I., and Gan S. H. – 5-Hydroxymethylfurfural (HMF) levels in honey and other food products effects on bees and human health, Chemistry Central Journal **12** (1) (2018) 1-18. https://doi.org/10.1186/s13065-018-0408-3
26. Rizve, S. N. R., Afzal, S., Khan, K. U. R., Aati, H. Y., Rao, H., Ghalloo, B. A., Shahzad, M. N., Khan, D. A., Esatbeyoglu, T., and Korma, S. A. – Chemical Characterisation, Antidiabetic, Antibacterial, and In Silico Studies for Different Extracts of Haloxylon stocksii (Boiss) Benth: A promising halophyte, Molecules **28** (9) (2023) 3847. https://doi.org/10.3390/molecules28093847
27. Bhatia, A., Singh, B., Arora, R., and Arora, S. - In vitro evaluation of the α-glucosidase inhibitory potential of methanolic extracts of traditionally used antidiabetic plants, BMC Complementary and Alternative Medicine **19** (1) (2019). https://doi.org/10.1186/s12906-019-2482-z
28. Choo, C. Y., Sulong, N. Y., Man, F., and Wong T. W. - Vitexin and isovitexin from the leaves of *Ficus deltoidea* with in-vivo α-glucosidase inhibition, Journal of Ethnopharmacology **142** (3) (2012) 776-781. https://doi.org/10.1016/j.jep.2012.05.062

29. Tjajaindra, A., Sari, A. K., Simamora, A., Timotius, K. H. J. M., and Sciences, C. B. - The stem infusate and ethanol extract of *Physalis angulata* inhibitory activities against a-glucosidase and xanthine oxidase, Molecular and Cellular Biomedical Sciences **5** (3) (2021) 115-20. http://dx.doi.org/10.21705/mcbs.v5i3.211

30. Szkudelski T. - The mechanism of alloxan and streptozotocin action in B cells of the rat pancreas. Physiol Research **50** (6) (2001) 537-46.

TÓM TẮT

ĐÁNH GIÁ HOẠT TÍNH CHỐNG OXY HÓA, ỨC CHẾ ENZYME α-AMYLASE, α-GLUCOSIDASE VÀ KHẢ NĂNG HẠ ĐƯỜNG HUYẾT CỦA CAO CHIẾT RỄ CÂY TẦM BÓP *Physalis angulata* L.

Ngô Văn Đức, Trương Ngọc Thiên Kim, Bùi Nguyễn Ngọc Ngân,
Định Thùy Ngọc Anh, Nguyễn Ngọc Ngoan, Trần Hoàng Ngâu*
Trường Đại học Công Thương Thành phố Hồ Chí Minh
*Email: *ngauth@huit.edu.vn*

Bệnh tiểu đường là một vấn đề sức khỏe toàn cầu và tỉ lệ bệnh này không ngừng tăng lên trên thế giới. Hiện tại, các loại thuốc điều trị tiểu đường bị hạn chế do tác dụng phụ hay giá thành điều trị. Một nhu cầu cần thiết là tìm kiếm các hợp chất có tác dụng điều trị tiểu đường ít hay không gây tác dụng phụ từ các cây dược liệu. Cây tầm bóp *Physalis angula* L. là một trong các cây dược liệu vùng nhiệt đới được dùng điều trị các bệnh như viêm gan, hen suyễn, thấp khớp và sốt rét. Nghiên cứu này tiến hành đánh giá thành phần hóa học của các hợp chất hiện diện trong cao chiết của cây tầm bóp và khảo sát hoạt tính sinh học trong điều kiện in vitro bao gồm chống oxy hóa, hoạt tính ức chế enzyme α-amylase và α-glucosidase và thử nghiệm khả năng hạ đường huyết trên mô hình in vivo chuột bị gây đái tháo đường. Bằng phương pháp sắc ký khí khối phổ, có 26 chất hóa học hiện diện trong thành phần cao chiết bao và hợp chất có hàm lượng cao nhất là 5-Hydroxymethylfurfural với 29,74%. Hoạt tính chống oxy hóa của cao chiết được xác định bằng phương pháp DPPH với giá trị nồng độ ức chế 50% (IC_{50}) là 962,8 µg/mL. Hoạt tính ức chế enzyme α-amylase và α-glucosidase xác định với giá trị IC_{50} lần lượt là 390,82 µg/mL và 90,82 µg/mL. Khả năng hạ đường huyết của các lô chuột điều trị uống cao chiết trên mô hình in vivo chuột bị gây đái tháo đường tại 200 mg/kg và 400 mg/kg lần lượt giảm đáng kể là 48,29% và 47,25% so với lô không điều trị. Ngoài ra, đường huyết của các lô chuột điều trị uống cao chiết cũng giảm đáng kể trong thử nghiệm dung nạp glucose. Kết quả thu nhận đã chứng minh cao chiết rễ cây tầm bóp với các hoạt tính chống oxy hóa, ức chế α-amylase và α-glucosidase, hạ đường huyết có thể được đánh giá là ứng viên tiềm năng cho các nghiên cứu liên quan đến điều trị tiểu đường.

Từ khóa: Physalis angulata, chống oxy hóa, ức chế α-amylase, tiểu đường, hạ đường huyết, cao chiết rễ.