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Investigation of the Anti-Diabetic and Antioxidant Activities of *Physalis angulata* Extract

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ABSTRACT

Physalis angulata (PA) is a plant under the *Solanaceae* family playing an important role in the remedy of inflammatory symptoms such as asthma, hepatitis, dermatitis, rheumatism and treatment of several health disorders, such as cold, cough, fever, pain, malaria and nervous diseases. Besides, *P. angulata* has been used as a traditional medicine for amelioration of hyperglycemia. Thus, this study aimed to investigate mechanism of action of *P. angulata* for its anti-diabetic activity using an *in vitro* model.

Ethanol extract of PA was prepared under the condition of ethanol 98% and ratio of ¼ (w/v) for 4 hours at 60°C. The concentrations of 50, 100, and 200 µg/mL of PA extract were used for all assays. The alpha-amylase inhibition was investigated via dinitrosalicylic acid assay. Glucose uptake was determined using LO-2 cell model. Radical scavenging activity was performed via 2,2-diphenyl-1-picryl-hydrazyl-hydrate (DPPH) and 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) $(ABTS)^+$ assays. Nitric oxide production was measured by Griess reaction, while cell viability was examined by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay.

The result showed that PA extract was able to inhibit alpha-amylase activity up to 56.6 ± 4.7% at the concentration of 200 µg/mL. Moreover, PA possessed glucose adsorption and glucose uptake capacities up to 2.2 ± 0.18 mM glucose/g extract and 156 ± 10.1%, respectively. In addition, PA extract scavenged 52.6 ± 3.5% DPPH and 59.7 ± 2.6% $ABTS^+$ radicals and reduced NO production to 34.2 ± 3.8% from RAW264.7 cells.

Consequently, *P. angulata* can be suggested as a pharmaceutical ingredient for the development of anti-diabetic agent.

***Keywords*:** Medicinal plant, Alpha-amylase, *Physalis angulata*, Biological activity, Glucose uptake.

Introduction

Diabetes is a common endocrine disorder that is characterized by chronic hyperglycemia due to dysregulation primarily of carbohydrate metabolism and deficiency of insulin secretion and insulin action.[1] Indeed, diabetes can be managed via proper diet, exercise and pharmacologic interventions.[2] Various drugs have been used for the treatment of diabetes by lowering blood glucose through different mechanisms. However, drugs for diabetes treatment have some disadvantages such as stomach upset, skin rash or itching, weight gain, and diarrhoea.[3] Nowadays, herbal plants have been recommended and preferred as a natural source of alternative medicine for the treatment of diabetes.[4] They are considered to be safe, effective, and less toxic and with strong antioxidant activity.[4,5]

Physalis angulata L. belongs to the *Solanaceae* family, which is locally named as "Tầm bóp, thù lù, lồng đèn" in Vietnam and found in the Brazilian Amazon Forest and other tropical countries of Africa, America and Asia. It grows up to 1 m with a small stem, cream-coloured flowers and light yellowish-orange edible fruits wrapped by a layer of leaves. Fruits from the *P. angulata* are used as food in several countries. *P. angulata* has an important role in the remedy of inflammatory conditions such as asthma, hepatitis, dermatitis, rheumatism and treatment of several health disorders, such as cold, cough, fever, pain, malaria and neurological diseases.[6-8] Recently, the phytochemistry of *P. angulata* has been reported and found to contain glucocorticoids, flavonoids, withanolides and physalins.[9] Similarly, numerous biological activities of *P. angulata* such as antinociceptive, immunosuppressive, anti-protozoal, antineoplastic and anti-inflammatory effects have been determined.[9] So far, *P. angulata* has been locally used as a traditional medicine for the remedy of hyperglycemia in Vietnam. Moreover, the anti-diabetic activity of *P. angulata* fruit has been demonstrated recently.[10] However, the studies regarding antidiabetic activity of the leaves and stem of *P. angulata* are still limited. Therefore, the present study was designed to investigate the role of the leaves and stem of *P. angulata* on anti-diabetic activity using an *in vitro* model.

Materials and Methods

Materials

The leaves and stem of *Physalis angulata* were collected from Tay Ninh province, Vietnam in March, 2019. *P. angulata* was authenticated by Do Huy Bich *et al*.[11] Alpha-amylase from *Bacillus licheniformis* (A4582) was purchased from Sigma-Aldrich (USA). Ethanol was purchased from Xilong (China). Acarbose and metformin were purchased from Long Chau pharmacy store at district 7, Ho Chi

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Minh City, Vietnam. All other reagents were purchased from Sigma–Aldrich (St. Louis, MO, USA).

Extraction
The plant materials including the leaves and stem of *P. angulata* were air-dried under the shade and powdered by a grinder. The powder (100 mg) was soaked in 10 mL of ethanol 98%. The mixture was maintained for 4 h at 60°C and the supernatant was collected after centrifugation. The ethanol extract was achieved after evaporation and kept at 4°C for future use.

Alpha-amylase inhibitory assay
The α-amylase inhibitory assay was performed as described by Bhutkar and Bhise.[12] Briefly, 1 mL of extract (50, 100, and 200 µg/mL) or acarbose (100 µg/mL) was pre-incubated with 1 ml of α-amylase (A4582, diluted for 10.000 in 20 mM sodium phosphate buffer, pH 6.9) for 30 min. Afterwards, 1 mL of starch solution (10 mg/mL) was added and incubated for 10 min at 37°C. Subsequently, 1 mL of DNS reagent was added and heated for 5 min. The absorbance was measured at 540 nm using a spectrophotometer (Genova Nano, Jenway, UK). The percentage of inhibition was calculated by the following equations (OD = optical density):

$$\alpha\text{-amylase inhibition (\%)} = \frac{[(ODC-ODB) - (ODT - ODB)}{ODC-ODB} \times 100\%$$

Where; C = starch 1% + alpha-amylase + distilled water, B = extract + alpha-amylase + distilled water, T = starch 1% + alpha-amylase + extract + distilled water

Determination of glucose adsorption capacity
The glucose adsorption capacity of extract was determined according to Ou *et al*.[13] The extract was added to 25 mL of glucose solution (10, 50, or 100 mM) to achieve a final extract concentration of 10 mg/mL. The mixture was mixed well, incubated in a shaker water bath at 37°C for 6 h, centrifuged at 4,000 g for 20 min and the glucose content in the supernatant was determined. The glucose adsorption was calculated using the following formula (G1 is the glucose concentration of the original solution; G2 is the glucose concentration after 6 h):

Glucose adsorption = [(G1 – G2) x Volume of mixture]/1 g extract

Glucose uptake in normal human hepatic (LO-2) cells
The glucose uptake in normal human hepatic (LO-2) cell line was determined as described by van de Venter *et al*.[14] In brief, LO-2 cells ($1x10^4$ cells/mL) were treated with the extract (50, 100, and 200 µg/mL) or metformin (20 µg/mL) for 48 h. The cell culture supernatant was then replaced by a 50 µL incubation buffer containing 8 mM of glucose and further maintained for 3 h at 37°C. Glucose concentration in the supernatant was measured by using a glucometer (Contour™ Plus Meter, Ascensia Diabetes Care, Switzerland). The amount of glucose uptake was calculated as a percentage compared to control C (The untreated cell group). The amount of glucose uptake was calculated using the following formula (T is glucose concentration in the supernatant of the treated cell group, while C is glucose concentration in the supernatant of the untreated cell group):

Glucose uptake (%) = [(8 – T)/(8 – C)]x100

1,1-Diphenyl-2-picryl-hydrazyl (DPPH) radical scavenging assay
The antioxidant activity of the extract was determined by the 1,1-diphenyl-2-picryl-hydrazyl (DPPH) radical scavenging assay as described by Vo *et al*.[15] Briefly, 100 µL of the extract (50, 100, and 200 µg/mL) was mixed with 100 µL of DPPH (0.3 mM) solution and incubated in the dark at room temperature for 30 min. The absorbance of the mixture was then measured at 490 nm by using a spectrophotometer (Genova Nano, Jenway, UK). Vitamin C (20 µg/mL) was used as a reference. The ability of the sample to scavenge DPPH radical was determined from:

DPPH scavenging effect (%) = $[(OD_{control} - OD_{extract})/OD_{control}] \times 100\%$

Azinobis-3-Ethyl benzothiazoline-6-sulfonic acid (ABTS) assay
This assay was performed as described by Vo *et al*.[15] Briefly, 0.9 mL of $ABTS^+$ solution was mixed with 0.1 mL of the extract (50, 100, and 200 µg/mL) or vitamin C (20 µg/mL) for 45 sec. The antioxidant effect of the extract was calculated with the following equation:

ABTS scavenging effect (%) = $[(OD_{control} - OD_{extract})/OD_{control}] \times 100\%$

Nitric oxide (NO) production assay
In order to investigate NO production, the RAW 264.7 macrophage cells were pre-treated with PA extract (50, 100, and 200 µg/mL) before stimulation with lipopolysaccharide (0.5 µg/mL) for 24 h. The NO level in the culture supernatant was measured by the Griess reaction as described by Vo *et al*.[16] Briefly, the mixture of 100 µL of cell culture medium and 100 µL of Griess reagent was incubated at room temperature for 10 min. The absorbance was then measured at 540 nm. The levels of NO production were calculated as a percentage compared to that of control (without extract treatment).

Release ratio (%) = (T – B)/(C – B) × 100

Where; B = extract; C = LPS; T = extract + LPS.

Cell viability assay
The viability levels of hepatic LO-2 cells and RAW 264.7 macrophage cells were determined by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay. In brief, the cells ($1x10^5$ cells/mL) were incubated with the extract (50, 100, or 200 µg/mL) for 24 h. The medium was removed and the cells were incubated with a solution of 1 mg/mL MTT for 4 h. Finally, the supernatant was removed and DMSO was added to solubilize the formed formazan salt. The amount of formazan salt was determined by measuring the absorbance at 540 nm using a microplate reader (Tecan Austria GmbH, Grodig/Salzburg, Austria). The cell viability was calculated as a percentage compared to blank.

Statistical analysis
Data were analyzed using the analysis of variance (ANOVA) test of statistical package for the social sciences (SPSS). The statistical differences among groups were assessed by using Duncan's tests. Differences were considered significant at $p < 0.05$.

Results and Discussion

Alpha-amylase inhibitory activity of PA extract
Hyperglycemia is seen in the development of diabetes and causes various complications in diabetic patients. Hyperglycemia relates to the excess activity of alpha-amylase, a carbohydrate digestive enzyme in the gastrointestinal system.[17] Therefore, the inhibition of α-amylase has been considered to be an effective strategy to block or slow the carbohydrate absorption after food intake, contributing to the control of hyperglycemia in diabetic patients.[18] In this study, the ethanol extracts of *P. angulata* (PA) were examined for its capability against α-amylase activity. It was found that PA extract possessed significant inhibition on the α-amylase enzyme in a dose-dependent manner ($p < 0.05$) (Figure 1). The inhibitory effect was observed up to 56.6 ± 4.7% at a concentration of 200 µg/mL. Its activity was comparable with acarbose 59.0 ± 5.3% at a concentration of 100 µg/mL. Currently, acarbose, miglitol and voglibose are anti-diabetic drugs that act mainly by inhibiting enzymes related to carbohydrate digestion such as α-amylase, sucrase, maltase and α-glucosidase.[19] However, their usage have been limited due to their side-effects such as flatulence and diarrhea. Hence, the inhibitory effect of PA extract on α-amylase may partly attenuate the hyperglycemia in type 2 diabetes.

The glucose adsorption capacity of PA extract
Besides carbohydrate digestive enzyme inhibition, the glucose adsorption capacity of bioactive agents also contributes to the

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prevention of carbohydrate absorption after food intake. Various herbal plants (ginseng, bitter melon, fenugreek, banaba, *Gymnema sylvestre* and *Coptis chinensis*) have been found as glucose adsorption agents and exhibited hypoglycemic effect in type 2 diabetes.[20] From the present study, the glucose adsorption capacity of PA extract was investigated via measuring the remaining glucose after mixing the extract with the indicated concentrations of glucose. This assay revealed that 1% (10 mg/mL) of PA extract exhibited glucose adsorption capacity at different glucose concentration (Figure 2). The adsorption capacity of the extract was directly proportional to the glucose concentration. The amount of glucose bound increased with increased glucose concentration. In particular, the adsorption capacity of PA was up to 0.30 ± 0.09 mM/g extract, 0.90 ± 0.11 mM/g extract and 2.20 ± 0.18 mM/g extract at glucose concentration of 10, 50, and 100 mM, respectively. From the results, the α-amylase inhibitory activity and the glucose adsorption capacity of the PA extract may contribute to the reduction of postprandial hyperglycemia in type 2 diabetic patients.

Glucose uptake capacity of PA extract

Glucose uptake into the tissue is an important process for the regulation of glucose homeostasis. In type 2 diabetic patients, insulin resistance or deficiency leads to decrease glucose uptake and increase endogenic hepatic glucose production, thus leading to hyperglycemia.[21] Bioactive agents which can stimulate glucose uptake might play an important role in reducing hyperglycemia in type 2 diabetic patients.[22] In this study, the stimulatory effect of PA extract on glucose uptake in liver LO-2 cells was examined. As shown in Figure 3, the treatment of PA extract increased glucose uptake in LO-2 cells as compared to the control. PA extract enhanced glucose uptake up to 156.0 ± 10.1% at the concentration of 200 µg/mL. Meanwhile, metformin significantly stimulated glucose uptake up to 197.0 ± 11.3%. Although this action was not as significant as that of metformin, the PA extract could still contribute to blood glucose-lowering effect when used as alternative medicine in diabetic patients.

Free radical scavenging activities of PA extract

Free radical causes oxidation of several cell components and biomolecules such as lipids, proteins and DNA by its unpaired electron.[23] These free radicals are evidenced to be associated with many diseases, including diabetes and its complications.[24] Moreover, long term complications of diabetes are associated with various oxidative reactions, increased free radical generation and subsequent increase in oxidative stress.[25] Studies have shown that antioxidants play an important role in the prevention of the pathogenesis as well as complications of diabetes by neutralizing the elevated amount of free radicals.[26] Therefore, the antioxidant activity of PA extract was also investigated via measuring its scavenging ability on DPPH and $ABTS^+$ radicals (Figure 4). The result showed that PA extract scavenged DPPH and $ABTS^+$ radicals up to 52.6 ± 3.5% and 59.7 ± 2.6% at the concentration of 200 µg/mL, respectively. Meanwhile, vitamin C significantly scavenged ($p < 0.05$) the free radicals at the concentration of 20 µg/mL (81.8% for DPPH and 98% for $ABTS^+$). These result indicated that the free radical scavenging activity of vitamin C was stronger than that of PA extract. It was shown that various phenolic acids (ellagic acid, caffeic acid, chlorogenic acid, and gallic acid) and flavonoids (kaempferol, isoquercitrin, rutin, quercitrin, and quercetin) have been found in the PA extract.[27,28] It has been confirmed that these antioxidant components possess multiple biological effects and play important roles in the treatment and management of diabetic-related diseases.[29] Thus, the antioxidant activity of PA extract may have contributed to the plant's role in the treatment of diabetes and its complications.

Inhibitory effect of PA extract on nitric oxide (NO) production

It has been well documented that nitric oxide (NO) has several benefits in physiological and cellular functions. However, high levels of NO may cause cell damage due to generation of peroxynitrite radical.[30] According to Adela *et al*, high NO production was observed in diabetic rats and patients with hyperglycemia as compared to controls.[31] Moreover, it has been reported that NO may contribute to the development of diabetes complications, both microvascular and cardiovascular.[32] Thus, NO was also suggested as potential target for the management of diabetes-associated complications. In this study, PA extract-pretreated cells can significantly decrease the NO production levels in a dose-dependent manner ($p < 0.05$) (Figure 5). The NO production levels were reduced to 88.0 ± 5.0%, 49.0 ± 7.2% and 34.2 ± 3.8% at 50, 100 and 200 µg/mL, respectively. Ibuprofen was effective in reduction of NO production to 22.0 ± 6.3% at the concentration of 100 µg/mL. This inhibitory activity of PA extract on the NO production was observed to be similar with that of oolong tea (*Camellia sinensis*) and Red Ginger (*Zingiber officinale*).[33,34] Flavonoids possess inhibitory activity on the expression levels of iNOS mRNA and protein and subsequently reduce the production of NO in several cell lines.[35] Notably, the central mechanism down-regulating iNOS expression of flavonoids was suggested due to inactivation of nuclear factor kappa B (NF-kB) and mitogen activated protein kinases (MAPK).[36,37]

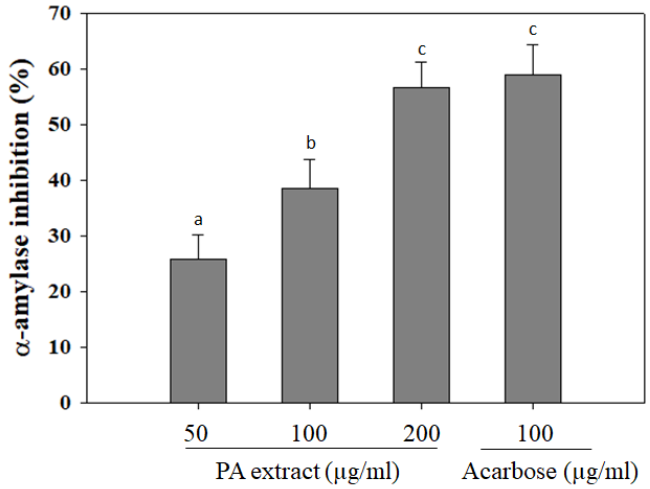

Figure 1: The inhibitory effect of *P. angulata* (PA) extract on α-amylase activity.
Acarbose was used as a reference. Each determination was made in three independent experiments and the data are shown as means ± SD. Different letters a–c indicate significant difference among groups ($p < 0.05$).

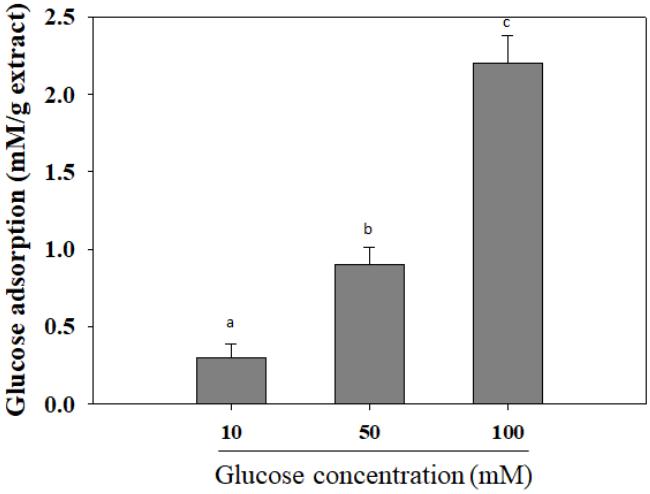

Figure 2: The capacity of *P. angulata* (PA) extract on glucose adsorption at the different glucose concentrations.
Each determination was made in three independent experiments and the data are shown as means ± SD. Different letters a–c indicate significant difference among groups ($p < 0.05$).

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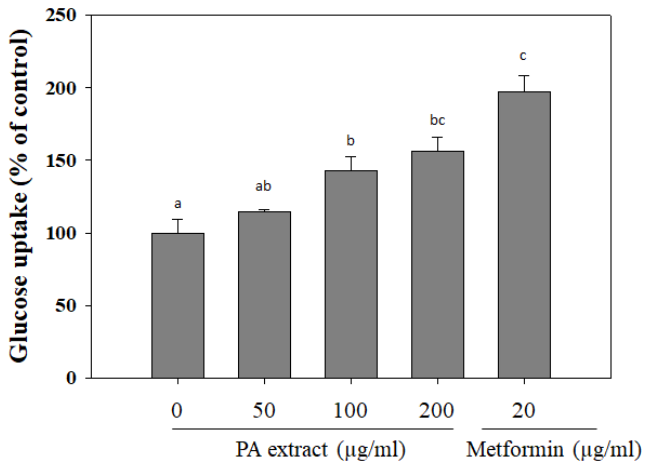

Figure 3: The stimulatory effect of *P. angulata* (PA) extract on glucose uptake in human hepatic LO-2 cells.
Metformin was used as a reference. The results were expressed as percentage of control. Each determination was made in three independent experiments and the data are shown as means ± SD. Different letters a–c indicate significant difference among groups ($p < 0.05$).

Flavonoids was suggested to be responsible for the inhibitory effect of the PA extract on NO production from RAW 264.7 cells. Subsequently, the free radical scavenging activity and NO production inhibition of PA extract may be useful for the prevention of complications in type 2 diabetes.

Effect of PA extract on cell viability

Many herbal plants have been known with therapeutic effects and can be used directly or in combination with other plants for medicinal purpose.[38] Besides, the knowledge related to the safety of these products is also important. Therefore, the preliminary studies related to the cytotoxic effect of herbal plants need to be evaluated to ensure their relatively safe use. For *in vitro* model, the MTT assay was used to measure cytotoxicity. This assay is based on the metabolic reduction of the soluble MTT salt, which reflects the normal function of mitochondria dehydrogenase activity and cell viability. Herein, the MTT assays were performed on hepatic LO-2 cells and RAW 264.7 macrophage cells pre-treated with different concentration of PA extract for 24 h. The results showed that cell viability levels were obtained in a range of 93 – 103% for LO-2 cells and 102 – 105% for RAW 264.7 macrophage cells as compared with the blank (Figure 6). It indicated that PA extract has no cytotoxic effect on hepatic cells and macrophage cells at the concentrations of 50, 100, and 200 µg/mL.

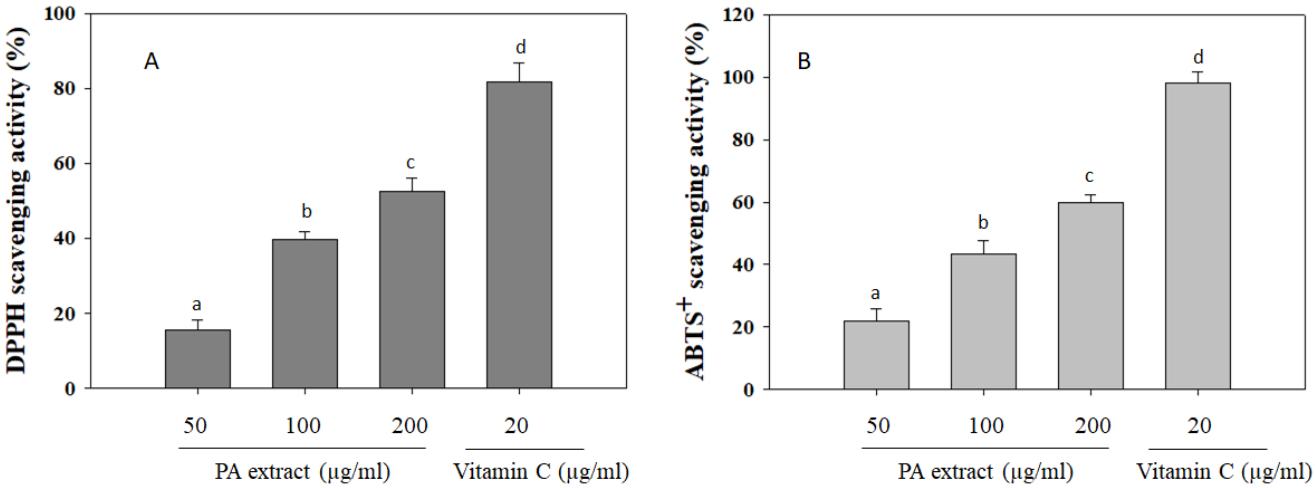

Figure 4: The antioxidant activity of *P. angulata* (PA) extract via scavenging DPPH (A) and $ABTS^+$ (B) radicals.
Vitamin C was used as a reference. Each determination was made in three independent experiments and the data are shown as means ± SD. Different letters a–d indicate significant difference among groups ($p < 0.05$).

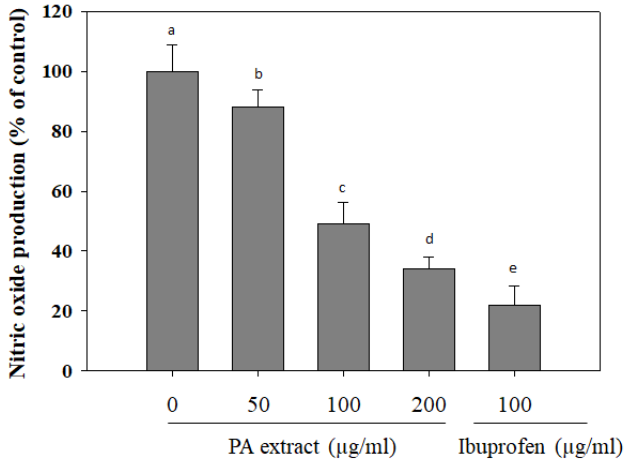

Figure 5: Inhibitory effect of *P. angulata* (PA) extract on NO production from LPS-stimulated RAW 264.7 cells.
Ibuprofen was used as a positive control. The results were expressed as percentage of control. Each determination was made in three independent experiments and the data are shown as means ± SD. Different letters a–e indicate significant difference among groups ($p < 0.05$).

Conclusion

In conclusion, *P. angulata* extract is a potential anti-diabetic agent due to its inhibitory effect on starch digestive enzyme (α-amylase), possessing glucose adsorption and glucose uptake capacity, ability to scavenge free radicals and an inhibitory effect on NO production.
Hence, *P. angulata* could be suggested as a promising material for the development of pharmaceutical products that may have the ability to lower hyperglycemia and prevent diabetes-related complication. However, further studies related to the safety and efficacy of *P. angulata* are necessary.

Conflict of interest

The authors declare no conflicting interest

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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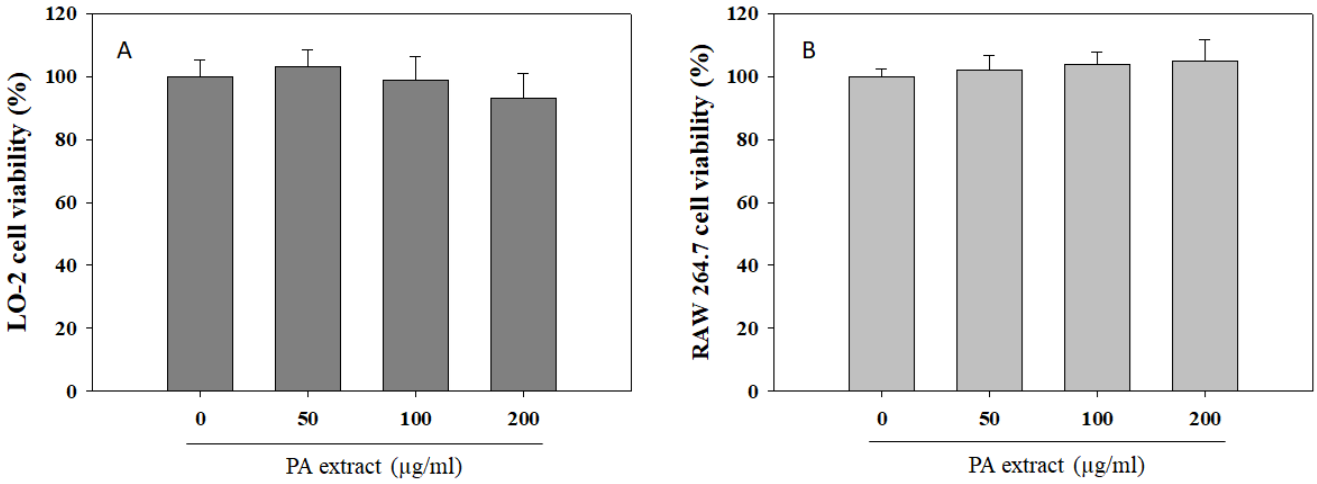

Figure 6: The cell viability of *P. angulata* (PA) extract on hepatic LO-2 cells (A) and RAW 264.7 macrophage cells (B).
Cell viability was assessed by MTT method and the results were expressed as percentage of surviving cells over blank cells. Each determination was made in three independent experiments and the data are shown as means ± SD.

Acknowledgements

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References

1. Kharroubi AT and Darwish HM. Diabetes mellitus: The epidemic of the century. World J Diabetes 2015; 6:850–867.
2. Li G, Zhang P, Wang J, Gregg EW, Yang W, Gong Q. The long-term effect of life style interventions to prevent diabetes in the China Da Qing diabetes prevention study: a 20-year follow-up study. Lancet 2008; 371:1783–1789.
3. Babiker A and Dubayee MA. Anti-diabetic medications: How to make a choice? Sudan J Paediatr. 2017; 17:11–20.
4. Kooti W, Farokhipour M, Asadzadeh Z, Ashtary-Larky D, Asadi-Samani M. The role of medicinal plants in the treatment of diabetes: a systematic review. Electron Physician 2016; 8:1832–1842.
5. George BO, Osioma E, Falodun A. Effect of Atiko (*Aframium Sceptrium*) and African Nutmeg (*Monodora Myristica*) on reduced glutathione Uric acid levels of liver marker enzymes in streptozotocin induced diabetic rats. Egy J Biochem Mol Biol. 2010; 28(2):67-78.
6. Mirzaee F, Hosseini AS, Askian R, Azadbakht M. Therapeutic activities and phytochemistry of *Physalis* species based on traditional and modern medicine. Res J Pharmacogn. 2019; 6:79-96.
7. Ruiz L, Ruiz L, Maco M, Cobos M, Gutierrez-Choquevilca AL, Roumy V. Plants used by native Amazonian groups from the Nanay River (Peru) for the treatment of malaria. J Ethnopharmacol. 2011; 133:917–921.
8. Bastos GN, Silveira AJ, Salgado CG, Picanço-Diniz DL, Nascimento JL. *Physalis angulata* extract exerts anti-inflammatory effects in rats by inhibiting different pathways. J Ethnopharmacol. 2008; 118:246–251.
9. Renjifo-Salgado E and Vargas-Arana G. *Physalis angulata*. L (*Bolsa Mullaca*): A review of its traditional uses, chemistry and pharmacology. Boletin Latino Americano Caribe Plant Med Aromat. 2013; 12:431–445.
10. Raju P and Mamidala E. Anti-diabetic activity of compound isolated from *Physalis angulata* fruit extracts in alloxan induced diabetic rats. The Ame J Sci Med Res. 2015; 1:40–43.
11. Do Huy Bich, Dang Quang Chung, Bui Xuan Chuong. Cây thuốc và động vật làm thuốc ở Việt Nam, Tập II. (1st ed.). Hà Nội: Nhà xuất bản Khoa học và Kỹ thuật; 2016; 792-793 p.
12. Bhutkar MA and Bhise SB. *In vitro* assay of alpha amylase inhibitory activity of some indigenous plants. Int J Chem Sci. 2012; 10:457–462.
13. Ou S, Kwok KC, Li Y, Fu L. *In vitro* study of possible role of dietary fiber in lowering postprandial serum glucose. J Agri Food Chem. 2001; 49:1026–1029.
14. van de Venter M, Roux S, Bungu LC, Louw J, Crouch NR, Grace OM, Maharaj V, Pillay P, Sewnarian P, Bhagwandin N, Folb P. Antidiabetic screening and scoring of 11 plants traditionally used in South Africa. J Ethnopharmacol. 2008; 119:81–86.
15. Vo TS, Le PU, Ngo DH. The increased gamma-aminobutyric acid content by optimizing fermentation conditions of bacteria from kimchi and investigation of its biological activities. EurAsian J BioSci. 2018; 12:369–376.
16. Vo TS, Le PU, Ngo DH. Free radical scavenging and anti-proliferative activities of avocado (*Persea americana* Mill.) seed extract. Asian Pac J Trop Biomed. 2019; 9:91–97.
17. Mahmood N. A review of α-amylase inhibitors on weight loss and glycemic control in pathological state such as obesity and diabetes. Comp Clin Path. 2016; 25:1253–1264.
18. Rehman K, Chohan TA, Waheed I, Gilani Z, Akash MSH. Taxifolin prevents postprandial hyperglycemia by regulating the activity of α-amylase: Evidence from an in vivo and in silico studies. J Cell Biochem. 2019; 120:425–438.
19. Agarwal P and Gupta R. Alpha-amylase inhibition can treat diabetes mellitus. J Med Health Sci. 2016; 5:1–8.
20. Prabhakar PK and Doble M. Mechanism of action of natural products used in the treatment of diabetes mellitus. Chin J Integr Med. 2011; 17:563–574.
21. Schinner S, Scherbaum WA, Bornstein SR, Barthel A. Molecular mechanisms of insulin resistance. Diabet Med. 2005; 22:674–682.
22. Xia EQ, Zhu SS, He MJ, Luo F, Fu C, Zou TB. Marine peptides as potential agents for the management of type 2 diabetes mellitus - A prospect. Mar Drugs 2017; 15:88.
23. Asmat U, Abad K, Ismail K. Diabetes mellitus and oxidative stress - A concise review. Saudi Pharm J. 2016; 24:547–553.
24. Chikezie PC, Ojiako OA, Ogbuji AC. Oxidative stress in diabetes mellitus. Int J Biol Chem. 2015; 9:92–109.

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

25. Singh R, Devi S, Gollen R. Role of free radical in atherosclerosis, diabetes and dyslipidaemia: larger-than-life. Diabetes Metab Res Rev. 2015; 31:113–126.
26. Zatalia SR and Sanusi H. The role of antioxidants in the pathophysiology, complications and management of diabetes mellitus. Acta Med Indones. 2013; 45:141–147.
27. Susanti RF, Kurnia K, Vania A, Reynaldo IJ. Total phenol, flavanoid and antioxidant activity of *Physalis angulata* leaves extract by subcritical water extraction. Mod Appl Sci. 2015; 9:190-198.
28. Akomolafe SA, Oyeleye SI, Olasehinde TA, Oboh G. Phenolic characterization, antioxidant activities, and inhibitory effects of *Physalis angulata* and *Newbouldia laevis* on enzymes linked to erectile dysfunction. Int J Food Prop. 2018; 21:645-654.
29. Aryaeian N, Sedehi SK, Arablou T. Polyphenols and their effects on diabetes management: A review. Med J Islam Repub Iran 2017; 31:1-14.
30. Pal P, Joseph SB, Lucas L. Nitric oxide and peroxynitrite in health and disease. Physiol Rev. 2007; 87:315–424.
31. Adela R, Nethi SK, Bagul PK, Barui AK, Mattapally S, Kuncha M, Patra CR, Reddy PN, Banerjee SK. Hyperglycaemia enhances nitric oxide production in diabetes: a study from south Indian patients. PLoS One 2015; 10:e0125270.
32. Giacco F and Brownlee M. Oxidative stress and diabetic complications. Circ Res. 2010; 107:1058–1070.
33. Novilla A, Djamhuri DS, Nurhayati B, Rihibiha DD, Afifah E, Widowati W. Anti-inflammatory properties of oolong tea (*Camellia sinensis*) ethanol extract and epigallocatechin gallate in LPS-induced RAW 264.7 cells. Asian Pac J Trop Biomed. 2017; 7:1005–1009.
34. Shimoda H, Shan SJ, Tanaka J, Seki A, Seo JW, Kasajima N, Tamura S, Ke Y, Murakami N. Anti-inflammatory properties of red ginger (*Zingiber officinale* var. Rubra) extract and suppression of nitric oxide production by its constituents. J Med Food 2010; 13:156–162.
35. Hämäläinen M, Nieminen R, Vuorela P, Heinonen M, Moilanen E. Anti-inflammatory effects of flavonoids: genistein, kaempferol, quercetin, and daidzein inhibit STAT-1 and NF-kappaB activations, whereas flavone, isorhamnetin, naringenin, and pelargonidin inhibit only NF-kappaB activation along with their inhibitory effect on iNOS expression and NO production in activated macrophages. Mediators Inflamm. 2007; 2007:1-10.
36. García-Mediavilla V, Crespo I, Collado PS, Esteller A, Sánchez-Campos S, Tuñón MJ, González-Gallego J. The anti-inflammatory flavones quercetin and kaempferol cause inhibition of inducible nitric oxide synthase, cyclooxygenase-2 and reactive C-protein, and down-regulation of the nuclear factor kappaB pathway in Chang Liver cells. Eur J Pharmacol. 2007; 557:221-229.
37. Li J, Xu L, Sang R, Yu Y, Ge B, Zhang X. Immunomodulatory and anti-inflammatory effects of total flavonoids of Astragalus by regulating NF-kB and MAPK signalling pathways in RAW 264.7 macrophages. Pharmazie 2018; 73:589-593.
38. Sen T and Samanta SK. Medicinal plants, human health and biodiversity: a broad review. Adv Biochem Eng Biotechnol. 2015; 147: 59–110.