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The Stem Infusate and Ethanol Extract of *Physalis angulata* Inhibitory Activities against α -Glucosidase and Xanthine Oxidase

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Background: Infusate of the whole plant of *Physalis angulata* is used traditionally for the remedy of various diseases including diabetes and gout. This study focused on the stem of *P. angulata*. The objectives of this study were to investigate the potential of the stem infusate (INPA) and ethanol extract (EEPA) of *P. angulata* as inhibitors of α -glucosidase and xanthine oxidase.

Materials and Methods: INPA and EEPA were determined for their α -glucosidase and xanthine oxidase inhibition activities *in vitro*, whereas antioxidant activity was determined by 2,2-diphenyl-1-picryl-hydrazyl-hydrate (DPPH) assay. Reference inhibitors were used for comparison. The total phenolic compounds were also estimated.

Results: EEPA had more concentrated phenolic than INPA which were 7.96 and 0.08 mgGAE/g dried biomass, respectively. INPA and EEPA inhibited α -glucosidase considerably, with IC₅₀ of 149.11 and 409.86 μ g/mL, respectively (acarbose was 130.66 μ g/mL). However, they inhibited xanthine oxidase weakly, with IC₅₀ of 0.546 and 2.643 mg/mL, respectively, compared with allopurinol 0.005 mg/mL. EEPA scavenged DPPH radicals very weakly (16.04 mg/mL) compared to BHT (0.021 mg/mL), whereas no activity was observed for INPA.

Conclusion: The stem infusate and ethanol extract of *P. angulata* are able to inhibit the activity of α -glucosidase, thus can be further explored for sources of bioactive compounds with α -glucosidase inhibition activity.

Keywords: α -glucosidase, infusate, ethanol extract, *Physalis angulata*, stem, xanthine oxidase

Introduction

Physalis angulata, (local name: ciplukan, nyoyoran, or cecendet) is a flowering plant of the family Solanaceae. It grows in the regions of South America and South East Asia. *P. angulata* is a shrub tree that can grow up to one meter. It has

yellowish bell-shaped flowers, and its fruits were covered in a lantern-shaped leaves. *P. angulata* is known for its various health benefits. Traditionally, infusate of *P. angulata* was reported to cure various diseases, such as asthma, malaria, hepatitis, and diabetes mellitus.¹ Pharmacological studies have been carried out to scientifically confirm the medicinal

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properties of *P. angulata*. Extracts of the aerial parts were reported for their antioxidant^{2,3}, antimicrobial⁴, antitumor⁵, and anti-inflammatory activities⁶⁻⁸.

Type 2 Diabetes mellitus (T2DM) is one of the global epidemics and is projected to affect 700 million by 2030.⁹ T2DM is a chronic metabolic disease characterized by high level of post prandial blood glucose. This can be caused by insulin resistance or impaired pancreatic β -cells or a combination of both. Therapeutic strategy in controlling post-prandial blood glucose level includes inhibition against enzymes responsible for the degradation of dietary polysaccharides into glucose in the intestine. Thus, α -glucosidase is targeted in the management of T2DM.

Cohort studies have demonstrated that hyperuricemia could increase the risk for developing T2DM.¹⁰ Hyperuricemia, a condition of high level of serum urate, may lead to gouty arthritis which is caused by the deposition of sodium urate in the joints. Xanthine oxidase is an enzyme involved in the oxidation of hypoxanthine to xanthine and from xanthine to uric acid. Inhibition on xanthine oxidase is the main therapeutic target in controlling serum urate.

As a home remedy, the whole plant of *P. angulata* is usually decocted or infused. However, the efficacy of the stem of *P. angulata* so far is not yet reported. Besides, studies are still few with regard to the antidiabetic and anti-hyperuricemia of the stem infusate of *P. angulata*. The present study describes the enzymes (α -glucosidase and xanthine oxidase) inhibitory and antioxidant activities of the stem extracts (infusate and ethanol extract) of *P. angulata*.

Materials and methods

Chemicals

Reagents used were of the highest purity, as follows: α -glucosidase from *Saccharomyces cerevisiae*, xanthine oxidase from bovine milk, 1,1-diphenyl-2-picrylhydrazyl (DPPH) radicals, *p*-nitrophenyl α -D-glucopyranoside, xanthine, acarbose, and allopurinol were obtained from Sigma-Aldrich (St Louis, USA). Solvents used were of analytical grade.

Sample Collection and Extract Preparation

P. angulata stems were collected from natural resources in Tangerang area (West Java) in May 2018. Stems were air-dried and pulverized and kept at 4°C until used. Infusate of *P. angulata* stem (INPA) was prepared by decocting the stem powder (5 g) in water (150 mL) at 90°C for 30 minutes.

Infusate was obtained by filtering the decoction, and the filtrate was freeze-dried to obtain solid material.

Ethanol extract of *P. angulata* (EEPA) was prepared by maceration of the stem powder (30.60 g) in ethanol for 72 h. The mixture was filtered through filter paper, and the filtrate was dried under reduced pressure using a rotary evaporator.

Stock solutions of INPA and EEPA were prepared by dissolving each dried filtrate in DMSO.

Determination of Total Phenolic Content (TPC)

INPA and EEPA were determined for their phenolic contents based on the Folin-Ciocalteu method as reported previously.¹¹ In brief, sample (0.5 mL) was mixed with Folin-Ciocalteu reagent (10% v/v, 2.5 mL), and left to stand for 10 mins. The mixture was then added with Na₂CO₃ (75 g/L, 2.5 mL) and incubated at room temperature for 2 h. The absorbance was measured at 765 nm. TPC was estimated using a gallic acid calibration curve (12.5–200 μ g/mL) and results were presented as mg gallic acid equivalent (mgGAE)/g dried biomass.

Determination of α -glucosidase Inhibition Activity

α -Glucosidase inhibition activity of INPA and EEPA was determined based on a reported method.¹¹ In this method, *p*-nitrophenyl glucopyranoside (*p*NPG) was used as a substrate and acarbose was used as a reference inhibitor. Sample of different concentrations (50 μ L) was mixed with α -glucosidase (0.5 U/mL, 50 μ L) and phosphate buffer (50 mM pH 6.8, 50 μ L). The mixture was pre incubated for 5 minutes at 37°C, thereafter *p*NPG (1 mM, 100 μ L) was added. The reaction mixture was further incubated for 20 minutes at 37°C. The reaction was interrupted by the addition of Na₂CO₃ (100 mM, 750 μ L) and the absorbance was measured on a spectrophotometer at 405 nm. Inhibition percentage was calculated by the following equation: $(A - B)/A \times 100\%$, where A is absorbance of control and B is absorbance of sample. Inhibition activity was presented as IC₅₀ value, calculated from a linear regression equation of inhibition % vs. sample concentration.

Determination of Xanthine Oxidase Inhibition Activity

Determination of xanthine oxidase inhibition activity was conducted following a reported method.¹² Xanthine was used as a substrate and allopurinol was used as a reference inhibitor. Sample of different concentrations (100 μ L) was mixed with xanthine oxidase (0.2 U/mL, 100 μ L) and

phosphate buffer (50 mM pH 7.4, 400 μ L). The mixture was pre incubated for 5 minutes at 37°C. Xanthine (0.3 mM, 200 μ L) was added to start the reaction and the reaction mixture was further incubated for 30 mins at 37°C. HCl (0.1 M, 200 μ L) was added to stop the reaction and the absorbance was read at 290 nm. Inhibition % was calculated as follows: $(A - B)/A \times 100\%$, where A is absorbance of control and B is absorbance of sample. Xanthine oxidase inhibition activity was presented as IC_{50} value which was calculated based on a regression equation (inhibition % vs. concentration).

Determination of DPPH Radical Scavenging Activity

Determination of DPPH radical scavenging activity was conducted according to a previous method.¹³ DPPH solution in ethanol (0.6 mM, 3 mL) was mixed with samples of different concentrations (1 mL in ethanol). The reaction mixture was incubated in the dark at room temperature for 30 mins, thereafter the absorbance was measured at 517 nm. The scavenging % was calculated by the following equation: $(A - B)/A \times 100\%$, where A is absorbance of control and B is absorbance of sample. DPPH radical scavenging activity was presented as IC_{50} , calculated from a regression equation of scavenging % plotted against sample concentrations.

Statistical Analysis

All experiments were conducted in three repetitions. Data were presented as mean \pm SD. Significance difference between samples were analysed using one-way ANOVA

(analyzed by an SPSS v 23 (IBM Corporation, Armonk, NY, USA)) followed by a Post Hoc test (Tukey test). Values of $p < 0.05$ were treated as significant.

Results

TPC

The TPC of INPA and EEPA were estimated by a spectrophotometric method, based on the complex formation of Prussian blue complex of $[(P\text{MoW}_{11}\text{O}_4)^{4-}]$ with phenolic compounds in the extracts.¹⁴ Linear regression of gallic acid standard curve obtained a good correlation coefficient with $R^2 = 0.998$ ($y = 0.008x + 0.034$).

EEPA was found to have higher content of phenolic (7.96 \pm 0.02 mgGAE/g dried biomass) compared to INPA (0.08 \pm 0.00 mgGAE/g dried biomass).

α -Glucosidase Inhibition Activity

Both INPA and EEPA inhibited α -glucosidase in a concentration-dependent manner. Inhibition activity (% inhibition) increased with increasing extract concentration, Tabel 1. However, INPA exhibited strong inhibition on α -glucosidase, comparable to that of the positive control acarbose. EEPA showed weaker activity compared to both INPA and acarbose ($p < 0.05$).

Xanthine Oxidase Inhibition Activity

Both INPA and EEPA were able to inhibit xanthine oxidase. Increased concentrations of extracts increased xanthine

Table 1. α -Glucosidase inhibition activity of *P. angulata* stem extracts.

| Samples | Concentrations (μ g/mL) | Inhibition (%) | IC_{50} (μ g/mL) |
|----------|------------------------------|-------------------|-------------------------|
| INPA | 90 | 9.28 \pm 5.80 | 149.11 \pm 7.46 |
| | 135 | 33.41 \pm 5.91 | |
| | 146 | 40.71 \pm 0.74 | |
| | 158 | 58.21 \pm 2.87 | |
| | 180 | 84.49 \pm 3.34 | |
| EEPA | 305.48 | 7.72 \pm 3.52 | 409.86 \pm 2.51 |
| | 374.46 | 13.57 \pm 2.27 | |
| | 443.44 | 84.36 \pm 11.21 | |
| | 522.27 | 97.38 \pm 0.55 | |
| Acarbose | 13 | 7.64 \pm 2.66 | 130.66 \pm 5.94 |
| | 26 | 19.54 \pm 3.00 | |
| | 52 | 30.18 \pm 2.77 | |
| | 208 | 66.66 \pm 1.44 | |

oxidase inhibition activity in a concentration-dependent manner, INPA showed higher inhibition activity than EEPA, yielding nearly five times higher activity for INPA than EEPA ($p < 0.05$) (Table 2). However, the activity of both extracts was much lower than allopurinol, the positive control.

DPPH Radical Scavenging Activity

DPPH assay was a versatile screening method in investigating the antioxidant activity of plant extracts. This antioxidant method is based on the scavenging activity of the extracts, probably by donation of hydrogen atom of the antioxidant compounds in the extracts to DPPH radicals. Increased INPA concentration (0.065 to 0.260 mg/mL) did not change inhibition percentage significantly, indicating a very weak activity. The IC_{50} could not be determined. For EEPA, percentage of inhibition increased as a function of concentration. However, EEPA showed very weak scavenging activity as seen in the obtained IC_{50} , compared to the positive control BHT (Table 3). BHT is a synthetic antioxidant and used as a food additive.

Discussion

Plant secondary metabolites, in particular polyphenolic compounds have been associated with the pharmacological activities of plant extracts, including antidiabetic¹⁵, anti-hyperuricemia¹⁶, and antioxidant¹⁷ activities.

Polarity of the solvent extractant is likely a decisive factor in the extraction of phenolic compounds in the plant

parts.¹⁸ Findings in this study indicate that ethanol is more efficient than water in extracting phenolic compounds from the stem of *P. acutangula*. It is likely that heterogenous polar moieties attached to the phenolic derivatives of the stem of *P. acutangula* influence the solubility of the compounds in different solvents.

Inhibition on α -glucosidase is one of important therapeutic strategies for the management of T2DM. Acarbose, the synthetic α -glucosidase inhibitor, suffers from adverse side effects including gastrointestinal discomfort that may interfere its clinical use.¹⁹ Plants are potential source of natural α -glucosidase inhibitor. For this purpose, *P. angulata* was investigated for its inhibition activity on α -glucosidase. A number of studies have reported marked inhibition activity on α -glucosidase by water²⁰ and polar solvent extracts (methanol and ethanol extracts)²¹.

The current study exhibited that both INPA and EEPA were able to inhibit α -glucosidase with comparable activity to acarbose, the reference standard. Antidiabetic activity of *P. angulata* has been studied previously using animal models, such as using whole plant, fruit, and leaves.²²⁻²⁴ The current study added to the literature in which stem extracts were studied. It was revealed that α -glucosidase inhibition activity of INPA and EEPA did not correlate with the phenolic contents of the stem extracts. These results suggest that the inhibition activity might also be attributed to the presence of non-phenolic compounds such as alkaloid and terpenoid, as also observed by others.^{25,26}

Gout is a globally distributed disease with increasing prevalence. Hyperuricemia is the major risk factor for gout

Table 2. Xanthine oxidase inhibition activity of *P. angulata* stem extracts.

| Samples | Concentrations ($\mu\text{g/mL}$) | Inhibition (%) | IC_{50} ($\mu\text{g/mL}$) |
|-------------|-------------------------------------|-------------------|--------------------------------|
| INPA | 0.200 | 24.37 \pm 4.31 | 0.546 \pm 0.03 |
| | 0.500 | 47.77 \pm 11.19 | |
| | 0.624 | 63.20 \pm 8.43 | |
| EEPA | 0.023 | 15.53 \pm 3.52 | 2.643 \pm 0.28 |
| | 0.375 | 47.82 \pm 2.23 | |
| | 6.757 | 80.81 \pm 3.25 | |
| Allopurinol | 0.003 | 8.24 \pm 4.87 | 0.005 \pm 0.00 |
| | 0.004 | 36.71 \pm 5.93 | |
| | 0.005 | 57.24 \pm 6.39 | |
| | 0.007 | 64.59 \pm 8.00 | |

Table 3. DPPH radical scavenging activity of *P. angulata* stem extracts.

| Samples | Concentrations (µg/mL) | Inhibition (%) | IC ₅₀ (µg/mL) |
|---------|------------------------|----------------|---------------------------|
| INPA | 0.065 | 27.66±0.49 | Not able to be determined |
| | 0.195 | 29.45±1.42 | |
| | 0.260 | 30.80±2.17 | |
| EEPA | 2.95 | 18.13±8.10 | 16.04±0.08 |
| | 5.91 | 22.43±1.50 | |
| | 11.82 | 41.00±0.22 | |
| | 17.73 | 53.69±1.59 | |
| BHT | 13 | 23.15±0.20 | 0.02±0.00 |
| | 26 | 40.99±0.59 | |
| | 52 | 56.27±1.86 | |
| | 208 | 60.58±1.47 | |

arthritis. Evidence from cohort study suggest association of hyperuricemia and diabetes mellitus.²⁷ Currently, allopurinol is the first line drug prescribed to lower serum urate level by the inhibition on xanthine oxidase. However, its use was reportedly caused hypersensitivity reactions.²⁸ The current study showed that INPA and EEPA were able to inhibit xanthine oxidase, although significantly more weakly than allopurinol. Similarly to α -glucosidase inhibition activity, the observed inhibition activity on xanthine oxidase did not relate to the phenolic content in the extracts. This indicates the presence of non-phenolic based inhibitors. To the best of our knowledge, the current study is the first report on xanthine oxidase inhibition activity of *P. angulata* stem extracts.

Oxidative stress is generally defined as an imbalanced formation and removal of reactive oxygen species (ROS). ROS are capable of damaging various biomolecules, including protein, lipid, and DNA. Oxidative stress has been associated with the onset and progression of DM. Further, xanthine oxidase has been reported to increase during oxidative stress.²⁹ Therefore, antioxidant is required to reduce the oxidative damage. INPA showed no radical scavenging activity, whereas EEPA had negligible activity.

The enzyme inhibition and radical scavenging activities observed in this study were weaker than those of standard compounds. This could be due to the crude extracts used in this study. The current study recommends for future studies to be directed on the use of successive extractions with solvents of varying polarity and the isolation of the bioactive compounds.

Conclusion

The study found that the ethanol extract of *P. angulata* (EEPA) had higher content of phenolic compounds than water extract (INPA). INPA and EEPA showed good inhibition activity on α -glucosidase, with INPA exhibited higher activity than EEPA, comparable to that of acarbose. Both INPA and EEPA were weak inhibitors of xanthine oxidase compared to allopurinol. INPA and EEPA were found to be weak antioxidants.

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