Effects of *Pseuderanthemum palatiferum* (Nees) Radlk. leaf ethanolic extract on α-amylase enzyme activity

ผลของสารสกัดใบพญาวานรด้วยเอทานอลต่อการทำงานของเอนไซม์ α-amylase

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**Introduction**

- *Pseuderanthemum palatiferum* (Nees) Radlk.
- พญาวานหรือฮว่านจองกี้
- Widely used as a folk medicine in Vietnam and Thailand
  - Peptic ulcer, dyspepsia
  - Hepatitis
  - Diarrhea
  - Thyroid dysfunction
  - Hypertension
  - *Diabetes mellitus*
Introduction

**Diabetes mellitus (DM)**

- Most common chronic metabolic disorder
- Major problems in both public health and economic aspects
- ↑ risk of cardiovascular disease
- 85-95% >>> Type 2 DM
- 171 millions in 2000
- 366 millions in 2030
- 3 millions in Thailand
Type 2 DM risk factors

- Age greater than 45 years
- Diabetes during a previous pregnancy
- Excess body weight (especially around the waist)
- Family history of diabetes
- Given birth to a baby weighing more than 9 pounds
- HDL cholesterol under 35 mg/dL
- High plasma TG (> 250 mg/dL)
- Hypertension
- Impaired glucose tolerance
- Low activity level (exercising less than 3 times a week)
- Metabolic syndrome
- Polycystic ovarian syndrome

“What fits your busy schedule better, exercising one hour a day or being dead 24 hours a day?”
Introduction

- **Treatment of DM**
  - Lifestyle modification (diet and exercise)
  - Anti-diabetic agents

- **Disadvantage of current anti-diabetic agents**
  - Limited efficacy
    - Single mechanism of action
  - ADRs
  - Cost
  - Accessibility
Previous studies of *Pseuderanthemum palatiferum* (Nees) Radlk. leaf ethanolic extract

- **Cytotoxicity test**
- Vero cells (african green monkey kidney) by a green fluorescent protein (GFP) -based assay
- No cytotoxicity was detected at the dose of 50 μg/mL
- Padee et al. (2009)
Previous studies of *Pseuderanthemum palatiferum* (Nees) Radlk. leaf ethanolic extract

- **In vivo acute toxicity test**
- Wistar rats, at 500, 1,000, 1,500 and 2,000 mg/kg
- No doses of extract produced any signs or symptoms of toxicity during the first 24 hrs and no rat died within 14 days.
- Longer acute toxicity test for 14 days, at 250, 500 and 1,000 mg/kg
- None of the dose levels showed any signs or symptoms of toxicity and no rat died.
- Padee et al. (2009)
Previous studies of *Pseuderanthemum palatiferum* (Nees) Radlk. leaf ethanolic extract

- **Hypoglycemic effect**
- At the doses of 250, 500 and 1,000 mg/kg for 14 days
- All doses significantly ↓ FPG
  - More effective than glibenclamide at the dose of 250 mg/kg
- All doses significantly ↑ insulin levels
- Padee et al. (2010)
What is the mechanism(s) of action of Hoan-ngoc?

Blood glucose-lowering medicines

**Mechanisms of action**

- (-) hepatic glucose production
- (-) GI glucose absorption
- (+) insulin sensitivity
- (+) insulin secretion

*Adapted from IDF Clinical Guidelines Task Force, 2005*
**Glucose absorption**

- **α-amylase enzyme**
  - Breaks **α-D-(1-4) glycosidic bond**
  - Salivary **α-amylase**, pancreatic **α-amylase**
- **α-glucosidase enzyme**
  - sucrase, maltase, glucoamylase, dextranase

![Chemical structure of starch and maltose](image)

- **α-amylase**
- **maltose**
- **starch**
Inhibition of carbohydrate hydrolyzing enzyme

- Delayed carbohydrate digestion
- Delayed carbohydrate absorption
- Control postprandial glucose***
- Postprandial glucose is linked with the risk of micro/macrovascular complications
- Postprandial glucose may targeted if HbA1C goals are not met despite reaching preprandial glucose goals
Objectives

- To investigate *in vitro* $\alpha$-amylase inhibitory effect of *Pseuderanthemum palatiferum* (Nees) Radlk. leaf ethanolic extract
Methods

1. Preparation of Pseuderanthemum palatiferum (Nees) Radlk. leaf ethanolic extract
   - 80% ethanol
   - 7-day maceration
   - Filtration
   - Rotary evaporator
   - Freeze dryer
   Conducted by Assistant Prof. Dr. Somsak Nualkaew
\(\alpha\)-amylase inhibitory assay

Pre-incubation method (Ali et al., 2006):

- Porcine pancreatic \(\alpha\)-amylase (4 unit/mL) 200 \(\mu\)L
- Plant extract (….. mg/mL) 40 \(\mu\)L
- Incubation at 25°C for 5 min
- Add 400 \(\mu\)L starch (0.5% w/v) + 160 \(\mu\)L distilled H\(_2\)O
Incubation at 25°C for 3 min,

Total volume = 800 μL = mixture

Take 200 μL of mixture then add 100 μL of 96 mM 3, 5-dinitrosalicylic acid (DNS) color reagent

Incubation at 85°C, 15 min (water bath)

Dilute with 450 μL distilled H₂O
Measure OD at 540 nm.

- **Negative control:** DMSO instead of plant extract.
- **Positive control:** α-amylase inhibitor from wheat seed *Triticum aestivum*.
- **Blank:** distilled water instead of enzyme.

- To subtract the OD of plant extract.

Brown color = maltose generated.
Maltose standard calibration

Maltose standard at concentration of $[X?]\times 100\mu L$

Incubation at $85^\circ C$, 15 min (water bath)

Dilute with $450\mu L$ distilled $H_2O$

Remove from water bath and measure OD at 540 nm
Calculation

- Maltose absorbance = A540 test – A540 blank
- % maltose generated is calculated from the standard curve
- % reaction = (mean maltose in sample) / (mean maltose in negative control) x 100
- % inhibition = 100 - % reaction
Maltose standard curve

\[ y = 10.097x - 0.0433 \]

\[ R^2 = 0.9961 \]
**Results**

- **Negative control (DMSO)**
  - % reaction = 100.00 ± 6.72 (n=3)
  - DMSO did not produce an inhibitory effect on $\alpha$-amylase enzyme

- **Positive control ($\alpha$-amylase inhibitor from wheat seed *Triticum aestivum*; 80 u/mL)**
  - % inhibition = 36.51 ± 8.13 (n=3)
Results

* $p < 0.05$; One-way ANOVA, Bonferroni post hoc test
## Results

<table>
<thead>
<tr>
<th>PPE extract concentration (mg/mL)</th>
<th>% inhibition (mean ± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.1</td>
<td>2.57 ± 3.67</td>
</tr>
<tr>
<td>0.5</td>
<td>2.96 ± 6.37</td>
</tr>
<tr>
<td>1</td>
<td>10.50 ± 5.28</td>
</tr>
<tr>
<td>5</td>
<td>28.88 ± 1.85*</td>
</tr>
<tr>
<td>10</td>
<td>51.72 ± 9.50*</td>
</tr>
<tr>
<td>20</td>
<td>59.86 ± 4.46*</td>
</tr>
<tr>
<td>50</td>
<td>59.04 ± 2.34*</td>
</tr>
</tbody>
</table>

*p<0.05; One-way ANOVA, Bonferroni posthoc test
Alpha-amylase inhibitory effect of PPE extract

*p<0.05; One-way ANOVA, Bonferroni posthoc test
**Results**

- $\text{IC}_{50} = 4.98 \pm 1.32 \text{ mg/mL}$

- Maximum inhibitory effect ($59.86 \pm 4.46\%$) reached at the concentration of $20 \text{ mg/mL}$
Discussion & Conclusion

- PPE extract produced an $\alpha$-amylase inhibitory action with the IC$_{50}$ of 4.98 ± 1.32 mg/mL
  - Other medicinal herbs used for the treatment of DM with $\alpha$-amylase inhibitory effect
    - Hexane extract of Phyllanthus amarus (ลูกใต้ใบ); IC$_{50} = 0.032$ mg/mL
    - Psidium guajava (ฝรั่ง) leaves, Syzygium cumini (หว้า) seed aqueous extract
    - Amaranthus caudatus (ผักขม) seed ethanol, ethyl acetate and hexane extract
    - Ocimum tenuflorum (ยี่หร่า) chloroform extract
**Discussion & Conclusion**

- Active compound(s) in PPE extract
  - Flavonoids,
  - \( \beta \)-sitosterol,
  - Stigmasterol,
  - Kaempferol,
  - Apigenin,
  - Phytol,
  - Triterpenoid saponin,
  - Salicylic acid
Discussion & Conclusion

- Active compound(s) acts as $\alpha$-amylase inhibitor
  - Triterpenoids: Triterpene acids
  - Oleanolic acid and ursolic acid mixture (2:1); IC = 2.01 $\mu$g/mL (Ali et al., 2006)

- Flavonoids
  - Flavones, Flavols
  - Kaemferol (Tadera et al., 2006; Lo Piparo et al., 2008)
Discussion & Conclusion

- Flavonoids as $\alpha$-amylase inhibitor
  - Number of $-\text{OH}$ group on ring B
  - Hydrogen bond formation
  - $-\text{OH}$ group of polyphenols vs. catalytic residues of $\alpha$-amylase enzyme (Lo Piparo et al., 2008)
Discussion & Conclusion

Other mechanisms of action of PPE extract are under investigation

- Effects on adipocyte lipolysis >>> finished
- Antioxidant effect
- Effects on insulin secretion
- Others >>>>>>
Suggestions

- Future experiments
- \textit{In vivo} \(\alpha\)-amylase inhibitory action
  - Oral starch tolerance test
- \textit{In vitro} \(\alpha\)-glucosidase inhibitory effect
- Identification of the active compounds
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