2nd Humboldt Kolleg in conjunction with International Conference on Natural Sciences, HK-ICONS 2014

An α-Glucosidase Inhibitor from *Drepananthus philippinensis*

Allan Patrick Gose Macabeo\(^a\)*, Peter Yuosef Moreno Rubio\(^a\), Grecebio Jonathan Duran Alejandro\(^b\), Matthias Knorn\(^c\)

\(^a\)Phytochemistry and Organic Synthesis Laboratory, Research Center for the Natural and Applied Sciences, University of Santo Tomas, Espana St., Manila 1015, Philippines
\(^b\)College of Science and Research Center for the Natural and Applied Sciences, University of Santo Tomas, Espana St., Manila 1015, Philippines
\(^c\)Institut fuer Organische Chemie, Universitaet Regensburg, Universitaetsstrasse 31, 93053 Regensburg, Germany

**Abstract**

Extraction, fractionation, depigmentation and chromatographic purification of the crude DCM-methanolic extract of the Philippine medicinal plant *Drepananthus philippinensis* resulted to the isolation and identification of trans-cinnamic acid (1) on the basis of spectroscopic evidences (\(^1^H\) NMR, \(^1^3^C\) NMR, COSY, HSQC and HMBC) and comparison with literature data. Microplate colorimetric anti-glucosidase assay showed compound 1 (IC\(_{50}\) 32 mM) to exhibit a moderately strong inhibitory activity against the test enzyme. This is the first report of 1 from *D. philippinensis* and its biological evaluation.

© 2015 The Authors. Published by Elsevier B.V. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

Peer-review under responsibility of the Scientific Committee of HK-ICONS 2014

**Keywords:** *Drepananthus philippinensis*; Annonaceae; α-glucosidase inhibitor; trans-cinnamic acid.

* Corresponding author. Tel.: +63 024 061 611 ext. 4057; fax: +63 027 314 031.

**E-mail address:** allanpatrick_m@yahoo.com.
1. Introduction

Diabetes mellitus is the world’s largest endocrine disease affecting 347 million people which involves abnormality in carbohydrate metabolism characterized by fast elevation of blood glucose level. Patients with diabetes experience notable morbidity and mortality from macrovascular and microvascular complications\(^1,2\). The fast elevation of blood glucose level can be controlled by inhibition of \(\alpha\)-glucosidase - an enzyme responsible for the hydrolysis of \(\alpha\)-glucosidic linkages of oligosaccharides that liberate monosaccharide units contributing to the sugar level of the blood. Thus, \(\alpha\)-glucosidase inhibitors delay release of monosaccharide units in the blood and managing diabetes that cause other serious complications\(^3\).

*Drepananthus philippinensis* (family Annonaceae) is a Philippine endemic species growing as small shrub in lowland forests and thickets of Samar, Philippines. So far, no report as to its phytochemistry and biological activity has been documented in the literature. This paper reports the isolation and identification of trans-cinnamic acid (I) as \(\alpha\)-glucosidase inhibitor constituent of *D. philippinensis*.

2. Material and methods

2.1 General

NMR spectra were recorded on a Bruker Avance 600 (600.13 MHz) spectrometer using the solvent peak as internal reference (CDCl\(_3\): \(\delta H\) 7.26; \(\delta C\): 77.0). Multiplicities are indicated, s (singlet), d (doublet), t (triplet), q (quartet), quint (quintet), m (multiplet); coupling constants \((J)\) are in Hertz (Hz). All reactions were monitored by thin-layer chromatography (TLC) using Merck silica gel plates 60 F\(_{254}\); visualization was accomplished with UV light and/or staining with vanillin-sulfuric acid followed by heating. Normal phase column chromatography was performed with silica gel 60 (Merck Art. 1.07734.1000).

2.2 Plant collection

*Drepananthus philippinensis* was collected from Balangiga, Eastern Samar, Eastern Visayas on May 2012. The voucher specimen (CY-001) was deposited at the University of Santo Tomas Herbarium, Research Center for the Natural and Applied Sciences. Identification based on morphology and molecular sequences was performed.

2.3 Extraction and fractionation

The ground, air-dried leaves of *D. philippinensis* (2.50 kg) were extracted with DCM-MeOH (1 : 1, 20 L) to give a green syrupy extract (DsD, 534 g). The aqueous suspension of a portion of this crude extract (300.0 g) was subjected to solvent partitioning with petroleum ether (1 L), DCM (1.3 L) and \(n\)-BuOH (800 mL) to afford the sub-extracts DsP (4.3 g), DsD (88.9 g) and DsB (17.0 g). A portion of the DsD sub-extract (100.0 g) was dissolved in EtOH (1.8 L) and 4 % of Pb(OAc)\(_2\) (200 mL) was added. The solution was placed on an ice bath to precipitate the polar components. The treated solution was filtered and concentrated *in vacuo* at 45 °C to give the crude extract DsDPb. The DsDPb (7.2 g) crude extract was subjected to gravity liquid chromatography and eluted with 10 % increments of ethyl acetate in hexanes to give 12 fractions. Fraction seven was subjected further to repeated silica gel column chromatography using 3 : 2 petroleum ether-ethyl acetate to yield I (78.9 mg) as amorphous solid which was identified by extensive NMR experiments as trans-cinnamic acid.

2.4 \(\alpha\)-Glucosidase inhibitory assay

\(\alpha\)-Glucosidase from yeast and substrate \(p\)-nitrophenyl \(\alpha\)-D glucopyranoside (\(p\)-NPG) were purchased from Sigma, USA. N-deoxynojirimycin was used as positive control. Sample solutions of the extracts Ds, DsD, DsB, DsP, DsDP and DsDPb fractions (1 mg · mL\(^{-1}\)) were prepared in dimethyl sulfoxide. Sodium phosphate (100 mM) buffer containing 50 mM NaCl was also prepared (pH 6.8). A standard enzyme inhibition protocol was adopted with
minor modifications. A 0.04 units $\text{mL}^{-1}$ enzyme and 0.7 mM of substrate were used for initial screening of plant extracts, fractions and pure compounds.

A 96-well plate was used to contain the $\alpha$-glucosidase solution. Plates were incubated at 37 °C. It was read at 400 nm using an ELISA reader (Biotek USA). An increase in reading indicated the activity of enzyme as it hydrolyzed $p$-NPG. Absorbance was initially read 15 min after adding the enzyme, and after indication of color change relative to positive control. The percent concentration was calculated as:

\[
\text{% inhibition} = \frac{\text{Abs of control} - \text{Abs of sample}}{\text{Abs of control}} \times 100
\]

### 3. Result and discussion

Extraction, solvent partitioning and lead acetate treatment afforded Ds, DsP, DsD, DsB and DsDPb extracts. Fractionation using silica gel chromatography yielded twelve fractions. Phytochemical analysis of DsDPb fractions indicated the presence of various types of secondary metabolites such as phenols, tannins, flavonoids, anthraquinones, coumarins, anthrones, terpenes, sterols, essential oils, flavonoids, indoles, sugars and steroids. Screening for $\alpha$-glucosidase inhibitory activity using a colorimetric microplate enzyme inhibition assay of the extracts showed the DsB sub-extract as the most inhibitory against $\alpha$-glucosidase with an IC$_{50}$ value of 0.94 $\mu$M (Table 1). The fractions of the depigmented extract, DsDPb showed almost similar activity for fraction four and eleven.

Isolation and chromatographic purification of fraction seven afforded an amorphous solid which was spectroscopically identified as $\text{trans}$-cinnamic acid (1) via extensive nuclear magnetic resonance (NMR) experiments such as $^1$H, $^{13}$C, HSQC, HMBC and, COSY. Compound 1 resulted to a moderately strong inhibitory activity against $\alpha$-glucosidase with IC$_{50}$ value of 32.

**Compound 1.** NMR data: $^1$H NMR (600 MHz, CDCl$_3$) $\delta$: 7.51 (2H, d, $J = 7.2$ Hz, H-2′,6′), 7.39 (1H, d, $J = 15.6$ Hz, H-3), 7.34 (2H, t, $J = 7.2$ Hz, H-4′), 7.30 (1H, t, $J = 7.2$ Hz, H-3′, 5′), 6.51 (1H, d, H-2). $^{13}$C NMR (150 MHz, CDCl$_3$) $\delta$: 127.0 (C-1′), 130.0 (C-2′,6′), 128.6 (C-3′,5′), 127.0 (C-4′), 137.6 (C-4), 140.8 (C-3), 175.8 (C = O).

![Fig. 1. Structure of trans-cinnamic acid (1) and correlation analysis via COSY and HMBC experiments.](image)

$\alpha$-Glucosidase inhibitors are important for treating various diseases. For example, the inhibition of $\alpha$-glucosidase decreases blood glucose levels which leads to reduction of glucose absorption. $\alpha$-Glucosidase inhibitors are also showing potential as anti-HIV agents, drugs for B- and C-type viral hepatitis. $\text{trans}$-cinnamic acids are known to exhibit a number of biological activities including hepatoprotective, anti-malaria land antioxidant activities. In this study, the authors have demonstrated the activity of $\text{trans}$-cinnamic acid (1) as potential drug compound for treating
diabetes by inhibiting α-glucosidase. This is the first report on the identification of 1 and biological evaluation of phytochemical constituents present in D. philippinensis.

Table 1. IC₅₀ values versus α-glucosidase.

| Extracts   | (IC₅₀ value ± se) μM |
|------------|----------------------|
| Crude      | 5.03 ± 3.24          |
| DsD        | 1.77 ± 2.59          |
| DsP        | 12.05 ± 3.00         |
| DsB        | 0.94 ± 1.83          |
| DsDPb      | 1.79 ± 6.43          |
| DsDPb1     | Not tested           |
| DsDPb2     | 500 ± 2.44           |
| DsDPb3     | 33.54 ± 3.04         |
| DsDPb4     | 4.83 ± 1.68          |
| DsDPb5     | 102.22 ± 2.58        |
| DsDPb6     | 150.52 ± 2.81        |
| DsDPb7     | 67.66 ± 2.28         |
| DsDPb8     | 65.34 ± 1.53         |
| DsDPb9     | 150.23 ± 1.75        |
| DsDPb10    | 76.38 ± 2.68         |
| DsDPb11    | 4.66 ± 2.22          |
| DsDPb12    | 130.58 ± 1.74        |

4. Conclusion

The current study underscored the identification of a cinnamic derivative 1 as anti-glucosidase principle of the endemic Philippine medicinal plant, Drepananthus philippinensis. Consequently, the degree of activity of the compound makes it a privileged scaffold to discover more active derivatives in on-going studies.

References

1. Rai M, Cordell GA, Martine JL, Marinoff M, Rastrelli L. Medicinal plants biodiversity and drugs. USA: CRC Press 2012.
2. Kardesler L, Buduneli N, Biyikoglu B, Cetinkalp S, KutukcuJ. Gingival crevicular fluid PGE2, IL-1b, t-PA, PAI-2 levels in type 2 diabetes and relationship with periodontal disease. Clin. Biochem. 2008:41: 863–868.
3. Lawag IL, Aguinaldo AM, Naheed S, Moshiuzzaman M. α-Glucosidase inhibitory activity of selected Philippine plants. J Ethnopharmacol 2012;144: 217–219.
4. Adisakwattana S, Sookkongwaree K, Roengsumran S, et al. Structure-activity relationships of trans-cinnamic acid derivatives on α-glucosidase inhibition. Bioorg Med Chem Let 2004;14:2893–2896.
5. Kasetti RB, Nabi SA, Swapna S, Apparao C. Cinnamic acid as one of the antidiabetic active principle(s) from the seeds of Syzygium alternifolium. Food Chem Toxicol 2012;50: 1425–1431.