

Identification of α -glucosidase inhibitors from the leaves of *Pluchea indica* (L.) Less., a traditional Indonesian herb: promotion of natural product use

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A promising approach for treating diabetes mellitus (DM) is to decrease postprandial hyperglycaemia by suppressing carbohydrate digestion using α -glucosidase inhibitors. *Pluchea indica* leaf extracts possess inhibitory activity against intestinal maltase. Enzyme assay-guided fractionation by chromatography yielded five active caffeoylquinic acid derivatives (1–5). Their structures were elucidated by mass spectrometry and NMR analysis and completed by comparison with reference data. 3,5-Di-*O*-caffeoylquinic acid (1), 4,5-di-*O*-caffeoylquinic acid methyl ester (2), 3,4,5-tri-*O*-caffeoylquinic acid methyl ester (3), 3,4,5-tri-*O*-caffeoylquinic acid (4) and 1,3,4,5-tetra-*O*-caffeoylquinic acid (5) were isolated. Comparison of the activities of each isolate suggested that both methyl esterification of quinic acid and the number of caffeate groups in the molecule were important for the inhibitory activity. This study provides basic information for further examination of the suitability of *P. indica* as a functional food and medicinal supplement for the treatment and prevention of diabetes.

Keywords: Indonesian herb; intestinal maltase inhibitor; diabetes mellitus; *Pluchea indica*; caffeoylquinic acid derivatives

1. Introduction

An effective tool in the management of diabetes mellitus (DM), and particularly that of non-insulin-dependent DM (NIDDM), is to decrease postprandial hyperglycaemia by inhibiting α -glucosidase in the digestive system. Many efforts have been made to identify α -glucosidase inhibitors from indigenous plants to develop physiologically functional foods and to discover compounds for use against diabetes (Kumar et al. 2011). Some indigenous plants from Indonesia possess α -glucosidase inhibitory activity, e.g. *Macaranga tanarius* leaves (Gunawan-Puteri & Kawabata 2010) and *Eleutherine americana* bulbs (Ieyama et al. 2011). We screened 25 Indonesian medicinal herbs for their α -glucosidase inhibitory activity, particularly intestinal maltase inhibitory activity. *Pluchea indica* (L.) Less. (Asteraceae) was found to be a promising source of α -glucosidase inhibitors.

P. indica is widely distributed in South-East Asia (Raharjo & Horsten 2001). Extracts of *P. indica* parts possess antioxidant, anti-ulcer, anti-nociceptive, anti-diuretic and anti-inflammatory properties (Sen & Chaudhuri 1991; Choi & Hwang 2005; Biswas et al. 2007; Buapool et al. 2013). In Thailand, *P. indica* leaves are used as tea because they are believed to possess an indigenous remedy due to their anti-diabetic properties. It has been reported for its anti-diuretic and anti-diabetic pharmacological effects in streptozocin-induced rats (Pramanik et al. 2006). Prior chemical investigations of *P. indica* have led to the isolation of several

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terpenes, lignin glycosides and terpenic glycosides from the aerial part (Uchiyama et al. 1991; Raharjo & Horsten 2001). The methanolic extract of the *P. indica* roots led to the isolation of an alkylnylthiophene derivative for its anti-amoebic activity (Biswas et al. 2007). 3,4,5-Tri-*O*-caffeoylquinic acid and 1,3,4,5-tetra-*O*-caffeoylquinic acid, herein referred to as compounds **4** and **5**, respectively, were recently isolated as constituents for this plant and have been reported to show collagenase inhibitory activity (Ohtsuki et al. 2008). However, *P. indica* is not used as an anti-diabetic agent in Indonesia. The compounds in *P. indica* responsible for α -glucosidase inhibition are unknown. Therefore, this study was performed to discover the compounds responsible for the inhibition of α -glucosidase, specifically of intestinal maltase, using a bio-assay-guided approach.

2. Results and discussion

Compounds were extracted from dried leaves of *P. indica* using 50% methanol. The methanol extract was partitioned between ethyl acetate (EtOAc) and water. The EtOAc fraction demonstrated a higher inhibitory activity against maltase than the water fraction. Further fractionation was carried out by chromatography and by guidance of the rat intestinal maltase inhibitory activity.

The EtOAc fraction was subjected to silica gel column chromatography using chloroform–methanol gradient and the active fraction was further fractionated using ODS column chromatography. The active ODS fractions were further purified using preparative HPLC resulting in the isolation of five caffeoylquinic acid derivatives: 3,5-di-*O*-caffeoylquinic acid (**1**), 4,5-di-*O*-caffeoylquinic acid methyl ester (**2**), 3,4,5-tri-*O*-caffeoylquinic acid methyl ester (**3**), 3,4,5-tri-*O*-caffeoylquinic acid (**4**) and 1,3,4,5-tetra-*O*-caffeoylquinic acid (**5**). The structure of all isolates (**1–5**) was determined using mass spectrometry and ^1H NMR spectra. The NMR pattern of all compounds revealed characteristic signals of caffeoylquinic acids and their structures were finally confirmed using published spectra: **1** (Basnet et al. 1996; Gao et al. 2008), **2** (Gao et al. 2008), **3** (Mertfort 1992), **4** (Islam et al. 2002) and **5** (Scholz et al. 1994).

This is the first report on the identification of compounds **1–3** from *P. indica*, whereas compounds **4** and **5** were recently isolated as constituents from this plant and have been reported to exhibit collagenase inhibitory activity (Ohtsuki et al. 2008). Interestingly, the 3,5-isomer of dicaffeoylquinic acid was isolated as a free form (**1**), whereas the 4,5-isomer was isolated only as a methyl ester (**2**). This indicates that methyl esterification selectively occurs in plant tissues. Compounds **1** and **4** have been previously reported as maltase inhibitors (Matsui et al. 2004). However, compounds **2**, **3** and **5** have not been evaluated for their intestinal maltase inhibitory activity.

The isolated caffeoylquinic acid derivatives were compared for their rat intestinal maltase inhibitory activity (Table 1). Half maximal inhibitory concentration (IC_{50}) values were used as measures of the effectiveness of each compound to inhibit maltase function. On the basis of IC_{50} values, compound **3** had the highest inhibition among the caffeoylquinic acid derivatives

Table 1. Yields and IC_{50} values of intestinal maltase inhibitory activity for compounds isolated from *P. indica*.

No.	Compounds	Yield (%)	IC_{50} (μM)
1	3,5-Di- <i>O</i> -caffeoylquinic acid	0.01	1166
2	4,5-Di- <i>O</i> -caffeoylquinic acid methyl ester	0.08	208
3	3,4,5-Tri- <i>O</i> -caffeoylquinic acid methyl ester	0.04	2
4	3,4,5-Tri- <i>O</i> -caffeoylquinic acid	0.02	13
5	1,3,4,5-Tetra- <i>O</i> -caffeoylquinic acid	0.04	11

isolated from *P. indica* leaves, followed by **4**, **5**, **2** and **1**, respectively. This suggests that, when comparing the inhibitory activity of compounds **1**, **4** and **5**, increasing numbers of caffeoyl groups attached to quinic acid moiety enhanced maltase inhibitory activity. This is consistent with a previous study in which the caffeoyl group played an important role in intestinal α -glucosidase inhibitory activity (Matsui et al. 2004). Among the compounds with the same number of caffeoyl groups, compounds **2** and **4** expressed five-fold higher inhibitory activities than compounds **1** and **3**, respectively. This suggests that methyl esterification of the carboxylic group in quinic acid has an additional effect of enhancing maltase inhibitory activity. This is the first report for the importance of the methyl esterification of caffeoylquinic acids with respect for their α -glucosidase inhibitory activity, although the mechanism still remains undetermined.

In this study, we demonstrated that increases in maltase inhibitory activity were due to the number of caffeoyl groups attached to the quinic moiety and the presence of methyl esterification on the carboxyl group. Considering the yields and IC_{50} values of the caffeoylquinic acid derivatives isolated, the inhibitory activity of *P. indica* leaves is likely to be due to compounds **3**, **4** and **5**, although compounds **1** and **2** may also contribute to the activity.

3. Conclusion

The caffeoylquinic acid derivatives (**1**–**5**) isolated from *P. indica* were intestinal maltase inhibitors. By comparing the inhibitory activities of the isolates, we determined that methyl esterification of the quinic acid moiety contributes to the inhibitory activity of the compound. We also determined that the number of caffeoyl groups in the molecule contributes to the inhibitory activity. Caffeoylquinic acid derivatives from this plant may be important medicinal substances, which may delay postprandial hyperglycaemia. *Pluchea indica* is an indigenous plant that may potentially be used as a functional food or as a medicinal supplement for DM treatment and prevention.

Supplementary material

Supplementary material relating to this article is available online.

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References

- Basnet P, Matsushige K, Hase K, Kadota S, Namba T. 1996. Four di-*O*-caffeoylquinic acid derivatives from propolis: potent hepatoprotective activity in experimental liver injury models. *Biol Pharm Bull.* 19:1479–1484.
- Biswas R, Dutta PK, Achari B, Bandyopadhyay D. 2007. Isolation of pure compound R/J/3 from *Pluchea indica* (L.) Less. and its anti-amoebic activities against *Entamoeba histolytica*. *Phytomedicine.* 14:534–537.
- Buapool D, Mongkol N, Chantimal J, Roytrakul S, Srisook E, Srisook K. 2013. Molecular mechanism of anti-inflammatory activity of *Pluchea indica* leaves in macrophages RAW 264.7 and its action in animal models of inflammation. *J Ethnopharmacol.* 146:495–504.
- Choi EM, Hwang JK. 2005. Screening of Indonesian medicinal plants for inhibitor activity on nitric oxide production of RAW264.7 cells and antioxidant activity. *Fitoterapia.* 76:194–203.
- Gao H, Huang YN, Gao B, Xu PY, Inagaki C, Kawabata J. 2008. α -Glucosidase inhibitory effect by the flower buds of *Tussilago farfara* L. *Food Chem.* 106:1195–1201.
- Gunawan-Puteri MDPT, Kawabata J. 2010. Novel α -glucosidase inhibitors from *Macaranga tanarius* leaves. *Food Chem.* 123:384–389.
- Ieyama T, Gunawan-Puteri MDPT, Kawabata J. 2011. α -Glucosidase inhibitors from the bulb of *Eleutherine americana*. *Food Chem.* 128:308–311.

- Islam MS, Yoshimoto M, Yahara S, Okuno S, Ishiguro K, Yamakawa O. 2002. Identification and characterization of foliar polyphenolic composition in sweet potato (*Ipomoea batatas* L.) Genotypes. *J Agric Food Chem.* 50:3718–3722.
- Kumar V, Prakash O, Kumar S, Narwal S. 2011. α -Glucosidase inhibitors from plants: a natural approach to treat diabetes. *Pharmacogn Rev.* 5:19.
- Matsui T, Ebuchi S, Fujise T, Abesundara KJM, Doi S, Yamada H, Matsumoto K. 2004. Strong antihyperglycemic effects of water-soluble fraction of Brazilian propolis and its bioactive constituent, 3,4,5-tri-*O*-caffeoylquinic acid. *Biol Pharm Bull.* 27:1797–1803.
- Merfort I. 1992. Caffeoylquinic acids from flowers of *Arnica Montana* and *Arnica chamissonis*. *Phytochemistry.* 31:2111–2113.
- Ohtsuki T, Yokosawa E, Koyano T, Preeprame S, Kowithayakorn T, Sakai S, Toida T, Ishibashi M. 2008. Quinic acid esters from *Pluchea indica* with collagenase, MMP-2 and MMP-9 inhibitory activities. *Phytother Res.* 22:264–266.
- Pramanik KC, Bhattacharya P, Biswas R, Bandyopadhyay D, Mishra M, Chatterjee TK. 2006. Hypoglycemic and antihyperglycemic activity of leaf extract of *Pluchea indica* Less. *Orient Pharm Exp Med.* 6:232–236.
- Raharjo I, Horsten SFAJ. 2001. *Pluchea indica* (L.) Less. [Internet] record from proseabase. In: van Valkenburg JLCH, Bunyapraphatsara N, editors. Bogor: PROSEA (Plant Resources of South-East Asia) Foundation. [cited 2013 Sept 24]. Available from: <http://www.proseanet.org>.
- Scholz E, Heinrich M, Hunkler D. 1994. Caffeoylquinic acids and some biological activities of *Pluchea symphytifolia*. *Plant Med.* 60:360–364.
- Sen T, Chauduri AKN. 1991. Antiinflammatory and evaluation of *Pluchea indica* root extract. *J Ethnopharmacol.* 33:135–141.
- Uchiyama T, Miyase T, Ueno A, Usmanghani K. 1991. Terpene and lignin glycosides from *Pluchea indica*. *Phytochemistry.* 30(2):655–658.