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Screening for potential α-glucosidase and α-amylase inhibitory constituents from selected Vietnamese plants used to treat type 2 diabetes

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ABSTRACT

Ethnopharmacological relevance: The 18 plant species investigated in this study have been used as herbal antidiabetic remedies in Vietnamese traditional medicines. This study aimed to evaluate their ability to inhibit α-glucosidase and α-amylase, two key enzymes involved in serum glucose regulation.

Materials and methods: Chloroform, ethanol and water extracts of 18 plants were screened for α-glucosidase and α-amylase inhibitory activity. Analytical-scale HPLC was subsequently used to investigate the most active extracts, where samples with low level of tannins were identified and fractionated into 96-well microplates, followed by α-glucosidase and α-amylase assessment of each well. High-resolution α-glucosidase and α-amylase inhibition profiles constructed from these assays allowed identification of HPLC peaks correlated with α-glucosidase and α-amylase inhibitory activity. The active constituents were subsequently isolated using preparative-scale HPLC and their structure was elucidated by HR-ESIMS and NMR.

Results: Ethanol extracts of *Nepenthes mirabilis*, *Phyllanthus urinaria*, and *Kandelia candel* significantly inhibited α-glucosidase with IC_{50} values of 32.7 ± 6.3, 39.7 ± 9.7, and 35.4 ± 13.9 μg/mL, respectively. Water extracts of *N. mirabilis*, *Phyllanthus amarus*, *P. urinaria*, *Lagerstroemia speciosa*, *Syzygium cumini*, *Rhizophora mucronata*, and *K. candel* showed IC_{50} values of 3.3 ± 0.8, 34.9 ± 1.5, 14.6 ± 4.6, 5.4 ± 0.5, 20.9 ± 1.8, 3.3 ± 0.6, and 4.0 ± 0.8 μg/mL, respectively. In the α-amylase inhibition assay, ethanol extracts of *K. candel* and *Ficus racemosa* showed IC_{50} of 7.6 ± 0.9 and 46.7 ± 23.6 μg/mL, respectively. Showing low tannin constituents as seen from HPLC profiles, *P. amarus* and *P. urinaria* water extracts and *F. racemosa* ethanol extract were subjected to microfractionation. Only high-resolution α-glucosidase inhibition profiles of *P. amarus* and *P. urinaria* water extracts showed several active compounds, which were isolated and identified as corilagin (**1**), repandusinic acid A (**2**), and mallotinin (**3**). IC_{50} of these compounds were 1.70 ± 0.03, 6.10 ± 0.10, and 3.76 ± 0.15 μM, respectively. Kinetics analysis revealed that **1** displayed a mixed type mode of inhibition with K_i and $K_{i'}$ values of 2.37 ± 0.90 and 2.61 ± 0.61 μM, respectively, whereas **2** and **3** competitively inhibited α-glucosidase with K_i values of 4.01 ± 0.47 and 0.65 ± 0.11 μM, respectively.

Conclusion: Corilagin (**1**), repandusinic acid A (**2**), and mallotinin (**3**) were potent α-glucosidase inhibitors contributing significantly to the inhibitory effect observed for the water extracts of *P. amarus* and *P. urinaria*.

1. Introduction

Diabetes mellitus, a chronic metabolic disease, has become a worldwide health problem. In 2010, 285 million people between the age of 20 and 79 were affected, and this number is predicted to increase to 439 million people by 2030 (Shaw et al., 2010). Treatment and strategies for prevention of diabetes amounted to 376 billion USD in 2010, and is expected to rise to 490 billion USD in the next two decades. (Zhang et al., 2010).

Accounting for roughly 90–95% of all diabetes cases worldwide, type 2 diabetes is characterized by high and/or fluctuating blood glucose due to insulin resistance. (Alberti et al., 2004; International Diabetes Federation, 2013). This is associated with severe complications such as high blood pressure, blindness, kidney

Abbreviations: HPLC, High-performance liquid chromatography; NMR, Nuclear Magnetic Resonance; HR-ESIMS, High-resolution electrospray ionization mass spectrometry

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failure, lower limb amputation, heart disease, and stroke (Fowler, 2008). One way of maintaining lower and more stable blood glucose is by inhibiting the carbohydrate hydrolyzing enzymes α-glucosidase and α-amylase in the digestive system (Toeller, 1994). Secreted from saliva and pancreas, α-amylase catalyzes the cleavage of α-1,4 glycosidic bonds to convert polysaccharides into smaller oligosaccharides such as maltose, maltotriose, and a number of α-1,4 and α-1,6-oligoglucans. These fragments are subsequently involved in further degradation by α-glucosidase located in the brush border of the small intestine. This enzyme is responsible in the hydrolysis of terminal non-reducing 1,4 linked α-glucose residues leading to the release of absorbable monosaccharides to enter the blood stream (Alagesan et al., 2012a, 2012b; Chiba, 1997; de Sales et al., 2012). Therefore, inhibition of these enzymes can delay digestion of carbohydrates causing a reduction in the rate of glucose absorption and consequently a suppression of postprandial hyperglycemia (Kumar et al., 2011).

In recent years, several studies have been focused on α-glucosidase and α-amylase inhibitors (AGIs and AAIs) from medicinal plants (Kasabri et al., 2011; Mojica et al., 2015; Nampoothiri et al., 2011; Thilagam et al., 2013; Wang et al., 2010). Although bioassay-guided fraction has been successfully applied for identification of AGIs and AAIs, this approach is time-consuming, and the repeated preparative-scale separations may cause minor constituents with interesting bioactivity to be missed (Pieters and Vlietinck, 2005). In this study, we therefore used high-resolution inhibition profiling, in which eluate from analytical-scale high-performance liquid chromatography is microfractioned into microplates followed by bioassaying of the material in all wells (Giera et al., 2009). High-resolution inhibition profiles constructed from these assays can allow fast pinpointing of individual chromatographic peaks responsible for the bioactivity, which is an essential step for targeting subsequent isolation and structure elucidation. High-resolution inhibition profiling has already proven a promising and efficient method for identification of the bioactive constituents from natural sources such as microorganisms (Wubshet et al., 2013a), food (Schmidt et al., 2014; Wiese et al., 2013; Wubshet et al., 2013b), and medicinal plants (Kongstad et al., 2015; Liu et al., 2015; Tahtah et al., 2015).

Vietnam is a tropical country with more than 10,000 plant species, many of which have been traditionally used to treat diabetes (Pham, 2007; Vo, 1997), yet little research has been carried out to evaluate the their ability to control hyperglycemia. Therefore, the aim of this study was to screen crude extracts of 18 Vietnamese medicinal plants for bioactive components against α-glucosidase and α-amylase in order to develop functional foods or identify lead compounds for use against type 2 diabetes.

2. Materials and methods

2.1. Chemicals

Dimethyl sulphoxide, methanol-d_4 (99.8% of deuterium), sodium phosphate monobasic dihydrate, sodium phosphate dibasic, sodium azide, sodium chloride, acarbose, *p*-nitrophenyl α-d-glucopyranoside (PNPG), α-glucosidase type I (EC 3.2.20, from *Saccharomyces cerevisiae*, lyophilized powder), NaCl, 2-chloro-4-nitrophenyl-α-d-maltotrioside (CNP-G3) and α-amylase type VI-B (EC 3.2.1.1, from porcine pancreas, lyophilized powder) were purchased from Sigma-Aldrich (St. Louis, MO). Calcium acetate and formic acid was from Merck (Darmstadt, Germany). HPLC-grade acetonitrile and chloroform were obtained from VWR International (Fontenay-sous-Bois, France). Water was purified by 0.22 μm membrane filtration and deionization (Milli-Q Plus system from Millipore, Billerica, MA, USA).

Table 1
Plants tested for α-glucosidase and α-amylase inhibitory activity

Scientific name	Family	Part used	Voucher #
Nepenthes mirabilis (Lour.) *Druce*	Nepenthaceae	Whole plant	VN-01
Ludwigia octovalvis (Jacq.) P.H. Raven	Onagraceae	Aerial part	VN-02
Phyllanthus amarus Schumach. & Thonn.	Phyllanthaceae	Whole plant	VN-03
Phyllanthus urinaria L.	Phyllanthaceae	Whole plant	VN-04
Phyllanthus reticulatus Poir.	Phyllanthaceae	Stem and leaf	VN-05
Scoparia dulcis L.	Scrophulariaceae	Whole plant	VN-06
Mirabilis jalapa L.	Nyctaginaceae	Whole plant	VN-07
Cassia fistula L.	Leguminosae	Leaf	VN-08
Lagerstroemia speciosa (L.) Pers.	Lythraceae	Leaf	VN-09
Syzygium cumini (L.) Skeels	Myrtaceae	Fruit	VN-10
Euphorbia hirta L.	Euphorbiaceae	Whole plant	VN-11
Rhizophora mucronata Lam.	Rhizophoraceae	Bark	VN-12
Kandelia candel (L.) Druce	Rhizophoraceae	Bark	VN-14
Cardiospermum halicacabum L.	Sapindaceae	Whole plant	VN-14
Pandanus odoratissimus L.f.	Pandanaceae	Fruit	VN-15
Morinda citrifolia L.	Rubiaceae	Fruit	VN-16
Ficus racemosa L.	Moraceae	Fruit	VN-17
Pithecellobium dulce (Roxb.) Benth.	Leguminosae	Stem and leaf	VN-18

2.2. Plant material

A list of 18 species of medicinal plants was obtained based on literature about plants that have been used widely in Vietnamese folk medicine to treat diabetes (Pham, 2007; Vo, 1997). Samples were collected from the Southern part of Vietnam in June 2014, and identified by Son V. Dang, Curator of the VNM Herbarium, Institute of Tropical Biology, Vietnam. Voucher specimens are stored at the General Herbarium of Vascular Plants, National History Museum, University of Copenhagen. All the plant species, the parts used and voucher numbers are presented in Table 1.

2.3. Plant extraction

The chloroform and ethanol extracts were prepared by sonicating 1 g of the dried and coarsely powdered plant material in 10 mL solvent each for 30 min and macerating the material overnight. After filtration through No. 4 filter paper (Whatman, USA), the filtrate was evaporated to dryness under reduced pressure at 35 °C by a Savant SPD121P speed vacuum concentrator (Thermo Scientific, Waltham, MA) coupled with an OFP400 vacuum pump and a RVT400 refrigerated vapor trap. The aqueous extracts were prepared by boiling 1 g of the dried and coarsely powdered plant material with 10 mL water in a flask fitted with reflux condenser for 30 min. The mixture was then filtered and concentrated as described above.

2.4. In vitro α-glucosidase inhibition assay

α-Glucosidase inhibition was assessed using the method described by Schmidt et al. (2012). In brief, 90 μL of 0.1 M phosphate buffer (pH 7.5, 0.02% NaN_3), 10 μL test sample dissolved in DMSO, and 80 μL of enzyme solution (well concentration 0.05 U/mL) were added to each well. The mixture was incubated at 28 °C for 10 min before adding PNPG to a final volume of 200 μL (final well concentration 1.0 mM). The blank was carried out in a similar manner, with the test sample replaced by solvent. The hydrolysis