IN VITRO ANTIHYPERGLYCAEMIC EFFECTS OF SOME MALAYSIAN PLANTS

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MOHAMAD JEMAIN MR, NIK MUSA'ADAH M, ROHAYA A, ABDUL RASHID L & NOR HADIANI I. 2011. *In vitro* antihyperglycaemic effects of some Malaysian plants. Uncontrolled postprandial hyperglycaemia increases the risk of vascular diabetic complications. One of the therapeutic approaches to control postprandial hyperglycaemia is by inhibiting α-amylase and α-glucosidase enzyme activities. In this study, 47 extracts from 22 Malaysian tropical plants were assayed for α-amylase and α-glucosidase inhibitory activities. Four plant species demonstrated potent α-amylase inhibition. The plants were *Burkillantus malaccensis* (stem), *Horsfieldia polyspherula* (leaf and stem), *Labisia pumila* (leaf and root) and *Phyllanthus pulcher* (stem). The IC_{50} values of the extracts ranged from 1.2 to 2.8 μg ml^{-1}. Four plant species could be classified as strong α-glucosidase inhibitors with IC_{50} values ranging from 2.4 to 6.1 μg ml^{-1}. The plants were *Gironniera parvifolia* (stem), *H. polyspherula* (leaf and stem), *P. pulcher* (leaf and stem) and *Rothmannia schoemanii* (stem). Extracts of *P. pulcher* (stem) and *H. polyspherula* (leaf and stem) showed potent inhibitory activity against both enzymes. The plant extracts are potentially useful in the development of new antidiabetic remedies.

Keywords: Diabetes, postprandial hyperglycaemia, α-amylase, α-glucosidase

MOHAMAD JEMAIN MR, NIK MUSA'ADAH M, ROHAYA A, ABDUL RASHID L & NOR HADIANI I. 2011. Kesan antihiperglisemik in vitro beberapa tumbuhan Malaysia. Hiperglisemia selepas makan yang tidak terkawal meningkatkan risiko komplikasi diabetik vaskular. Salah satu pendekatan terapeutik untuk mengawal hiperglisemia selepas makan adalah dengan merencat aktiviti enzim α-amilase dan α-glukosidase. Dalam kajian ini, 47 ekstrak daripada 22 tumbuhan tropika Malaysia dikaji aktiviti perencatan enzim α-amilase dan α-glukosidase. Sebanyak empat spesies menunjukkan perencatan α-amilase yang poten. Tumbuhan tersebut ialah *Burkillantus malaccensis* (batang), *Horsfieldia polyspherula* (daun dan batang), *Labisia pumila* (daun dan akar) dan *Phyllanthus pulcher* (batang). Nilai IC_{50} bagi ekstrak tersebut berjulat antara 1.2 μg ml^{-1} hingga 2.8 μg ml^{-1}. Sebanyak empat spesies boleh dikelaskan sebagai perencat α-glukosidase yang kuat dengan IC_{50} berjulat antara 2.4 μg ml^{-1} hingga 6.1 μg ml^{-1}. Tumbuhan tersebut ialah *Gironniera parvifolia* (batang), *H. polyspherula* (daun dan batang), *P. pulcher* (daun dan batang) dan *Rothmannia schoemanii* (batang). Ekstrak *P. pulcher* (batang) and *H. polyspherula* (daun dan batang) menunjukkan aktiviti perencatan yang poten bagi kedua-dua enzim. Ekstrak tumbuhan ini berpotensi untuk digunakan dalam pembangunan ubat antidiabetik yang baharu.

INTRODUCTION

Investigations into sources of natural antidiabetic agents are an important research area in order to provide humankind with new and safer therapeutic products. Diabetes is a well-known disorder of carbohydrate metabolism characterised by hyperglycaemia, which results from defects in insulin secretion, insulin action or both (Henquin 2000). Statistically, more than 180 million people worldwide are suffering from diabetes with 5% deaths globally each year. Without intervention, this number may double by 2030 (World Health Organization 2005). Plant-based materials have been used in the treatment of diabetes since ancient times. More than 1200 plants have been used traditionally as antidiabetic remedies and approximately 30% of the plants have been scientifically investigated (Alarcon-Aguilar et al. 2002). However, there are many plants being used in the treatment of diabetes, especially in traditional medicine, without any scientific evidence.

Natural antidiabetic remedies from plants are gaining popularity because of fewer side effects as compared with modern drugs (Modak et al. 2007). This has attracted scientists to explore natural antidiabetic remedies. Numerous research studies have been carried out on plant extracts that demonstrated various activities related to

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diabetes, for example the ability to increase insulin secretion, improve glucose uptake in peripheral tissues and inhibit digestive enzyme activity (Bnouham et al. 2006). *Andrographis paniculata* was reported to inhibit α-amylase and α-glucosidase enzymes *in vitro*. It significantly reduced blood glucose level in normal and streptozocin-induced diabetes rats, illustrating the potential of plants as sources of compounds useful as antidiabetic agents (Subramanian et al. 2008).

Discovery of natural antidiabetic agents from higher plants could be based on random selection of plants followed by chemical screening together with one or more biological assays (Pieters & Vlientinck 2005). In this study, 22 randomly selected Malaysian tropical plants were screened for antidiabetic properties using *in vitro* α-amylase and α-glucosidase inhibition assays.

MATERIALS AND METHODS

Plant materials

Plant samples were collected randomly during a research expedition at Kuala Keniam, Taman Negara Pahang, Malaysia. The plants were identified by K Shamsul of the Institute Bioscience, Universiti Putra Malaysia. The stems, leaves and roots of the plants were used in this study. Plant samples were cut into small pieces and dried for one week at room temperature. The dried parts were ground into powder using a milling machine with pore size 10 mm. The ground samples (15 g) were soaked in dichloromethane (100 ml) at room temperature for 72 hours. The solvent was evaporated to dryness under vacuum at 40 °C. Dichloromethane was chosen as the extraction solvent in order to avoid extracting the more polar glycosidic compounds. Presence of glycosides in the extract may interfere with the assay as they complicate the quantification of maltose, produced in the α-amylase inhibition assay (Hasenah et al. 2006).

Alpha-amylase inhibitory activity assay

The α-amylase inhibition assay was adopted from Hasenah et al. (2006) with slight modifications. Stock solutions of plant extracts in dimethyl sulfoxide (DMSO) were prepared at concentration of 156 μg ml^{-1}. The sample solution (40 μl) and porcine pancreatic α-amylase enzyme (type VI, Sigma) at concentration of 4 unit ml^{-1} (200 μl) were mixed in 1.5 ml Eppendof tube and incubated at 25 °C. After 5 min, 160 μl of distilled water and 400 μl of starch solution (ACS reagent, 0.5% w/v in 20 mM phosphate buffer, pH 6.9) were added to give a final concentration of plant extract of 7.8 μg ml^{-1}. The reaction mixture was then incubated at 25 °C for 7 min. After incubation, 200 μl of the reacted mixture were transferred into another Eppendof tube containing 100 μl of 3, 5-dinitrosalicylic acid colour reagent solution (96 mM 3, 5-dinitrosalicylic acid and 5.31 M sodium potassium tartrate in 2 M NaOH). All Eppendof tubes were placed in a water bath at 80 °C. After 15 min, 900 μl deionised water were added. The absorbance of the mixture was measured using a spectrophotometer (λ = 540 nm). For positive control, the plant extract was replaced with acarbose, while for control (represents 100% enzyme activity) the sample was replaced with DMSO. For blank, the α-amylase enzyme solution was replaced with deionised water. The final control ($A^{control}$) and test sample ($A^{test\ sample}$) absorbance values were obtained by subtracting their corresponding sample blank readings. The percentage inhibition of the α-amylase inhibitory activity was calculated as

$$\frac{A^{control} - A^{test\ sample}}{A^{control}} \times 100$$

A is the absorbance of the test mixture at wavelength of 540 nm.

Alpha-glucosidase inhibitory activity assay

The α-glucosidase enzyme inhibitory activity assay was adopted from Lee et al. (2008) with slight modifications. Stock solutions of plant extracts were prepared in DMSO at concentration of 100 μg ml^{-1}. The sample solution (10 μl), α-glucosidase enzyme, type 1 from Baker's yeast (20 μl), phosphate buffer saline (pH 6.5) (40 μl) and deionised water (20 μl) were mixed in 96-well plate. The mixture was incubated at 37 °C. After 10 min, 10 μl of 20 mM p-nitrophenyl-α-D-glucopyranoside solution were added into the mixture to give a final concentration of 10.0 μg ml^{-1} plant extract. The absorbance of the reaction mixture was measured immediately using spectrophotometer (λ = 405 nm). The reaction mixture was incubated at 37 °C. After 30 min, the absorbance was measured

again. The percentage inhibitory activity was calculated as

$$\frac{\Delta A^{control} - \Delta A^{test\ sample}}{\Delta A^{control}} \times 100$$

A is the absorbance of the test mixture at wavelength of 405 nm.

Statistical analysis

IC_{50} values were calculated using GraphPad Prism 5 software. The data were expressed as mean ± SEM duplicate measurement in each of the three independent experiments.

RESULTS AND DISCUSSION

Alpha-amylase inhibitor is one of the therapeutic agents used in the treatment of diabetes (Boivin et al. 1988). It functions by inhibiting the production of glucose through inhibiting the action of salivary and pancreatic α-amylase enzyme and breaking down long-chain carbohydrates to simpler sugars. Inhibitors help in lowering glucose production, regulating proper glucose metabolism and lowering postprandial glycaemic level, thus contributing to the management of diabetes. In our search for new natural inhibitors, we screened 22 Malaysian tropical plants for α-amylase inhibitory activity. Screening results of the 47 extracts at final concentration of 7.8 μg ml^{-1} for *in vitro* porcine pancreatic α-amylase inhibitory activity are shown in Table 1. Seven extracts exhibited no α-amylase inhibitory activity while 40 extracts showed percentage inhibition ranging from 2.0 to 97.5%. Six extracts representing various parts of four plant species were identified as potent α-amylase inhibitor with percentage inhibition above 70%. The plant species were *Burkillantus malaccensis* (stem 95.3%), *Horsfieldia polyspherula* (leaf 90.4% and stem 96.4%), *Labisia pumila* (leaf 97.5% and root 97.2%) and *Phyllanthus pulcher* (stem 91.4%).

The IC_{50} of acarbose (positive control) was 11.4 μg ml^{-1} while the IC_{50} values of all the test extracts ranged from 1.2 to 2.8 μg ml^{-1}, showing that they were stronger α-amylase inhibitor compared with the positive control (Table 2). The root extract of *L. pumila* showed the strongest inhibitory activity with IC_{50} value 1.2 μg ml^{-1}, followed by *L. pumila* leaf (1.5 μg ml^{-1}), *H. polyspherula* stem (1.6 μg ml^{-1}), *B. malaccensis* stem (1.8 μg ml^{-1}), *H. polyspherula* leaf (2.4 μg ml^{-1}) and *P. pulcher* stem (2.8 μg ml^{-1}). The inhibitory activity may be due to the presence of inhibitors, capsulation of starch and enzymes, reduced accessibility of the enzyme to starch or direct adsorption of enzymes on extracts leading to decrease in α-amylase activity (Isaksson et al. 1982, Gourgue et al. 1992, Ou et al. 2001, Chau et al. 2004). The plant extracts with potent α-amylase inhibition property may have potential application in effective management of postprandial hyperglycaemia.

Besides α-amylase, α-glucosidase enzyme is also involved in carbohydrate metabolism to form glucose as the ultimate end-product. Inhibition of this enzyme is another useful approach in the management of diabetes (Stuart et al. 2004). Plants have also been recognised as a source of α-glucosidase inhibitor (Shim et al. 2003). In this study, the plants were screened for α-glucosidase inhibitory activity. The α-glucosidase inhibitory activities of the 47 extracts at final concentration of 10.0 μg ml^{-1} are shown in Table 1. Twenty-four extracts showed α-glucosidase inhibitory activity with percentage inhibition varying from 1.8 to 97.0%. Six extracts possessed potent inhibitory activity with percentage inhibition above 70%. The extracts were *Gironniera parvifolia* (stem 89.4%), *H. polyspherula* (leaf 90.0% and stem 91.1%), *P. pulcher* (leaf 84.2% and stem 97.0%) and *Rothmannia schoemanii* (stem 75.2%). The IC_{50} values of these plant extracts are shown in Table 2. The IC_{50} values exhibited by the extracts ranged from 2.4 to 6.1 μg ml^{-1} (Table 2). *Horsfieldia polyspherula* stem extract showed the lowest IC_{50} value (2.4 μg ml^{-1}), followed by *P. pulcher* stem (3.7 μg ml^{-1}), *H. polyspherula* leaf (4.2 μg ml^{-1}), *P. pulcher* leaf (5.5 μg ml^{-1}), *G. parvifolia* stem (6.1 μg ml^{-1}) and *R. schoemanii* stem (6.1 μg ml^{-1}). The extracts may contain valuable compounds able to inhibit α-glucosidase enzyme from hydrolysing oligosaccharides to non-reducing α-D-glucose. If this inhibition activity takes place *in vivo*, the glucose production and its absorption in blood stream will be delayed, resulting in lowering of postprandial glucose levels. Extracts of *Commelina communis* were reported showing potent *in vitro* α-glucosidase inhibitory activity which reduced blood glucose level in diabetic mice after maltose loading, illustrating the correlation between *in vitro* and *in vivo* activities (Youn et al. 2004).

Table 1 Inhibitory activities of 47 extracts from 22 Malaysian tropical plants

Species / Family	Plant part	% Inhibition[a]	
		α-amylase[b]	α-glucosidase[c]
Burkillanthus malaccensis	Stem	95.3 ± 2.1	33.6 ± 1.9
Rutaceae			
Cananga odorata	Leaf	22.6 ± 1.3	Nil [d]
Annonaceae	Stem	25.3 ± 3.3	Nil
Ficus deltoidea	Leaf	56.9 ± 2.7	10.0 ± 0.9
Moraceae	Stem	61.4 ± 5.1	18.9 ± 0.9
Gironniera parvifolia	Leaf	24.6 ± 3.5	Nil
Ulmaceae	Stem	52.6 ± 5.3	89.4 ± 4.3
Goniothalamus macrophyllus	Leaf	4.8 ± 1.1	Nil
Annonaceae	Stem	Nil	6.4 ± 0.1
Goniothalamus scortechinii	Leaf	29.5 ± 2.8	Nil
Annonaceae	Stem	17.6 ± 4.6	14.8 ± 1.0
Horsfieldia polyspherula	Leaf	90.4 ± 3.8	90.0 ± 3.0
Myristicaceae	Stem	96.4 ± 2.7	91.1 ± 4.9
Labisia pumila	Leaf	97.5 ± 1.9	38.7 ± 0.8
Myrsinaceae	Root	97.2 ± 1.0	29.1 ± 2.8
Lasianthus constrictus	Leaf	Nil	Nil
Rubiaceae	Stem	16.3 ± 3.8	Nil
Lasianthus maingayi	Leaf	58.8 ± 2.8	Nil
Rubiaceae	Stem	34.0 ± 1.5	Nil
	Root	2.0 ± 4.8	13.4 ± 2.5
Leea indica	Leaf	30.3 ± 4.2	Nil
Leeaceae	Stem	25.5 ± 3.5	Nil
Mycetia malayana	Leaf	22.3 ± 1.1	Nil
Rubiaceae	Stem	52.7 ± 3.7	52.7 ± 2.5
	Root	11.4 ± 0.9	15.1 ± 1.7
Myristica cinnamomea	Leaf	Nil	1.8 ± 2.0
Myristicaceae	Stem	Nil	Nil
Ophiorrhiza communis	Leaf	50.9 ± 3.5	Nil
Rubiaceae	Stem	12.5 ± 1.4	32.2 ± 1.3
	Root	13.3 ± 1.4	14.6 ± 1.4
Oxyspora curtisii	Leaf	24.8 ± 1.8	Nil
Melastomataceae	Stem	26.3 ± 7.5	22.7 ± 3.6
Phyllanthus pulcher	Leaf	36.0 ± 2.0	84.2 ± 2.4
Euphorbiaceae	Stem	91.4 ± 2.9	97.0 ± 1.0
Psychotria malayana	Leaf	15.7 ± 0.8	Nil
Rubiaceae	Stem	18.6 ± 1.1	Nil
	Root	19.5 ± 2.6	Nil
Psychotria sp.	Leaf	44.9 ± 1.8	Nil
Rubiaceae	Stem	19.1 ± 4.2	32.9 ± 2.4
Rothmannia schoemanii	Leaf	47.1 ± 3.4	45.2 ± 2.2
Rubiaceae	Stem	47.2 ± 1.8	75.2 ± 6.9
Sterculia coccinea	Leaf	Nil	Nil
Sterculiaceae	Stem	32.0 ± 2.3	43.8 ± 3.9
Urophyllum hirsutum	Leaf	38.1 ± 2.3	Nil
Rubiaceae	Stem	36.9 ± 1.5	Nil
Urophyllum villosum	Leaf	Nil	3.0 ± 0.8
Rubiaceae	Stem	Nil	Nil
Acarbose (positive control)		32.2 ± 0.26	Nil

[a] Inhibition (%) ± SEM duplicate measurement in each of three independent experiments.
[b] Percentage inhibition of α-amylase activity was measured at extract concentration of 7.8 μg ml^{-1}.
[c] Percentage inhibition of α-glucosidase activity was measured at extract concentration of 10.0 μg ml^{-1}.
[d] Nil represents no inhibition.

Table 2 IC_{50} data of promising plant species with their plant parts that produced high inhibitory activities

Species	Plant part	IC_{50} (μg ml^{-1}) ± SEM	
		α-amylase	α-glucosidase
Burkillanthus malaccensis	Stem	1.8 ± 0.3	nt
Gironniera parvifolia	Stem	[a] nt	6.1 ± 0.7
Horsfieldia polyspherula	Leaf	2.4 ± 0.4	4.2 ± 0.9
	Stem	1.6 ± 0.2	2.4 ± 0.4
Labisia pumila	Leaf	1.5 ± 0.1	nt
	Root	1.2 ± 0.1	nt
Phyllanthus pulcher	Leaf	nt	5.5 ± 1.0
	Stem	2.8 ± 0.6	3.7 ± 0.4
Rothmannia schoemanii	Stem	nt	6.1 ± 0.1
Acarbose (positive control)		11.4 ± 0.5	2.8 ± 0.2

[a] nt = extract not tested; IC_{50} refers to the concentration of inhibitors that produces 50% inhibition of the initial rate of reaction.

The present study revealed that two plant species possessed strong inhibitory activities against both α-amylase and α-glucosidase enzymes. They were *H. polyspherula* (leaf and stem) and *P. pulcher* (stem). This paper is the first to report the α-amylase and α-glucosidase inhibitory activities of these plants. *Horsfieldia polyspherula* has not been studied previously for biological activity and no traditional medicinal uses have been reported. *Phyllanthus pulcher* has been used traditionally for treating toothache, nose ulcer, swelling, abscesses, carbuncles, pruritus, fever, renal disorders and gastalgia (Ami et al. 2002). Dichloromethane extract of *P. pulcher* has been reported to show antitumour activity (Stanslas et al. 2008).

The results of this study have demonstrated the potential of Malaysian tropical plants as sources of α-amylase and α-glucosidase inhibitors. In the screening phase, four extracts were identified to possess significant α-amylase inhibitory activity and six extracts showed significant α-glucosidase inhibitory activity. *Phyllanthus pulcher* (stem) and *H. polyspherula* (leaf and stem) showed strong inhibition for both enzymes. The chemical constituent of *H. polyspherula* has never been studied. The roots of *P. pulcher* were reported to contain pentacyclic triterpenes with cytotoxic properties (Bagalkotkar et al. 2011). However, the antidiabetic properties has never been reported. In conclusion, *H. polyspherula* and *P. pulcher* are promising sources of α-amylase and α-glucosidase inhibitors worthy of further investigations. These two plants may also become potential candidates for development of new botanical drugs or functional food products.

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