Natural Product Sciences
13(4) : 311-316 (2007)

α-Amylase and Protein Tyrosine Phosphatase 1B Inhibitory of Some Vietnamese Medicinal Plants Used to Treat Diabetes

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Abstract – In this study, the twenty-four ethyl acetate extracts of twenty-two medicinal plants, traditionally used in Vietnam as anti-diabetes agents, were investigated for α-amylase and protein tyrosine phosphatase 1B (PTP1B) enzymes inhibitory activity *in vitro*. The results indicated that, twelve materials (50.0%) showed moderate to strong inhibitory activity in α-amylase inhibitory activity with IC_{50} values ranging from 2.5 to 48.8 μg/mL; meanwhile, ten extracts (41.6%) could demonstrate PTP1B activity with IC_{50} values less than 30.5 μg/mL. Some plants presented interesting activities against both of α-amylase and PTP1B enzymes such as *Catharanthus roseus, Carthamus tinctorius, Momordica charantia, Gynostemma pentaphyllum, Glycyrrhiza glabra, Smilax glabra, Psidium guajava* (leave), and *Rehmannia glutinosa*. The study may provide a proof, at least in a part, for the ethno-medical use in diabetes disease of these plants.
Keywords – Vietnamese medicinal plants, diabetes, α-amylase, protein tyrosine phosphatase 1B

Introduction

Diabetes mellitus is only major diseases that is becoming more common, in part because of the ageing population, a lifestyle that promotes obesity, a growing Hispanic community that has a particularly prevalence of diabetes, and more poor people than the national average (King *et al*., 1998). The real problem of diabetes is that the condition brings with it a train of chronic complications including accelerated arteriosclerosis, and disease of the eye, foot, and kidney, each of which is costly to manage and can be devastating from the patient. More than 80% of people with type 2 diabetes will live in developing countries (White and Rafique, 2002). Those of under-developed countries including Vietnam cannot afford the increasing burden of chronic renal failure and blindness. The large poor populations has lower prevalence rates than the rich, but have higher rates of complications because of later diagnosis, inaction on risk factors, and poor management. In 1990s, about 1-1.5% Vietnamese people were affected by diabetes which would increased to 4% by 2001 but at least 70% patients were clearly not excavated and had right treatment (Vietnam National Diabetes Federation Conference, June 2003). Therefore, there is an urgent need to develop necessary therapies for these diseases.

One of the therapeutic approaches for treating diabetes is to decrease the postprandial hyperglycemia. This is done by retaining the absorption of glucose through the inhibition of the carbohydrate-hydrolyzing enzymes, α-amylase and α-glucosidase, in the digestive tract (Ali *et al*., 2006). Inhibitors of these enzymes delay carbon-hydrate digestion and prolong overall carbohydrate digestion time, causing a reduction in the rate of glucose absorption and consequently blunting the post-prandial plasma glucose rise (Defronzo *et al*., 2004). Recently, protein tyrosine phosphatase1B (PTP1B) has been shown to be a negative regulator of the insulin signaling pathway, suggesting that inhibitors of this enzyme may be a promising therapeutic target in effective treatment of type 2 diabetes. PTP1B is a major nontransmembrane phosphotyrosine phosphatase in human tissues and was one of the earliest PTP identified. PTP1B inhibitors would increase insulin sensitivity by blocking the PTP1B-mediated negative insulin signaling pathway and might be an attractive target in type 2 diabetes mellitus and obesity

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(Kennedy, 1999; van Huijsduijnen *et al*., 2002).

Research on plants used in traditional medicine as anti-diabetes offers an alternative to the development of new drugs and/or validation of their use in folk medicine (Grover *et al*., 2002). In Vietnam, no less than 2500 species have been used in ethno medicine and approximately 30 species were used for treatment of diabetes (Loi, 2001). Although these have been used as folk medicines for a long time, they are still untapped source for potential bioactive agent. Therefore, it is expected that medicinal plants not only continue to be used traditionally but also be a useful source of drug discovery. In this study, 22 Vietnamese anti-diabetes plants belong to 16 families were investigated on the basic of their used in traditional medicine with the aim of characterizing on inhibition of α-amylase and PTP1B.

Experimental

Plant material – Most of the medicinal plants were collected in the North of Vietnam in spring, 2006. Some of them were purchased at Dongxuan oriental herbarium market in Hanoi, Vietnam at the same time. The plants were botanically identified by Professor Pham Thanh Ky, Department of Pharmacognosy, Hanoi College of Pharmacy, Vietnam where the voucher specimens were deposited.

Preparation of plant extracts – The collected plants were dried and powdered. Twenty gram of each material was extracted with boiled ethyl acetate for 2 hours under reflux two times. The obtained extracts were filtered through Whatman No. 2 filter paper, and then freeze-dried at 40 °C Both extracts were stored at –4 °C until used. The ethyl acetate extract was selected and tested for inhibitory effects because of the containing negligible amount of sugar compounds, which would cause complication in the maltose detection.

α-amylase inhibitory activity – The α-amylase assay was performed using the chromogenic method adopted from Sigma-Aldrich with slight modification. A potato starch solution (0.5% w/v) was obtained by stirring potato starch in 20 mM sodium phosphate buffer with 6.7 mM sodium chloride, pH 6.9 at 65 °C for 15 min (R1). The enzyme solution was prepared by mixing α-amylase in ice-cold distilled water to give a concentration of 4 unit/mL (R2). Those extract of collected plants were dissolved in DMSO to give various concentrations. The colorimetric reagent (DNS) solution was prepared mixing 96 mM 3,5-dinitrosalicylic acid and 5.31 M sodium potassium tartrate in 2 M NaOH (R3). In the experiment, 40 μL of extract, 160 μL distilled water, 400 μL R1 and 200 μL R2 were mixed and incubated at 25 °C for 3 min. After incubation, 200 μL of mixture was removed and added into other separate tube which containing 100 μL R3. The mixture was boiled at 85 °C for 15 min, and then diluted with 900 μL distilled water. The reaction was detectable at 540 nm. The control incubations were conducted with the same method but replacing extract samples with 40 μL DMSO. The blank incubation tubes were carried out as above but replacing extract samples by 40 μL DMSO and R2 was replaced by distilled water. Ursolic acid was used as positive control (Ali *et al*., 2006).

Protein tyrosine phosphatase 1B (PTP1B) inhibitory activity – PTP1B (human, recombinant) was purchased from BIOMOL® International LP (USA) and the enzyme activity were measured using *p*-nitrophenyl phosphate (*p*-NPP) as a substrate (Na *et al*., 2006). To each 96-well (final volume: 200 μL) were added 2 mM *p*-NPP and PTP1B (0.05 - 0.1 μg) in a buffer containing 50 mM citrate (pH 6.0), 0.1 M NaCl, 1 mM EDTA, and 1 mM dithiothreitol (DTT) with or without test compounds. Following incubation at 37 °C for 30 min, the reaction was terminated with 10 M NaOH. The amount of produced *p*-nitro phenol was estimated by measuring the absorbance at 405 nm. The nonenzymatic hydrolysis of 2 mM *p*-NPP was corrected by measuring the increase in absorbance at 405 nm obtained in the absence of PTP1B enzyme. RK-682 used as a positive control (Hamaguchi *et al*., 1995).

Statistics analysis – The results were expressed as mean ± S.D. of three determinations at each concentration for each sample. The inhibitory concentration 50% (IC_{50}) was calculated using Microsoft Excel program. Statistical significance was calculated by one-way analysis of variance (ANOVA), followed by Dunnett's test.

Results and discussion

The plants are listed in alphabetical order of their family name, followed by the scientific name, vernacular name, as well as part used (Table 1). In the present study, twenty-two plant species which belonging to sixteen families were selected, and total of twenty-four extracts were investigated based on their ethno-medical use for the treatment of diabetes and other diseases by the natives of Vietnamese folk medicinal systems (Loi, 2001). The inhibitory effect of twenty-four extracts on α-amylase and PTP1B activities are summarized (Table 2).

About α-amylase inhibitory activity, the results indicated that, the ethyl acetate extract of 12 materials (50.0%)

Table 1. Vietnamese medicinal plants in this study

No.	Family/scientific name	Vernacular name	Part used	Extract yielded (%w/w)[a]
	Amaryllidaceae			
01	*Allium cepa*	Hanh tay	Root	0.63
	Apocynaceae			
02	*Catharanthus roseus*	Dua can	Whole plant	5.52
	Araliaceae			
03	*Panax ginseng* C. A. Mey	Nhan sam	Root	0.71
	Asteraceae			
04	*Atractylodes macrocephala*	Bach truat	Rhizome	1.10
05	*Carthamus tinctorius* L	Hong hoa	Flower	2.35
06	*Stevia rebaudiana* Bertoni	Co ngot	Whole plant	1.27
	Convolvulaceae			
07	*Ipomoea batatas* (L.) Lam.	Khoai lang	Rhizome	0.66
	Cucurbitaceae			
08	*Momordica charantia*	Muop dang	Fruit	1.57
09	*Trichosanthes japonica* Regel	Thien hoa phan	Root	1.15
10	*Gynostemma pentaphyllum*	Giao co lam	Whole plant	1.28
	Dicksoniaceae			
11	*Cibotium barometz* (L) J.Sm	Cau ki	Rhizome	1.10
	Dioscoreaceae			
12	*Dioscorea persimilis*	Hoai son	Rhizome	1.15
	Dilleniaceae			
13	*Tetracera scandens* (L.) Merr.	Chac chiu	Stem	2.37
	Fabaceae			
14	*Glycyrrhiza glabra*	Cam thao	Root	5.85
	Nymphaeaceae			
15	*Euryale ferox* Salisb	Khiem thuc	Seed	1.30
	Myrtaceae			
16	*Psidium guajava*	Oi	Flower	6.60
17			Leaf	2.82
18			Stem	0.37
	Liliaceae			
19	*Asparagus cochinchinensis*	Thien mon	Root	0.85
20	*Polygonatum odoratum*	Ngoc truc	Rhizome	0.95
21	*Smilax glabra*	Tho phuc linh	Rhizome	1.72
	Scrophulariaceae			
22	*Rehmannia glutinosa* Libosch	Sinh dia hoang	Rhizome	0.15
	Solanaceae			
23	*Lycium chinensis* Mill	Dia cot tu	Rhizome	0.78
	Verbenaeae			
24	*Lantana camara*	Bong oi	Flower	3.41

[a] Percentage extract yield (w/w) was calculated as (dry extract weight/dry starting material weight) × 100.

showed moderate to strong inhibitory activity with IC_{50} values ranging from 2.5 to 48.8 μg/mL in the comparison with ursolic acid (IC_{50} = 5.3 ± 0.4 μg/mL) which was used as positive control. These plants include *Allium cepa, Catharanthus roseus, Panax ginseng*, *Carthamus tinctorius, Stevia rebaudiana, Momordica charantia, Gynostemma pentaphyllum, Glycyrrhiza glabra*, *Psidium guajava* (leaf), *Smilax glabra, Rehmannia glutinosa,* and *Lycium chinensis*. Among them, extract of *Catharanthus roseus* and *Carthamus tinctorius* exhibited strongest activity with IC_{50} values of 2.5 ± 0.5 and 2.5 ± 0.4 μg/mL, respectively, followed by the extract of *Momordica charantia* (IC_{50} = 4.1 ± 0.8 μg/mL), *Allium cepa* (IC_{50} = 9.8 ± 1.4 μg/mL) and *Panax ginseng* (IC_{50} = 11.7 ± 2.1 μg/mL).

Of the 24 extracts assayed, only 10 extracts (41.6%) could demonstrate PTP1B activity with IC_{50} values less than 30.0 μg/mL (Table 2). The result indicated that the extracts of *Glycyrrhiza glabra* possessed the most potent effect with IC_{50} as 3.7 ± 0.3 μg/mL, followed by *Tetracera scandens* (IC_{50} = 5.2 ± 0.7 μg/mL), *Catharanthus roseus* (IC_{50} = 7.5 ± 0.7 μg/mL), and *Carthamus tinctorius* (IC_{50} = 10.5 ± 0.8 μg/mL). Some other plants as *Gynostemma*

Table 2. Inhibition activity of extracts

Plant	Inhibitory activity (IC_{50} μg/mL)[a]	
	α-amylase	PTP1B
Allium cepa	9.8 ± 1.4[†]	–
Catharanthus roseus	2.5 ± 0.5[†]	7.5 ± 0.7[*]
Panax ginseng	11.7 ± 2.1[†]	–
Carthamus tinctorius	2.5 ± 0.4[†]	10.5 ± 0.8[*]
Stevia rebaudiana	28.6 ± 4.0[†]	–
Momordica charantia	4.1 ± 0.8[†]	16.2 ± 1.4[*]
Gynostemma pentaphyllum	25.7 ± 2.1[†]	12.1 ± 1.0[*]
Tetracera scandens	–	5.2 ± 0.7[*]
Glycyrrhiza glabra	36.2 ± 4.1[†]	3.7 ± 0.3[*]
Psidium guajava (leaf)	48.8 ± 3.6[†]	15.2 ± 1.1[*]
Smilax glabra	15.7 ± 3.9[†]	28.6 ± 2.6[*]
Rehmannia glutinosa	35.5 ± 2.9[†]	28.2 ± 2.5[*]
Lycium chinensis	42.1 ± 3.6[†]	–
Lantana camara	–	16.0 ± 1.4[*]
Ursolic acid[b]	5.3 ± 0.4	ND
RK-682[b]	ND	4.5 ± 0.5

[a] IC_{50} values were determined by the regression analyses and expressed as means ± SD of three replicates.
[b] Positive controls.
[†] $p < 0.05$ vs. ursolic acid.
[*] $p < 0.05$ vs. RK-682.
(–) Very weak inhibitory or no activity.
ND Not determine.

pentaphyllum, Momordica charantia, Psidium guajava (leaf), *Smilax glabra, Rehmannia glutinosa* and *Lantana camara* expressed inhibitory activity with IC_{50} values ranging from 12.1 to approximately 30.0 μg/mL. In this experiment, RK-682 (IC_{50} = 4.5 ± 0.5 μg/mL) was used as positive control. The other plants were apparently inactive or very weak activity with IC_{50} > 30.0 μg/mL).

Catharanthus roseus is an important medicinal plant which can against normal and streptozotocin-induced diabetic rat models by blood sugar lowering capacity, and its antidiabetic activity seems to be a result of increase in glucose utilization. (Chattopadhyay, 1999; Singh *et al*., 2001). The rich of flavonoids and indole alkaloids containing in this plant may have revealed corresponding for these activity (Schroder *et al*., 2004; Tikhomiroff and Jolicoeur, 2002). For the plant *Carthamus tinctorius*, the anti-diabetic composition comprises shows a high anti-hyperglycemic effect and a maltase-inhibitory or -reducing effect (Satoru *et al*., 2005). These activities could be linked to the presence and structural transformation of lignans, quinochalcone and flavonoid in the petals (Nose *et al*., 1992; Meselhy *et al*., 1993).

Some of the other plants presented interesting activities against both of α-amylase and PTP1B enzymes such as *Momordica charantia, Gynostemma pentaphyllum, Glycyrrhiza glabra, Smilax glabra, Psidium guajava,* and *Rehmannia glutinosa. Momordica charantia* was used in various systems of traditional medicine for diabetes and its complications as nephropathy, cataract, insulin resistance (Grover and Yadav, 2004; Ahmed *et al*., 2001; Welihinda *et al*., 1986). *Gynostemma pentaphyllum* is a traditional agent for treatment of elevated cholesterol (Djang, 2005). Its extract inhibited α-glucosidase activity, and has effectiveness in obese Zucker fatty diabetic rat model (Megalli *et al*., 2006). Recent published paper showed that phanoside, a gypenoside isolated from this plant, stimulates insulin secretion from rat pancreatic islets (Hoa *et al*., 2007). *Glycyrrhiza glabra* is the licorice plant, and has a history of consumption like one of the most frequently employed botanicals in traditional medicines. Glabridin, the major flavonoid of licorice was investigated on abdominal fat accumulation and blood glucose level in obese diabetic KK-Ay mice (Nakagawa *et al*., 2004). The hypoglycemic effect of the rhizomes of *Smilax glabra* was also investigated in normal and KK-Ay mice by reduced the blood glucose (Fukunaga *et al*., 1997). In the case of *Psidium guajava,* its leaves presented hypoglycemic activity on alloxan-induced diabetic rats in both acute and sub-acute tests (Mukhtar *et al*., 2004) and possessed antidiabetic effect in type 2 diabetic mice model via the inhibition of PTP1B (Oh *et al*., 2005). The potent antiglycative agent and anticoagulant of guava leaves, which can be great value in the preventive glycation-associated cardiovascular diseases in diabetes, due to its α-dicarbonyl compounds (Hsieh *et al*., 2007). The radix of *Rehmannia glutinosa* is one component of the Seishin-kanro-to which used in patients with diabetes traditional medicine (Miura *et al*., 1997). In alloxan-induced diabetic rats, its oligosaccharide showed a significant decrease in blood glucose level, hepatic glucose-6-phosphatase activity with an increase in hepatic glycogen content, raised plasma insulin level and lowered plasma corticosterone level (Zhang *et al*., 2004).

In Vietnam, the use of those medicinal plants and herbal therapy has been practiced long before recorded history. However, the potential use of them or other anti-diabetes plants as the source of new drugs is still poorly explored. In most case, only pharmacological screening or preliminary studies have been carried out. It is possible to note that alkaloids, flavonoids, terpenoids, lignans and/or other phytochemical constituents containing in those plant play important role for their biological activities. In this study, some of the selected plants could not manifest the

ability effects but some of them presented interesting *in vitro* activities against one kind of enzyme or both of α-amylase and PTP1B enzymes. This may suggest that these plant extract might be interacting with the enzymes in different mechanisms. The action of some plant extracts on the inhibition of α-amylase enzyme is somewhat well established, however, the inhibitory ability in PTP1B enzyme did not presented clearly. Except the extract of *Psidium guajava* leaves, which could present anti-diabetic effect via the inhibition of PTP1B (Oh *et al*., 2005), this is the first time the inhibitory activity of *Catharanthus roseus, Carthamus tinctorius, Momordica charantia, Gynostemma pentaphyllum, Tetracera scandens, Glycyrrhiza glabra, Smilax glabra, Rehmannia glutinosa,* and *Lantana camara* in PTP1B were exhibited. Specially, *Gynostemma pentaphyllum* extract reportedly have many effects, such as lowered cholesterol, immunopotentiation, as well as antitumor, antioxidant, and hypoglycemic effects. A large group of substances in this extract is saponins as gypenosides which can be representatives of gypenosides from ginseng (Norberg *et al*., 2004). Other example may be listed with *Tetracera scandens* which are mainly used for treatment of inflammation; it is possible that this extract may reduce the effect of inflammatory cytokine release during diabetes, one of the causative agents for the tissue distraction and insulin resistance. However, no report on principles of this plant has been documented.

It is intended to provide an anti-diabetic drug containing active ingredient originating in natural plant which can be obtained and taken in daily diet, inhibits hyperglycemia after eating to thereby efficaciously relieve the onset of diabetes or the symptoms thereof, and is highly safe and less expensive. In conclusion, we have carried out a systematic investigation of some Vietnamese medicinal plants for α-amylase and PTP1B inhibitory activity. The results indicate a number of medicinal plants that may be useful for the treatment of diabetes, and provide the basis for further investigation on these medicinal plant species to isolate active constituents and drug development.

Acknowledgments

We are grateful to Pharmacist Vu Thi Hang for her kind help in collecting and providing plant materials.

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(Accepted October 14, 2007)