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Original research article

In vitro Inhibitory Efficiency of *Ventilago denticulata* Willd. Dried Leaves Extract on Alpha-glucosidase, Alpha-amylase and Lipase and Antioxidant Activities

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ABSTRACT

Ventilago denticulata Willd. has been used in many countries in Southeast Asia and South Asia for the folk medicine to decrease blood sugar and cholesterol. Therefore, the inhibition efficiencies against α-glucosidase, α-amylase and lipase of ethanolic extract of *V. denticulata* dried leaves was evaluated using *in vitro* enzyme kinetic assays. The results showed that the inhibitory efficiency of α-amylase was significantly better than α-glucosidase and lipase ($P \leq 0.05$) (IC_{50}=3.29 (3.11-4.11), 11.08 (11.67-13.04) and 27.21 (19.33-28.81) mg/mL, respectively). However, the IC_{50} values of the extract were larger than that of acarbose and orlistat ($P \leq 0.05$). The enzyme kinetic studies were analyzed using the Lineweaver-Burk plots. The results demonstrated that the inhibition behavior of standard acarbose and orlistat were competitive inhibition. On the contrary, the inhibition of the extract against α-glucosidase and α-amylase were mixed-type inhibition and that of lipase was non-competitive inhibition. Hence, the extract is less inhibitory efficacies to the enzymes than the standard inhibitors. In addition, the extract showed high DPPH antioxidant capacity (IC_{50} and TEAC are 0.0332 (0.0329-0.0333) mg/mL and 100.72 (100.38-101.83) mg Trolox/g dry weight, respectively), total phenolic, total tannin and total flavonoid contents (90.83 (88.10-94.25) mg GAE/g dry weight, 90.11 (89.34-91.66) mg TE/g dry weight, 62.71 (62.57-63.09) mg RE/g dry weight, respectively). The most abundant phenolic acids were protocatechuic acid, vanillic acid, sinapic acid, ferulic acid and gallic acid, whereas the major flavonoids were quercetin, rutin and catechin. Phenolic and flavonoid compounds, especially phenolic acids, quercetin and rutin, were responsible for the antioxidant capacity and enzymes inhibition because of the abilities to bind with the active sites, allosteric sites and enzyme-substrate complex. In conclusion, these findings were the first scientific data supporting to the

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folk medicine use of *V. denticulata* in order to decrease lipids and glucose levels in blood. Although, it cannot be replaced the drugs acarbose and orlistat, it may be applied as the functional food additive or developed to the herbal tea for the diabetes and obesity patients or the person who is in the glucose and lipid level controlling period. However, animal and human studies are required before implementing this plant as a functional food.

Keywords: *Ventilago denticulata* Willd.; α-glucosidase; α-amylase; Lipase; Enzyme inhibition

1. Introduction

Diabetes mellitus is a chronic disease associated with hyperglycemia that has become a public health problem worldwide. The type 2 diabetes or insulin independence diabetes is due to the disorder of insulin receptors in target cells, resulting in the insulin resistance and blood glucose level rise. It is found in more than 90% of all patients. The enzymes which play important roles in the carbohydrate digestion and glucose absorption are α-glucosidase and α-amylase. Alpha-amylase from pancreas and saliva glands hydrolyzes starch at α-1,4 glycosidic bonds to disaccharides such as maltose and smaller oligosaccharides, which are further degraded into glucose by α-glucosidase in the small intestine. Thus, the inhibition of these enzymes by the specific inhibitors results in a stable level of blood glucose [1]. A synthetic drug named acarbose is used to inhibit the activities of α-glucosidase and α-amylase in type 2 diabetes patients. As a result, carbohydrate hydrolysis and glucose absorption are lessened, which causes a decrease in postprandial hyperglycemia.

Like diabetes, obesity is a significant global public health, especially in developing countries. It is a condition of the high level of lipids such as cholesterol and triglyceride in blood or dyslipidemia. These lipids accumulate in coronary and cerebral arteries and cause hypertension, atherosclerosis, cardiovascular and cerebrovascular disease. The enzyme involved in lipids digestion is lipase, which is secreted from the small intestine and pancreas, and hydrolyzes lipids into glycerol and fatty acids. Inhibition of lipase activity using the drug orlistat leads to the reduction of lipid digestion and absorption in the small intestine, resulting in the lower level of lipids profile in blood [2].

Although acarbose and orlistat are effective inhibitors for diabetes and obesity, the long term use of these drugs leads to undesirable and adverse side effects. Toxicity and irritation to liver and gastrointestinal system are commonly found [3]. Therefore, α-glucosidase, α-amylase and the lipase inhibitory effect of many plants have been studied in order to replace or support the use of synthetic drugs for diabetes and obesity patients. Natural herbs are of interest to many researchers since they generally contain a wide variety of antioxidants such as phenolic and flavonoid compounds, which have been known as the natural enzyme inhibitors with safer and fewer side effects than synthetic inhibitors [4].

Ventilago denticulata Willd., commonly known as Rangdaeng, is in the family of Rhamnaceae. It is a perennial, semi-shrub and vine wood plant with climbing and hanging branches. Mature stems are cracked with red and narrow patterns which are the red substance called ventilagin [5]. It has been used in many countries in Southeast Asia and South Asia such as Thailand, Myanmar, Vietnam, India and Bhutan as the folk medicine to decrease blood sugar and cholesterol [6]. In Thailand, it is mostly found in Koh Kret district, Nonthaburi province. According to the traditional uses, it is the longevity

medicine which can treat several disorders of the body such as cachexia, fatigue and muscle pains. Drinking of *V. denticulata* dried leaves as a tea could reduce lipids and glucose levels in the blood and also renal dialysis treatment [7]. The previous studies noted that the extracts of *V. denticulata* showed the presence of tannins, phenolic acids, flavonoids, saponins, alkaloids, steroids, cardiac glycosides and quinone compounds [8-9]. It had phosphodiesterase inhibiting effects [10], antioxidant activities and antimicrobial potential against various bacterial and fungal species such as *Escherichia coli*, *Bacillus subtilis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Proteus vulgaris*, *Salmonella sp.*, *Trichoderma viride*, *Penicillium sp.* and *Aspergillus niger* [8,11]. Cytotoxic studies of other plants in the genus of *Ventilago* revealed that *V. leiocarpa* Benth. inhibited the K562-cancer cells and tumors growth [12-13] and *V. harmandiana* Pierre. provided the anti-inflammatory effect [14].

From the data described above, *V. denticulata* was not reported in pharmacological activities related to anti-diabetes and anti-obesity activities. So, the objectives of this study were to evaluate the abilities of *V. denticulata* dried leaves extract to inhibit α-glucosidase, α-amylase and lipase enzyme *in vitro*. Inhibition kinetics of the extract were investigated to identify the inhibition type. Total phenolics, total flavonoids, total tannins, antioxidant activities, phenolic acid and flavonoid compositions of the extract were also determined.

2. Materials and Methods

2.1 Samples preparation and extraction

Ventilago denticulata Willd. young leaves were derived from the local micro-community enterprises in Koh Kret district, Nonthaburi Province, Thailand. Samples (voucher specimen R. Srimoon 1) were identified and deposited at the Department of Plant Production Technology and Landscape, Faculty of Agro-Industrial Technology, Rajamangala University of Technology Tawan-Ok, Chanthaburi Campus, Chanthaburi Province, Thailand. Samples were processed using the method, according to Srimoon [15]. The leaves were washed, blanched with boiled water for a minute, spread for withering and then dried in a non-stick pan at 50°C for 2 hr. Dried leaves were further dried in a hot air oven at 50°C for 2 hr. The completely dried samples were kept in a plastic bag in a refrigerator prior to extraction.

Maceration of dried samples was performed with 70% ethanol (1:5 w/v) for 24 hr and filtered. The residue was re-macerated for 24 hr and filtered. The filtrates were combined and evaporated using a rotary evaporator until they became a crude extract.

2.2 Chemicals and equipments

Chemical reagents in enzyme inhibition and antioxidant assays were analytical reagent grade. Tannic acid, protocatechuic acid, vanillic acid, caffeic acid, coumaric acid, ferulic acid, sinapic acid, catechin, rutin, orlistat, 95% acarbose, polysorbate (Tween 40) and *p*-nitrophenyl-α-D-glucopyranoside (PNPG) were products of Alfa-Aesar (UK). Pancreatin porcine enzyme (lipase), α-glucosidase from *Saccharomyces cerevisiae* (19.3 units/mg solid), dinitrosalicylic acid (DNS), glyceryltrioleate (triolein), 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (trolox), 2,2-diphenyl-1-picryl hydrazyl (DPPH), quercetin and gallic acid were purchased from Sigma–Aldrich (USA). Folin-Ciocalteu phenol reagent

and α-amylase from malt-diastase (minimum activity 1:2,000 I.P.unit) were products of Loba Chemie (India). Soluble starch, sodium carbonate (Na_2CO_3) and aluminium chloride ($AlCl_3$) were products of Univar Ajax Finechem (New Zealand). Phenolphthalein, sodium hydroxide (NaOH), sodium chloride (NaCl), sodium nitrite ($NaNO_2$), sodium dihydrogen phosphate dihydrate ($NaH_2PO_4.2H_2O$) and disodium hydrogen phosphate (Na_2HPO_4) were purchased from Univar Ajax Finechem (Australia). Potassium acetate (CH_3COOK) was a product of Unilab (Australia). Potassium sodium tartrate ($KNaC_4H_4O_6$) was purchased from QRëC (New Zealand). Ethanol and methanol were products of Merck (Germany). Chemical reagents in HPLC assay were HPLC grade. Acetic acid, acetonitrile, formic acid and methanol were products of Merck (Germany). The spectrophotometer was a product of Libra S22 Biochrom (UK). The rotary evaporator was a product of Labora 4003 Heidolph (Germany). High performance liquid chromatography (HPLC, Thermo Separation Products; USA) was assisted by the Faculty of Medicine, Thammasat University, Pathumthani Province, Thailand.

2.3 *In vitro* α-glucosidase, α-amylase and lipase inhibition assay

2.3.1 Enzymes inhibition

Inhibition of α-glucosidase activity was performed using the colorimetric method modified from Hemalatha et al. [4] and Adefegha et al. [16]. The solutions were mixed in the reaction tube as follows: 1) 0.20 mL of 0.1 M phosphate buffer pH 6.9, 2) 1.0 mL of the extract (0-5.0 mg/mL)/or standard acarbose (0-1.0 mg/mL)/or 0.1 M phosphate buffer pH 6.9 blank solution, and 3) 0.2 mL of 1.0 unit/mL α-glucosidase. The mixture was incubated at 37ºC for 10 min. After that, 0.2 mL of 0.5 mM PNPG was added, and further incubated at 37ºC for 30 min before terminating the reaction with 1.40 mL of 0.67 M Na_2CO_3. The absorbance of the mixture was measured at 405 nm. The percentage of α-glucosidase inhibition and IC_{50} (the concentration of the extract that inhibited 50% of enzyme activity) were calculated using linear regression of the plots between the inhibition percentages and sample concentrations. Acarbose was used as the standard.

Inhibition of α-amylase activity was assessed by using soluble starch as a substrate and measured by the colorimetric method, according to Hemalatha et al. [4] and Adefegha et al. [16]. The solutions were mixed in the reaction tube as follows: 1) 0.25 mL of 0.1 M phosphate buffer containing 6.7 mM NaCl (PBS) pH 6.9, 2) 0.25 mL of the extract (0-1.0 mg/mL)/or standard acarbose (0-0.1 mg/mL)/or 0.1 M PBS pH 6.9 blank solution, and 3) 0.25 mL of 0.15 unit/mL α-amylase. The reaction mixture was incubated at 37ºC for 10 min before adding 1% soluble starch to start the reaction. After incubating at 37ºC for 30 min, 0.5 mL of dinitrosalicylic acid (DNS) was added to the mixture and it was further incubated at 100ºC in a water bath for 5 min to stop the reaction. After cooling at room temperature, 1.50 mL of distilled water was added and the absorbance of the mixture was measured at 540 nm. Percentages of α-amylase inhibition and IC_{50} were calculated. Acarbose was used as the standard.

Inhibition of lipase was determined using the titrimetric method as described by Sharma et al. [17] and Huerta et al. [18]. Briefly, 2.5 mL of the extract (0-10.0 mg/mL)/or standard orlistat (0-60.0 mg/mL)/or 50 mM sodium phosphate buffer pH 8 blank solution was mixed with 3.0 mL of 1% triolein in tween 40, followed by adding of 1.0 mL of 50 mM sodium phosphate buffer pH 8. After incubating at 37ºC for 30 min, 1.0 mL of pancreatic lipase was added and re-incubated at 37ºC for 30 min. A 3.0 mL

amount of 95% ethanol was added to stop the reaction. The mixture was titrated with 0.025 M NaOH using phenolphthalein as an indicator. Percentages of lipase inhibition and IC_{50} were calculated. Orlistat was used as the standard.

2.3.2 Enzymes inhibition kinetics

Enzyme inhibition kinetics of the extract were conducted with the same methods as the enzyme inhibition assay, except that the different concentrations of substrate were incubated with the enzyme in the absence, or presence of the extract at various concentrations equivalent to IC_{50}. For α-glucosidase, substrate was [PNPG]=0.5-5.0 mM, positive control was [acarbose]=0.1-1.0 mg/mL, negative control was no inhibitor, [extract]=5.0-15.0 mg/mL. For α-amylase, substrate was [starch]=0.5-5.0 mM, positive control was [acarbose]=0.01-0.1 mg/mL, negative control was no inhibitor, [extract]=1.0-5.0 mg/mL. For lipase, substrate was [triolein]=0.5-2.5 mM, positive control was [orlistat]=10-20 mg/mL, negative control was no inhibitor, [extract]=20.0-30.0 mg/mL.

Type of inhibition, maximum rate (V_{max}) and Michaelis-Menten constant (K_m) were determined using the double-reciprocal Lineweaver-Burk plots Eq.(1) that rearranges from the Michaelis-Menten equation Eq.(2) as follows:

$$\frac{1}{V} = \frac{K_m}{V_{max}[S]} + \frac{1}{V_{max}}, \quad \ldots(1)$$

$$V_0 = \frac{V_{max}[S]}{K_m + [S]}, \quad \ldots(2)$$

where [S] is substrate concentrations, V is the reaction rate, V_0 is the initial rate, and V_{max} is the maximum rate. From the Lineweaver-Burk plots data, the secondary plots between the slope and concentrations of the extract, and the plots between the intercepts and concentrations of the extract were evaluated the inhibition constant for enzyme-inhibitor [EI] complex (K_i) and for enzyme-substrate-inhibitor [ESI] complex (K_i'), respectively. The Lineweaver-Burk plots were plotted with Microsoft Excel 2003 for Windows.

2.4 DPPH radical scavenging activity assay

A DPPH assay was performed with the method modified from Shimada et al. [19]. An aliquot of 0.1 mg/mL standard trolox/or the extract was mixed with 4.5 mL of 0.04 mg/mL DPPH and then diluted to 5.0 mL with distilled water. After shaking in the dark for 20 min, the absorbance of the mixture was measured at 515 nm. Percentage of DPPH inhibition, IC_{50} (the concentration of the extract that inhibited 50% of DPPH radicals) and TEAC (trolox equivalent antioxidant capacity) were calculated compared with standard trolox.

2.5 Total phenolics, total tannins and total flavonoids analysis

Total phenolic content was analyzed using the Folin–Ciocalteu phenol reagent method, according to Wong et al. [20]. The 2.0 mL of the extract/or standard gallic acid was mixed with 5.0 mL of 10% Folin-Ciocalteu phenol reagent. After shaking for 3 min, 2.0 mL of 7.5% Na_2CO_3 was added and the mixture was left for an hour at room temperature. The absorbance of the mixture was measured at 765 nm. Total phenolic content was calculated and expressed as gallic acid equivalent (mg GAE/g dry weight).

Total tannin content was estimated using the Folin–Ciocalteu phenol reagent method modified from Shad et al. [21]. A 2.0 mL of the extract/or standard tannic acid was mixed with 1.0 mL of 10% Folin-Ciocalteu phenol reagent, followed by 2.0 mL of saturated Na_2CO_3 and 5.0 mL of distilled water. After standing for an hour at room temperature, the absorbance of the solution was measured at 725 nm. Total

tannin was calculated and expressed as tannic acid equivalent (mg TE/g dry weight).

Total flavonoid content was determined using aluminium chloride method as described by Malla et al. [22]. A 0.5 mL of the extract/or standard rutin was diluted with 2.0 mL of distilled water, mixed with 0.15 mL of 5% $NaNO_2$ and left for 6 min. Then, 0.15 mL of 10% $AlCl_3$ was added, left for 6 min, 2.0 mL of 4% NaOH was added and the solution was diluted to 5.0 mL with distilled water. The absorbance of the solution was read at 510 nm. Total flavonoid content was calculated and expressed as rutin equivalent (mg RE/g dry weight).

2.6 Phenolic acid and flavonoid compositions analysis

Phenolic acid compositions were assessed using the chromatographic method according to Butsat and Siriamornpun [23]. High-performance liquid chromatography (HPLC, Thermo Separation Products, USA) included pump (Spectra System P-4000 Thermo Separation, USA) with diode array detector (Spectra System UV-2000, wavelength=280 nm). Chromatographic separation was performed on a C18 4.6x150 mm (Luna 5 µm, Phenomenex, USA) with guard column of which the temperature was ambient temperature, and carried out with 1% acetic acid (A) and acetonitrile (B) as mobile phases with the gradient elution (0-5 min=linear gradient from 5% to 9% solvent B, 5-15 min=hold at 9% solvent B, 15-22 min=linear gradient from 9% to11% solvent B, 22-38 min=linear gradient from 11% to 18% solvent B, 38-43 min=from 18% to 23% solvent B, 43-44 min=from 23% to 90% solvent B, washing period of 6 min=80% solvent B, re-equilibration period between individual runs of 15 min=5% solvent B). The separation flow rate was 1 mL/min, the autosampler was Spectra System AS3500, and the injection volume was 10 µL. Chrome-Quest software was used for data acquisition and processing. Phenolic acid compositions were identified by the comparison of the retention time and UV spectra of the extract with 7 standard phenolic acids (gallic acid, protocatechuic acid, vanillic acid, caffeic acid, coumaric acid, ferulic acid and sinapic acid).

Flavonoid compositions were assessed by a chromatographic method, according to Zhang et al. [24]. HPLC analysis was performed the same as the phenolic acid analysis, except that the mobile phases consisted of formic acid pH 2.5 (C) and methanol (D) with the gradient elution (0-20 min=linear gradient from 80% to 30% solvent C, 20-22 min=isocratic at 90% solvent D and re-equilibration period between individual runs of 8 min=80% solvent C). Flavonoid compositions were identified by the comparison of the retention time and UV spectra of the extract with 3 standard flavonoids (catechin, rutin and quercetin).

2.7 Statistical analysis

The results were presented as median and range of triplicates. The statistical analysis was evaluated by Mann-Whitney U-test at $P \leq 0.05$ of significance.

3. Results and Discussion

3.1 *In vitro* inhibition of α-glucosidase, α-amylase and lipase enzyme

Extraction of *Ventilago denticulata* Willd. dried leaves samples was performed using 70% ethanol (1:5 w/v) and the extraction yield was 43.16%. The *in vitro* inhibition efficiencies of the extract on α-glucosidase, α-amylase and lipase were demonstrated in Table 1 and Fig. 1-3. The results showed that the inhibition efficiency of α-amylase was higher than that of α-glucosidase and lipase, significantly ($P \leq 0.05$). However, the IC_{50}

values of the extract against α-glucosidase and α-amylase were larger 25.89 and 58.33 folds than standard acarbose, respectively, and the IC_{50} values of the extract against lipase were larger 1.55 folds than standard orlistat ($P \leq 0.05$). Plant extracts which can be considered to have the high enzymes inhibition should have the IC_{50} values below the IC_{50} values of the standard. From our results, *V. denticulata* dried leaves extract had less inhibition efficacy against α-glucosidase, α-amylase and lipase when compared to the standard inhibitors and was also higher than the IC_{50} of quinoa (0.062-0.182 mg/mL for α-glucosidase and 0.108-0.241 mg/mL for α-amylase), some berry fruit (0.004-0.021 mg/mL for α-amylase) and red grape extract (0.014 mg/mL for lipase) [4,25-26].

Table 1. The *in vitro* inhibitory efficiencies of standard inhibitors and *V. denticulata* Willd. dried leaves extract on α-glucosidase, α-amylase and lipase (n=3), data was presented as median (range).

Enzyme inhibition efficiencies	IC_{50} (mg/mL)	
	Standard inhibitors*	*V. denticulata* extract
α-glucosidase	0.47^{a} (0.46-0.47)	$11.08^{b,x}$ (11.67-13.04)
α-amylase	0.064^{a} (0.063-0.065)	$3.29^{b,y}$ (3.11-4.11)
lipase	16.42^{a} (14.34-17.75)	$27.21^{b,z}$ (19.33-28.81)

*standard inhibitor for α-glucosidase and α-amylase was acarbose and standard inhibitor for lipase was orlistat.
The superscript letters (^{a,b}) in each rows showed the significant differences ($P \leq 0.05$)
The superscript letters (^{x,y,z}) in the last column showed the significant differences ($P \leq 0.05$)

3.2 Enzymes inhibition kinetics

Inhibition kinetics of standard inhibitors and *V. denticulata* dried leaves extract against α-glucosidase, α-amylase and lipase were analyzed using the Lineweaver-Burk plots as displayed in Figs. 4-6 and all kinetic parameters (V_{max}, K_m and K_i) are presented in Table 2. The result showed that the inhibition of standard inhibitors, acarbose and orlistat, were competitive manner (V_{max} were unchanged, K_m were increased and the intersections were at the Y-axis). This type of inhibition revealed that standard inhibitors [I] have a similar structure to the substrates [S] and compete with the substrates for the active site of the enzymes. Competitive inhibitors bind to the free enzyme [E] and form an enzyme-inhibitor complex [EI]. They prevent the enzyme from binding with their substrates; hence, the inhibition efficiencies of standard inhibitors; acarbose and orlistat; were higher than the extract with reference to the IC_{50} values.

On the other hand, the inhibition of the extract against α-glucosidase and α-amylase was mixed-type inhibition. Although the V_{max} and K_m values were closed, they showed the decreasing of V_{max} and the increasing of K_m trends, and the intersections of the Lineweaver-Burk plots occurred in the second quadrant. These results have similar trends as the extracts of Propolis and some of Labiatae plants [27-28]. The secondary plots between the slopes of the Lineweaver-Burk plots and concentrations of the extract were carried out to obtain the inhibition constant for enzyme-inhibitor [EI] complex (K_i), and the plots between the intercepts and concentrations of the extract were carried out to obtain the inhibition constant for enzyme-substrate-inhibitor [ESI] complex (K_i'). From the data, a smaller K_i value than K_i' value for α-glucosidase and α-amylase indicated that the inhibition of the extract was dominant in competitive manner, or preferred [EI] to [ESI]. A mixed-type inhibitor is mostly from allosteric inhibition which occurs when the inhibitor binds to the allosteric site of the enzyme and alters the shape of the active site. Thus, the enzyme is inactive.

In contrast, the inhibition behavior of the extract against lipase was non-competitive behavior because the V_{max}

values were reduced, K_m values were unchanged and the intersections of the Lineweaver-Burk plots were at the negative x-axis. This implies that the extract and substrate do not compete to bind at the same active sites of lipase or the extract has binding affinities to both of the enzyme [E] and the enzyme-substrate [ES] complex. This inhibition kinetic pattern has similar pattern to the lipase inhibition of *Dendrobium formosum* extract [29]. Although the extract has less inhibitory efficacies on the enzymes than acarbose and orlistat, it has some advantages over the standard inhibitors. It may not be affected by the substrate concentrations and still may be effective at lower concentrations [27-28].

3.3 DPPH radical scavenging activity, total phenolics, total tannins, total flavonoids and phenolic acid and flavonoid compositions

DPPH radical scavenging activity, total phenolics, total tannins and total flavonoids of *V. denticulata* dried leaves extract are shown in Table 3. Phenolic acid and flavonoid compositions are shown in Table 4. HPLC chromatograms of standard phenolic acids, standard flavonoids and the extract are illustrated in Figs. 7-8.

The results showed that *V. denticulata* extract had high DPPH antioxidant activity and high concentration of total phenolics, total tannins and total flavonoids. The most abundant phenolic acids were protocatechuic acid, vanillic acid, sinapic acid, ferluric acid and gallic acid, while protocatechueic acid and vanillic acid were the first two dominant phenolic acids. The major flavonoid compounds were quercetin, rutin and catechin, whereas quercetin was found to be the highest content of flavonoids.

Previous phytochemical studies demonstrated that a crude extract of *V. denticulata* showed the presence of tannins, phenolic acids, flavonoids, saponins, alkaloids, steroids and cardiac glycosides and exhibited anti-herpes simplex virus type 1 *in vivo* [8,30]. Seven quinone compounds; ventiloquinone I, 2–hydroxyislandicin, chrysophanol, physcion, emodin, questin and ventilatone A; were screened from the root extract of *V. denticulata* which showed the anti-alzheimer property against acetylcholinesterase enzyme [9].

Although the previous studies reported that *V. denticulata* composed of various groups of bioactive compounds, phenolics and flavonoids were the major constituents which played the important roles in α-glucosidase, α-amylase and lipase inhibition. Many reports noted that chlorogenic acids, protocatechuic acid, ferulic acid, caffeic acid, rosmarinic acid, resveratrol, rutin, catechin and quercetin showed high α-glucosidase and α-amylase inhibition [16,31], while coumaric acid, caffeic acid, gallic acid, chlorogenic acid, naringenin, quercetin, catechin, rutin and proanthocyanidins were the good inhibitors of lipase [32-34]. Shobana et al. [35] also found the relations between high levels of phenolic and flavonoid compounds in finger millet (*Eleusine coracana* L.) and the inhibition of α-glucosidase and α-amylase. Moreover, the study of Srisawat [36], of *Cassia siamea* Lam. and *Piper sarmentosum* Roxb. leaves extracts which contained high concentration of antioxidants, such as phenolics and flavonoids, also showed high inhibition capacities to α-glucosidase, α-amylase and lipase enzyme. From the results of our research and the supporting information from the former studies, it can be suggested that phenolic and flavonoid compounds were responsible for the α-glucosidase, α-amylase and lipase inhibition due to their abilities to bind with the active sites, allosteric sites and enzyme-substrate complex [27,37-38].

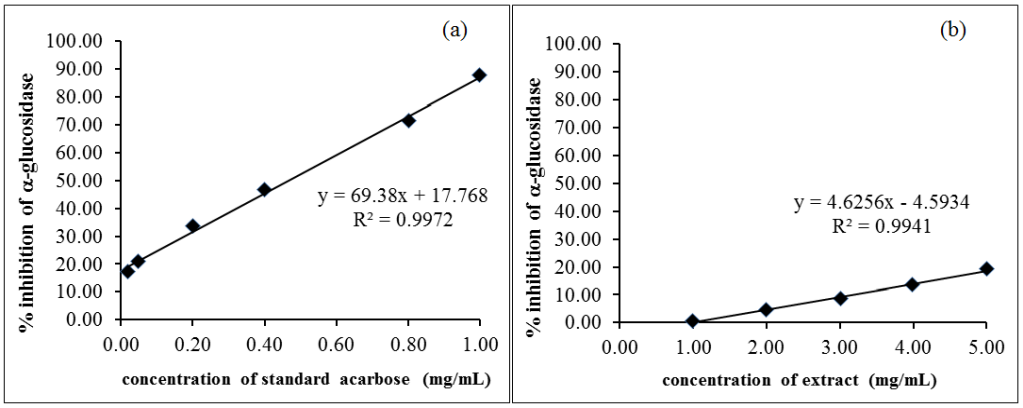

Fig. 1. Alpha-glucosidase inhibitions of (a) standard acarbose and (b) *V. denticulata* extract.

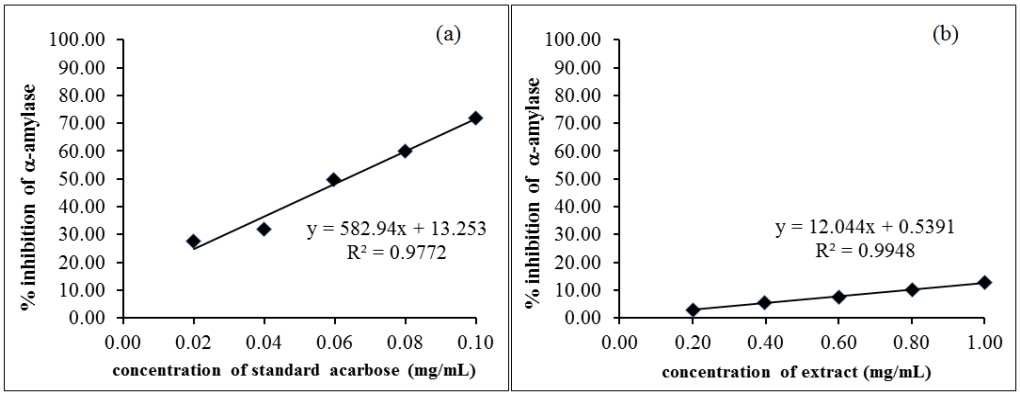

Fig. 2. Alpha-amylase inhibitions of (a) standard acarbose and (b) *V. denticulata* extract.

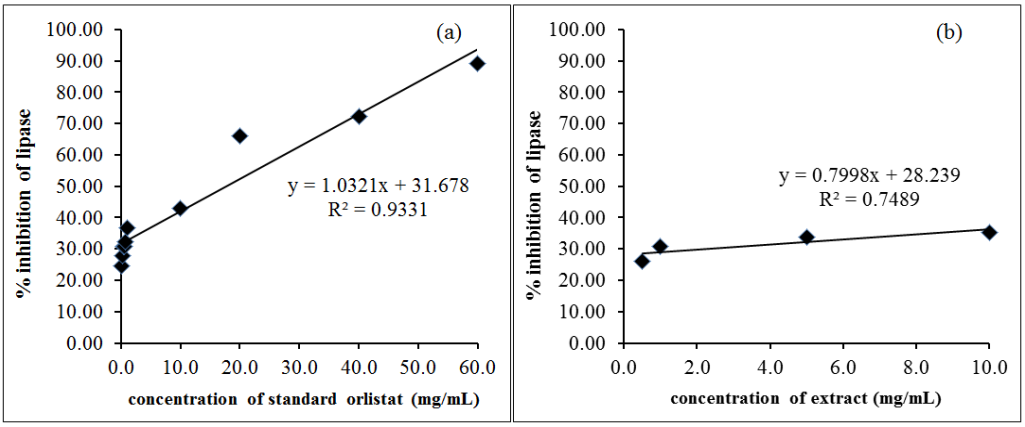

Fig. 3. Lipase inhibitions of (a) standard orlistat and (b) *V. denticulata* extract.

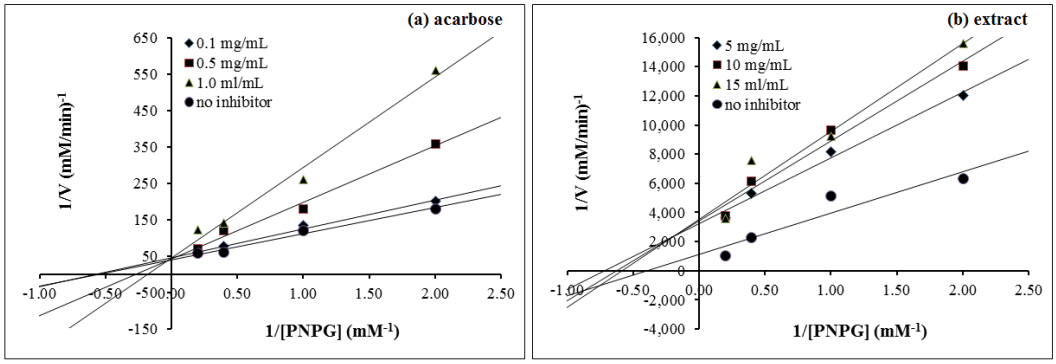

Fig. 4. Lineweaver-Burk plots of α-glucosidase by (a) acarbose and (b) *V. denticulata* extract.

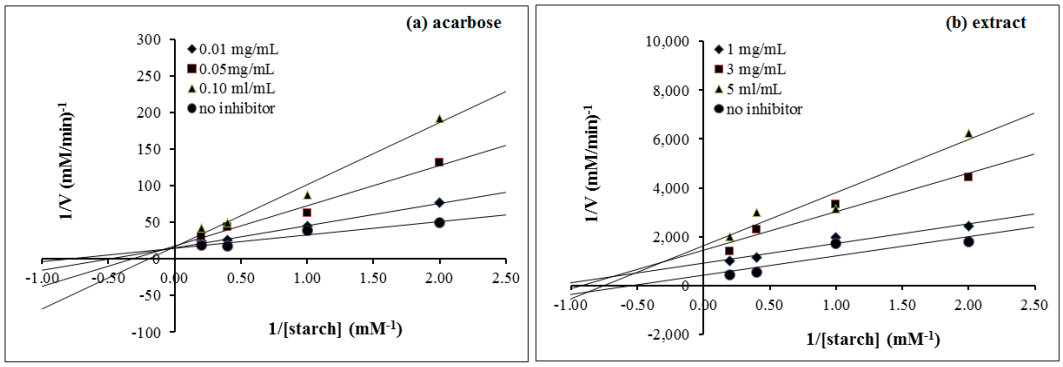

Fig. 5. Lineweaver-Burk plots of α-amylase by (a) acarbose and (b) *V. denticulata* extract.

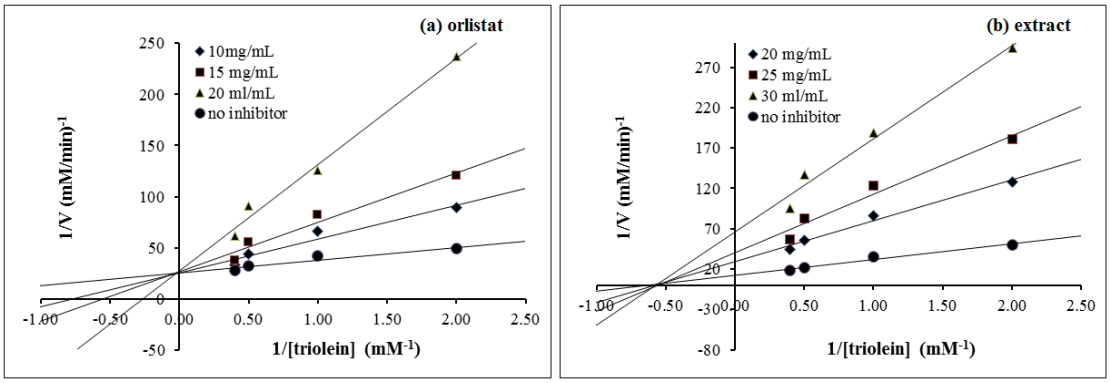

Fig. 6. Lineweaver-Burk plots of lipase by (a) orlistat and (b) *V. denticulata* extract.

Table 2. Kinetic parameters for α-glucosidase, α-amylase and lipase inhibition from the Lineweaver-Burk plots of *V. denticulata* dried leaves extract (n=3), data was presented as median (range).

Concentration (mg/mL)		V_{max} (mM/min)	K_m (mM)	K_i (mg/mL)	Type of inhibition
α-glucosidase					
acarbose	0.10	0.02335 (0.02172-0.02432)	1.87471 (1.71912-1.92467)	0.33456 (0.32278-0.33521)	competitive
	0.50	0.02389 (0.02325-0.02518)	3.70932 (3.60902-3.90921)		
	1.00	0.02227 (0.02129-0.02894)	5.53219 (5.28796-7.18646)		
extract	5.00	0.00032 (0.00031-0.00033)	1.47025 (1.41367-1.52048)	27.32778 (26.93215-27.41007) K_i'= 88.32350 (87.19812-96.20722)	mixed
	10.00	0.00029 (0.00028-0.00029)	1.58002 (1.51397-1.59049)		
	15.00	0.00028 (0.00022-0.00028)	1.67283 (1.34068-1.70053)		
α-amylase					
acarbose	0.01	0.06689 (0.06425-0.07109)	2.03298 (1.95252-2.16049)	0.04120 (0.04059-0.04335)	competitive
	0.05	0.06828 (0.05619-0.06934)	3.75197 (3.08772-3.80989)		
	0.10	0.06922 (0.06143-0.06935)	5.86571 (5.20547-5.87724)		
extract	5.00	0.00112 (0.00109-0.00115)	0.90138 (0.87795-0.92552)	1.43611 (1.42152-1.4447) K_i'=4.41895 (4.40175-4.45327)	mixed
	10.00	0.00066 (0.00062-0.00069)	1.02881 (0.97698-1.08384)		
	15.00	0.00062 (0.00061-0.00066)	1.34594 (1.32969-1.42652)		
Lipase					
orlistat	10.00	0.03084 (0.03009-0.03868)	1.01679 (0.99223-1.27524)	6.29997 (6.26788-6.31416)	competitive
	15.00	0.03989 (0.03713-0.03999)	1.92575 (1.79238-1.93053)		
	20.00	0.04029 (0.03601-0.04100)	4.18685 (3.74221-4.26041)		
extract	20.00	0.03437 (0.03336-0.03623)	1.74132 (1.69012-1.83579)	12.89452 (12.74422-13.16789)	non-competitive
	25.00	0.02529 (0.02475-0.02822)	1.83113 (1.79235-2.04351)		
	30.00	0.01508 (0.01315-0.01551)	1.74313 (1.52022-1.79299)		

Table 3. DPPH radical scavenging activity, total phenolics, total tannins and total flavonoids of *V. denticulata* dried leaves extract (n = 3), data was presented as median (range).

Antioxidants and antioxidant activities	Contents
IC_{50} (mg/mL)	0.0329-0.0333 (0.0332)
TEAC* (mg trolox/g dw)	100.38-101.83 (100.72)
Total phenolics (mg GAE/g dw)	88.10-94.25 (90.83)
Total tannins (mg TE/g dw)	89.34-91.66 (90.11)
Total flavonoids (mg RE/g dw)	62.57-63.09 (62.71)

Table 4. Phenolic acid and flavonoid compositions of *V. denticulata* dried leaves extract (n = 3), data was presented as median (range).

Phenolic group	Compounds	Concentration (µg/g dry weight)
Phenolic acids	Gallic acid	13.57 (13.57-14.27)
	Protocatechuic acid	497.12 (492.24-499.88)
	Vanillic acid	352.69 (348.50-371.07)
	Caffeic acid	ND*
	Coumaric acid	ND*
	Ferulic acid	21.10 (20.75-21.72)
	Sinapic acid	37.04 (36.85-38.55)
Flavonoids	Catechin	165.90 (160.18-165.90)
	Rutin	829.46 (826.74-831.91)
	Quercetin	2,817.60 (2,809.02-2,825.33)

*ND = not detected

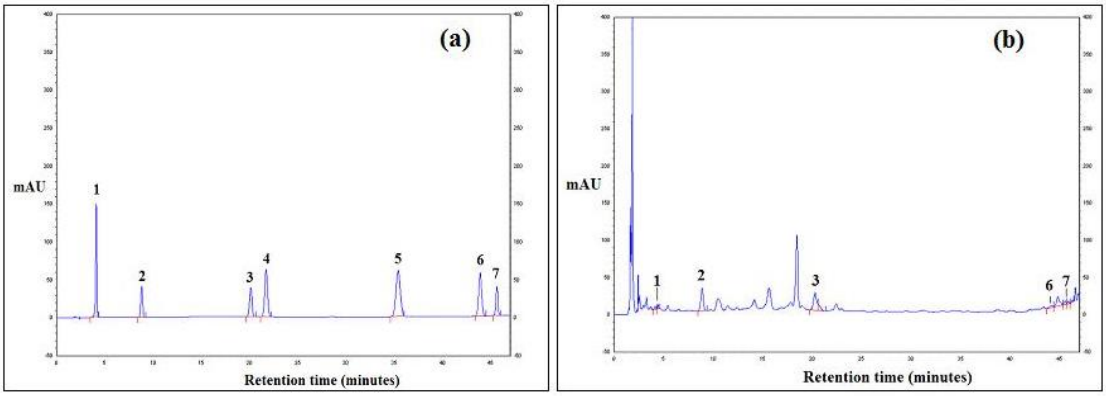

Fig. 7. HPLC chromatograms of (a) standard phenolic acids (50 µg/mL) and (b) *V.denticulata* dried leaves extract (100 mg/mL)(1=gallic acid, 2=protocatechuic acid, 3=vanillic acid, 4=caffeic acid, 5=coumaric acid, 6=ferulic acid, 7=sinapic acid).

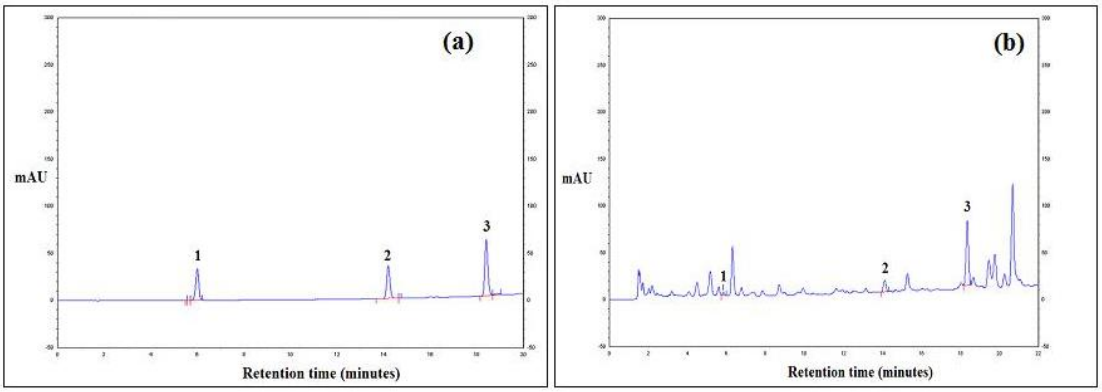

Fig. 8. HPLC chromatograms of (a) standard flavonoids (50 µg/mL) and (b) *V. denticulata* dried leaves extract (100 mg/mL) (1=catechin, 2=rutin, 3=quercetin).

4. Conclusion

This study is the first finding to describe the anti-diabetes and anti-obesity activities of *Ventilago denticulata* Willd. dried leaves extract. Experimental data show that the extract inhibits the α-glucosidase, α-amylase and lipase enzyme *in vitro* and contains high phenolic, tannin and flavonoid compounds which promote the antioxidant activities and the enzymatic inhibition. A plant extract with α-glucosidase and α-amylase inhibition activities promotes the reduction of carbohydrate digestion and glucose absorption, which prevents the hyperglycemia. While the extract with lipase inhibition activity reduces the hydrolysis of lipids into fatty acids and glycerol, decreasing in dyslipidemia. These results are the scientific data supporting to the traditional use of dried *V. denticulata* to decrease glucose and lipids levels in blood. Even though it cannot replace the synthetic drugs, it may be developed as one of the alternative functional foods or as herbal tea for people who wants to control the blood glucose and lipid level. However, even though the natural inhibitors have less adverse side effects than the synthetic inhibitors, further animal and human studies are required before developing this plant as a functional food.

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