

## Full Length Research Paper

# Anti-lipolytic, $\alpha$ -amylase inhibitory and antioxidant activities of *Pseuderanthemum palatiferum* (Nees) Radlk. leaf ethanolic extract

Pawitra Pulbutr\*, Achida Jaruchotikamol, Benjamart Cushnie, Sakulrat Rattanakiat and Somsak Nualkaew

Pharmaceutical Chemistry and Natural Product Research Unit, Faculty of Pharmacy, Mahasarakham University, Maha Sarakham, 44150, Thailand.

Received 30 May, 2014; Accepted 7 July, 2014

*Pseuderanthemum palatiferum* (Nees) Radlk. has been used extensively as a medicinal plant for the treatment of diabetes mellitus in various parts of Southeast Asia. However, the mechanism of its anti-diabetic action has not been fully characterized yet. The objectives of this study were to investigate the *in vitro* effects of *P. palatiferum* leaf ethanolic extract (PPE extract) on adipocyte lipolysis,  $\alpha$ -amylase enzyme activity and antioxidant activity. The PPE extract at every concentration tested (10, 25, 50, 100, 250, 500  $\mu$ g/ml) caused a significant inhibition on basal lipolysis in adipocytes of the high fat diet-fed rats. The PPE extract also produced a concentration-dependent anti-lipolytic action against isoprenaline (0.1  $\mu$ M)-induced lipolysis in the normal pellet diet-fed rat derived adipocytes. Inhibition of  $\alpha$ -amylase was detected using the 3,5-dinitrosalicylic acid assay, the PPE extract having an  $IC_{50}$  of 4.99 mg/ml. An antioxidant activity, as evaluated by the DPPH assay ( $IC_{50}$ ), was 58.13  $\mu$ g/ml. In the ferric reducing power assay, the extract exerted antioxidant activity with a vitamin C equivalent antioxidant capacity (VCEAC) and a trolox equivalent antioxidant capacity (TEAC) of 2.33 and 2.04 g/100 g extract, respectively. The anti-lipolytic,  $\alpha$ -amylase inhibitory and antioxidant activities of PPE extract detected here help justify the traditional use of this plant in the treatment of diabetes mellitus.

**Key words:** *Pseuderanthemum palatiferum*, diabetes mellitus, adipocyte lipolysis,  $\alpha$ -amylase, 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay, ferric reducing power assay.

## INTRODUCTION

Diabetes mellitus (DM) is a chronic metabolic disorder caused by impairment in insulin secretion and/or insulin

\*Corresponding author. E-mail: ppawitra@yahoo.com. Tel/Fax: +66-43-754-360.

Author(s) agree that this article remain permanently open access under the terms of the [Creative Commons Attribution License 4.0 International License](http://creativecommons.org/licenses/by/4.0/)

action. The incidence of DM, especially type 2 DM has increased substantially in modern society in which people live with a sedentary lifestyle. Treatment of type 2 DM includes both drug therapy and lifestyle modifications (ADA, 2014). Although several groups of anti-diabetic agents with various mechanisms of action have been developed, certain treatment problems, including unresponsiveness and adverse drug reactions, still arise regularly. Additionally, the high cost of some novel anti-diabetic agents is another main obstacle in diabetic treatment, especially in developing countries. Therefore, other treatment options, particularly medicinal plants, have been used widely as alternative anti-diabetic therapies.

*Pseuderanthemum palatiferum* (Nees) Radlk. has been used traditionally as a medicinal plant for the treatment of DM in several Southeast Asian countries, especially in Vietnam and Thailand. The leaf ethanolic extract of the plant produced a significant plasma glucose-lowering action in streptozotocin-induced diabetic rats without causing any acute toxicity (Padee et al., 2010). However, the mechanism(s) of its anti-diabetic action have not been clearly established yet. Chronic elevations of plasma free fatty acid (FFAs) and postprandial plasma glucose contribute to the pathogenesis of insulin resistance and type 2 diabetes (Shulman, 2000; Bensellam et al., 2012; Rachek, 2014). Additionally, the increased plasma levels of both FFAs and postprandial glucose are linked to the production of reactive oxygen species which play a key role in pancreatic  $\beta$ -cell destruction and diabetic complications (Bakker et al., 2000; Karunakaran and Park, 2013). The plasma level of FFAs is primarily controlled by adipocyte lipolysis (Zhai et al., 2010). Meanwhile, the level of postprandial plasma glucose is regulated chiefly by the activity of intestinal carbohydrate-digesting enzymes, including  $\alpha$ -amylase (Nolte Kennedy, 2012).

Since adipocyte lipolysis,  $\alpha$ -amylase enzyme activity and oxidative stress are associated with the development of diabetes and its complications, proper regulation of these biological functions may impede pathogenesis of the disease. The objectives of this study were to investigate the *in vitro* effects of *P. palatiferum* leaf ethanolic extract (PPE extract) on three diabetes-related biological activities, adipocyte lipolysis,  $\alpha$ -amylase enzyme activity and antioxidant activity.

## MATERIALS AND METHODS

### Preparation of *P. palatiferum* leaf ethanolic extract (PPE extract)

*P. palatiferum* (Nees) Radlk. was planted and harvested in Roi Et province, Thailand. The specimens were authenticated by the Plant Varieties Protection Division, Department of Agriculture, Ministry of Agriculture and Cooperatives. A voucher is deposited at the Herbarium of Pharmaceutical Chemistry and Natural Product Research Unit, the

Faculty of Pharmacy, Mahasarakham University, Thailand (code: MSU.PH-ACA-P1). The leaves were dried by hot-air oven at a temperature of 50°C for 45 h. The dried leaves (100 g) were macerated with 80% ethanol (1,000 ml) for 7 days. The extract was then dried using a rotary evaporator (Heidolph Laborota 4000, Germany) followed by a freeze dryer (Christ Alpha 1-4, Germany). The leaf ethanolic extract of *P. palatiferum* (PPE extract) was then obtained with % yield of 12.7% w/w. The PPE extract was stored at -20°C until use.

### Study on lipolysis in rat adipocytes

#### Preparation of adipocytes

Male Wistar rats (150 to 170 g), obtained from the National Laboratory Animal Center Mahidol University, Thailand were kept at a constant temperature (25 ± 1°C) with a 12 h dark-light cycle. The rats were fed *ad libitum* with free access to water. After 1 week acclimatization, the rats (n = 16) were randomly assigned into two groups (8 rats per group) and fed with two different diet types, normal pellet diet (NPD) and high fat diet (HFD). NPD used was CP mice feed (food no.082, Bangkok, Thailand), whilst HFD was prepared following the formulation of Srinivasan et al. (2005). The amount of fat in the NPD and HFD are approximately 12 and 56%, respectively. After 3 weeks, the rats were sacrificed and the epididymal fat pads were collected for preparation of adipocyte suspension. All procedures with the animals were approved by the animal research ethics committee, Mahasarakham University, Thailand (no.0127/2555). Adipocyte suspensions were prepared by collagenase digestion (collagenase type II, Sigma, 0.25 mg/ml), according to the method of Rodbell (1964), with slight modification. Hanks buffer containing 5 mM glucose and 0.5% bovine serum albumin (BSA, with less than 0.005% (w/w) free fatty acid) (Sigma) was used in the preparation of adipocyte suspension. The adipocytes were resuspended with the buffer into 1:1 (v/v) proportion and stored at 37°C before performing experiments.

#### Adipocyte lipolysis assay

Adipocytes were incubated for 60 min in microfuge tubes at 37°C with Hanks buffer, pH 7.4, containing 5 mM glucose, 0.5% BSA and various concentrations of the PPE extract at 10, 25, 50, 100, 250 and 500 µg/ml. Adipocyte lipolysis was examined both in the absence (basal lipolysis) and the presence of 0.1 µM isoprenaline (isoprenaline-induced lipolysis). After 1 h incubation, the cell free incubation media were collected and the concentrations of glycerol were measured using a glycerol assay kit from Sigma.

#### *In vitro* study on $\alpha$ -amylase enzyme activity

The  $\alpha$ -amylase enzyme activity assay was performed according to the method of Ali et al. (2006). Freshly prepared porcine pancreatic  $\alpha$ -amylase enzyme (200 µl, 4 unit/ml) was pre-incubated at 25°C for 5 min with 40 µl of the PPE extract, DMSO (a negative control) or  $\alpha$ -amylase inhibitor from wheat seed (*Triticum aestivum*) (a positive control). A 400 µl volume of potato starch solution (0.5% w/v) and 160 µl of distilled water were subsequently added and incubated at 25°C for further 3 min. A 200 µl volume of the mixture was taken into a new microfuge tube containing 100 µl of 3,5-dinitrosalicylic acid (DNS) color reagent solution (96 mM DNS and 5.31 M sodium

potassium tartrate in 2 M NaOH) and then incubated at 85°C for 15 min.

After the incubation, the mixture was diluted with 450 µl of distilled water and the optical density was measured at the wavelength of 540 nm. The obtained absorbance was subtracted with the absorbance of the blank in order to deduct the absorbance produced by the plant extract. For blank incubations, the enzyme solution was replaced with an equal amount of distilled water. The % (w/v) of maltose generated was calculated from the maltose standard calibration curve (0 to 0.1% w/v). Percent reaction was obtained from the following equation:

$$\% \text{ Reaction} = (\text{Mean maltose in sample} / \text{Mean maltose in negative control}) \times 100$$

Percent inhibition was calculated as 100% reaction. The median inhibitory concentration (IC<sub>50</sub>) was obtained from the concentration-inhibitory response curve plotted by using GraphPad Prism software version 6.0.

### ***In vitro* studies on antioxidant activity**

#### ***DPPH* assay**

The 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging assay was performed according to the method of Bonina et al. (2000). A 180 µl volume of DPPH solution (63.4 µM) was mixed with the PPE extract (20 µl), DMSO (a negative control) or ascorbic acid (a positive control). After 30 min-incubation in the dark, the absorbance was measured at 515 nm. The scavenging capacity was expressed as % of inhibition as calculated by the following equation:

$$\% \text{ inhibition} = [(\text{Absorbance of negative control} - \text{Absorbance of sample}) / \text{Absorbance of negative control}] \times 100$$

The median inhibitory concentration (IC<sub>50</sub>) was obtained from the concentration-inhibitory response curve plotted by using GraphPad Prism software version 6.0.

#### ***Ferric reducing power* assay**

The ferric reducing power assay is based upon the conversion of the ferric tripyridyltriazine (Fe<sup>3+</sup>-TPTZ) complex into the ferrous tripyridyltriazine (Fe<sup>2+</sup>-TPTZ) in the presence of antioxidants. The PPE extract at various concentrations (20 µl) was mixed with the acetate buffer-TPTZ-FeCl<sub>3</sub> reagent (180 µl). The absorbance of the mixture was measured at 593 nm after 5 min-incubation. Negative and positive controls of the assay were DMSO and ascorbic acid (vitamin C) or trolox, respectively. The ferric reducing power was expressed as vitamin C equivalent antioxidant capacity (VCEAC) and trolox equivalent antioxidant capacity (TEAC).

#### **Statistical analysis**

All data were expressed as mean ± standard deviation (SD), except the results from the adipocyte lipolysis study which were expressed as mean ± standard error of mean (SEM). The data were statistically analyzed using one-way analysis of variance (ANOVA) followed by the Bonferroni post-hoc test. Statistically significant differences were indicated by a *p*-value of < 0.05.

## **RESULTS**

### **Effects of the PPE extract on adipocyte lipolysis**

#### ***NPD-fed rat derived adipocyte lipolysis***

In basal lipolysis, the glycerol concentration released from adipocytes of the NPD-fed rats was 86.17 ± 2.10 µg ml<sup>-1</sup>/ml packed cell volume (PCV)/h (n = 4). The PPE extract at any concentration tested (10, 25, 50, 100, 250 and 500 µg/ml) did not cause any significant change in the basal lipolysis as shown in Figure 1A. Isoprenaline at a concentration of 0.1 µM significantly increased adipocyte lipolysis with a glycerol level of 177.27 ± 28.21 µg ml<sup>-1</sup>/ml PCV/h (*p* < 0.05; n = 4), calculated as 205.72 ± 32.74% of the basal lipolysis. The PPE extract at concentrations of 25, 50, 100, 250 and 500 µg/ml had a significant inhibitory action on the isoprenaline-induced lipolysis (*p* < 0.05; n = 4) (Figure 1B). The median concentration of the PPE extract which inhibited the isoprenaline-induced lipolysis (IC<sub>50</sub>) was 67.19 µg/ml (pIC<sub>50</sub> = 4.17 ± 0.31).

#### ***HFD-fed rat derived adipocyte lipolysis***

The glycerol level in the basal lipolytic condition of the HFD-fed rat derived adipocytes was 66.12 ± 9.15 µg ml<sup>-1</sup>/ml PCV/h (n = 4). The PPE extract at every concentration tested significantly decreased the basal lipolysis (*p* < 0.05; n = 4) (Figure 1C). Isoprenaline at a concentration of 0.1 µM only slightly increased lipolysis in adipocytes derived from the HFD-fed rats, with the glycerol level of 71.47 ± 2.72 µg ml<sup>-1</sup>/ml PCV/h (n = 5). This level was not statistically different from that of the basal lipolysis. The PPE extract at any concentration used in the experiment did not produce any significant change in the isoprenaline-induced lipolysis (Figure 1D).

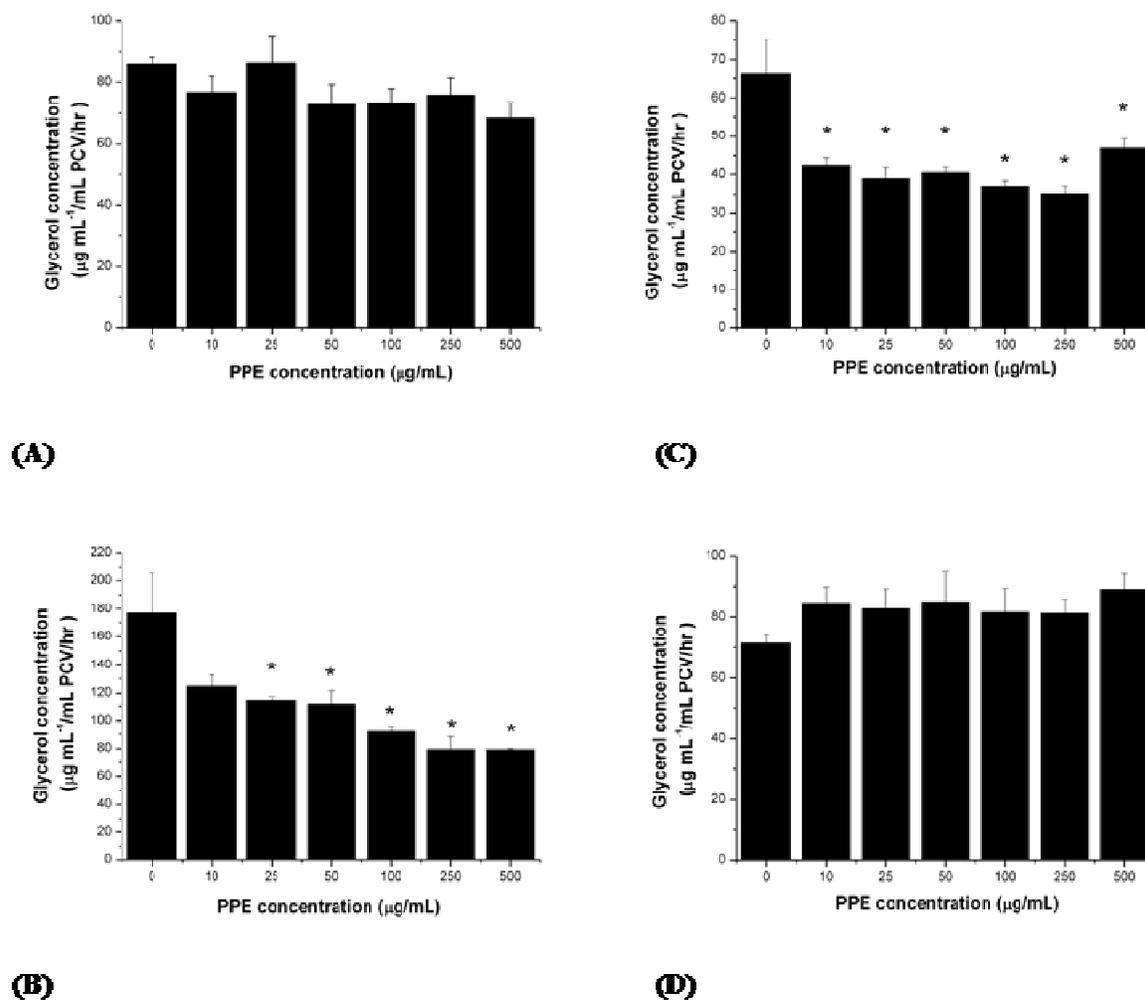
### **Effects of the PPE extract on *in vitro* α-amylase enzyme activity**

DMSO (negative control) did not inhibit α-amylase enzyme (% inhibition = 0.00 ± 1.98%). The α-amylase inhibitor from wheat seed (positive control) significantly inhibited α-amylase enzyme activity with the % inhibition of 70.38 ± 7.40%. The PPE extract at concentrations of 5, 10, 20 and 50 mg/ml produced a significant inhibition against α-amylase enzyme activity (*p* < 0.05; n = 3) as shown in Table 1. The maximal α-amylase inhibition of 59.86 ± 4.46% was found at the PPE extract concentration of 20 mg/ml. The PPE extract caused a concentration-dependent inhibitory action on α-amylase

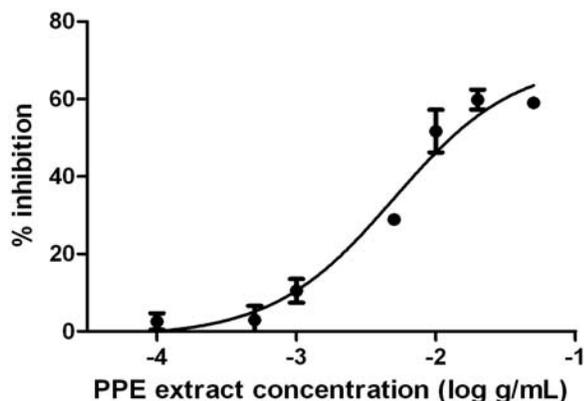
**Table 1.** *In vitro* effects of the PPE extract on  $\alpha$ -amylase enzyme activity.

PPE extract concentration (mg/ml)	% inhibition (mean $\pm$ SD)
0	0.00 $\pm$ 1.98
0.1	2.57 $\pm$ 3.67
0.5	2.96 $\pm$ 6.37
1	10.50 $\pm$ 5.28
5	28.88 $\pm$ 1.85*
10	51.72 $\pm$ 9.50*
20	59.86 $\pm$ 4.46*
50	59.04 $\pm$ 2.34*

\* $p$ <0.05; One-way ANOVA, Bonferroni post-hoc test; n = 3



**Figure 1:** Effects of the PPE extract on adipocyte lipolysis. (A) Basal lipolysis in adipocytes derived from the NPD-fed rats. (B) Isoprenaline (0.1 µM)-induced lipolysis in adipocytes derived from the NPD-fed rats. (C) Basal lipolysis in adipocytes derived from the HFD-fed rats. (D) Isoprenaline (0.1 µM)-induced lipolysis in adipocytes derived from the HFD-fed rats. The data are expressed as the mean  $\pm$  SEM (n=4). \* $p$ <0.05 when compared with the control (One-way ANOVA followed by Bonferroni post hoc test).



**Figure 2.** Concentration-inhibitory response curve of the PPE extract against *in vitro*  $\alpha$ -amylase enzyme activity. The % inhibition was expressed as the mean  $\pm$  SD (n=3).

enzyme activity with an  $IC_{50}$  of 4.99 mg/ml ( $pIC_{50}$  of  $2.30 \pm 0.12$ ) (Figure 2).

### ***In vitro* antioxidant activity of the PPE extract**

#### ***DPPH* assay**

The PPE extract produced scavenging activity against DPPH free radical with an  $IC_{50}$  of 58.13  $\mu$ g/ml ( $pIC_{50}$  of  $4.24 \pm 0.08$ ) (n = 5). Ascorbic acid, positive control, exerted antioxidant activity with an  $IC_{50}$  of 4.16  $\mu$ g/ml ( $pIC_{50}$  of  $5.38 \pm 0.05$ ) (n = 5).

#### ***Ferric reducing power* assay**

The PPE extract exerted ferric reducing power with a vitamin C equivalent antioxidant capacity (VCEAC) and a trolox equivalent antioxidant capacity (TEAC) of 2.33 and 2.04 g/100 g extract, respectively.

## **DISCUSSION**

### **Effects of the PPE extract on adipocyte lipolysis**

Both basal lipolysis and isoprenaline (0.1  $\mu$ M)-stimulated lipolysis were investigated in adipocytes derived from the NPD- or HFD-fed rats. HFD feeding in rodents have been known to induce obesity and insulin resistance, which are a crucial pathogenesis of type 2 DM (Buettner et al., 2006). The basal lipolysis in adipocytes derived from the NPD-fed rats was not affected by the PPE extract. On the contrary, the PPE extract caused a significant inhibition of

basal lipolysis in the HFD-fed rat adipocytes in a concentration-independent manner (Figure 1C). The elevation of basal lipolytic level was reported in adipocytes of the rats fed with HFD for 7 to 8 weeks (Portillo et al., 1999; Gaidhu et al., 2010). However, the difference in the basal lipolytic levels between the NPD- and the HFD-fed rat adipocytes was not observed in this study. This may be due to the shorter duration of HFD-feeding (3 weeks). The changes in several basal lipolytic-related proteins have been described in adipocytes of the HFD-fed rats (Portillo et al., 1999; Gaidhu et al., 2010). The expressions of adipose triglyceride lipase (ATGL) and comparative gene identification-58 (CGI-58) were increased in the HFD-fed rat adipocytes, whilst the level of perilipin expression was decreased (Gaidhu et al., 2010). A target site of the PPE extract may be over-expressed in HFD-fed rat adipocytes. Thus, the anti-lipolytic action of the PPE extract can only be found obviously in these adipocytes, but not in adipocytes isolated from the NPD-fed group. Nonetheless, further experiments are needed to investigate the exact anti-lipolytic site of action of the PPE extract.

The PPE extract caused a concentration-dependent inhibition of isoprenaline-induced lipolysis in adipocytes isolated from the NPD-fed rats ( $IC_{50}$  = 67.19  $\mu$ g/ml), but it did not cause any significant change in the HFD-fed rat adipocytes (Figure 1B and D). In this study, the lipolytic response to isoprenaline in adipocytes of the HFD group was dramatically lower than that of adipocytes isolated from the NPD-fed rats. This is in agreement with the previous studies in which adipocytes of the HFD-fed rats had a lower degree of  $\beta$ -agonist-induced lipolysis (Matsuo et al., 1995; Portillo et al., 1999; Gaidhu et al., 2010). The reduction in lipolytic response to  $\beta$ -agonists in adipocytes of the HFD-fed rats was found to be associated with an alteration in  $\beta$ -adrenoceptor binding affinity (Matsuo et al., 1995). The expressions of some essential proteins involved in the  $\beta$ -agonist-stimulated lipolytic pathway, such as perilipin and adipose phospholipase A2, were modified in the HFD-fed rat adipocytes as well (Gaidhu et al., 2010). Thus, the difference in isoprenaline-induced lipolytic response to the PPE extract between the NPD- and HFD-fed rat adipocytes is likely to be explained by the alteration in isoprenaline-induced lipolytic pathway.

The anti-lipolytic action of the PPE extract is reported for the first time in this study. The anti-lipolytic action of the PPE extract demonstrated in this study is likely to be associated with its plasma glucose-lowering action reported previously by Padee et al. (2010). A reduction in adipocyte lipolysis leads to a lower amount of released glycerol, which is a major precursor in hepatic gluconeogenesis. Additionally, FFAs, another product of adipocyte lipolysis, also play a key role in the regulation of plasma glucose level and glucose uptake by interfering

with glucose transporter 4 (GLUT4) functions (Shulman, 2000). FFAs are also precursors of hepatic very low density lipoprotein (VLDL) synthesis (Malloy and Kane, 2012). The plasma levels of VLDL and high-density lipoprotein (HDL-C) are linked, since triglyceride in VLDL can be transferred into HDL-C. Subsequently, HDL-C becomes triglyceride-rich HDL-C, which can be easily degraded (Taskinen, 2003). Thus, the anti-lipolytic effects of the PPE extract is likely to be implicated in its plasma triglyceride-lowering and HDL-C-increasing actions reported previously as well (Padee et al., 2010). Moreover, FFAs released during adipocyte lipolysis are also involved in pancreatic  $\beta$ -cell apoptosis and insulin resistance (Shulman, 2000; Cernea and Dobreanu, 2013). The negative regulation of basal adipocyte lipolysis by the PPE extract thus potentially provides beneficial impacts especially in persons having a high amount of fat deposition or taking a high-fat diet, who are at high risk of type 2 DM. Nonetheless, further experiments are required to prove whether the anti-lipolytic action of the PPE extract can still be found *in vivo* where several factors implicated in the regulation of adipocyte lipolysis exist.

#### Effects of the PPE extract on *in vitro* $\alpha$ -amylase enzyme activity

The PPE extract produced a significant  $\alpha$ -amylase enzyme inhibition in a concentration-dependent manner with an  $IC_{50}$  of 4.99 mg/ml. A maximal  $\alpha$ -amylase enzyme inhibition of approximately 60% was found at a concentration of 20 mg/ml. A similar level of inhibition was observed at the PPE extract concentration of 50 mg/ml, the highest concentration tested. The achievement of the plateau inhibition was thus suggested at these concentrations. To our knowledge, this is the first report of  $\alpha$ -amylase inhibitory activity from a PPE extract. It was reported that the methanolic extract of *P. palatiferum* dry leaves (1 mg/ml) exerted an *in vitro* inhibitory action against  $\alpha$ -glucosidase enzyme, with 7% inhibition (Rungprom et al., 2010). This is somewhat similar to our results in which 1 mg/ml of the PPE extract produced approximately 10% inhibition on  $\alpha$ -amylase enzyme activity (Table 1). However, the  $IC_{50}$  of the PPE extract against  $\alpha$ -glucosidase enzyme activity has not been reported yet. Thus, the potency of the extract against these two enzymes cannot be compared. Both  $\alpha$ -amylase and  $\alpha$ -glucosidase enzymes are essential for intestinal carbohydrate digestion. The inhibition of both enzymes would hence produce an advantage over the inhibition of each enzyme alone. Further research thus should be performed to investigate the effects of the PPE extract on  $\alpha$ -glucosidase enzyme activity.

It is still not known which phytochemical components of the PPE extract the active compounds are acting against the  $\alpha$ -amylase enzyme. Several phytochemicals, including  $\beta$ -sitosterol, stigmasterol and kaempferol, were isolated from the PPE extract (Giang et al., 2003). The *in vitro* inhibitory activity of these phytochemicals against  $\alpha$ -amylase was demonstrated previously (Tadera et al., 2006; Nkobole et al., 2011; Takahama and Hirota, 2013). Additionally, it was reported that  $\beta$ -sitosterol, stigmasterol and kaempferol significantly decreased the plasma glucose levels in streptozotocin- or alloxan-induced diabetic rats (de Sousa et al., 2004; Kumar et al., 2013). These phytochemicals thus possibly play a role in the plasma glucose-lowering action of the plant extract via the inhibition of carbohydrate-digesting enzymes. Nonetheless, additional experiments will be required to identify the specific phytochemicals in the PPE extract inhibiting  $\alpha$ -amylase.

The  $\alpha$ -amylase inhibitory action of the PPE extract potentially acts as one of its mechanisms of action against DM. However, the postprandial plasma glucose level, an indicator of carbohydrate digestion and absorption, after the treatment with the PPE extract has not been investigated yet. *In vivo* carbohydrate tolerance tests are therefore needed to determine whether the extract controls plasma glucose level mainly via its action against carbohydrate-digesting enzymes.

Postprandial hyperglycemia is implicated in the destruction of pancreatic  $\beta$ -cells via induction of glucotoxicity (Dubois et al., 2007; Bensellam et al., 2012). The high level of plasma glucose after meals is also linked to oxidative stress, inflammation, and endothelial dysfunction (Ceriello et al., 2005). These derangements are a crucial pathophysiology of atherosclerosis, which leads to the development of coronary artery disease and myocardial infarction, the major cause of death in diabetic patients. Several epidemiological studies indicated postprandial hyperglycemia as the direct and independent risk factor of cardiovascular diseases in diabetic patients (Cariello et al., 2005). Control of postprandial plasma glucose using inhibitors of carbohydrate-digesting enzymes is thus likely to reduce the risk of cardiovascular complications.

#### *In vitro* antioxidant activity of the PPE extract

The PPE extract scavenged DPPH free radicals in a concentration-dependent manner with an  $IC_{50}$  of 58.13  $\mu$ g/ml. The DPPH scavenging potency of the extract was lower than that of ascorbic acid ( $IC_{50}$  of 4.16  $\mu$ g/ml). The aqueous extract of *P. palatiferum* also showed *in vitro* antioxidant activity, with an  $IC_{50}$  of DPPH radical scavenging activity of 221.14  $\mu$ g/ml (Chayarop et al., 2012).

Thus, it suggests that the active antioxidative agents are more efficiently extracted with a less polar solvent (ethanol). Various ethanolic plant extracts also possessed higher DPPH scavenging potency than that of their aqueous counterparts (Nguyen and Eun, 2011). The phenolic compounds, especially flavonoids, a group of well known antioxidants, have been identified in both ethanolic and aqueous extracts of *P. palatiferum* (Nguyen and Eun, 2011; Chayarop et al., 2012). It was reported that the level of total phenolic contents in the ethanolic extract of *P. palatiferum* was higher than in the aqueous extract (Nguyen and Eun, 2011). This is in agreement with the higher DPPH scavenging potency of the ethanolic extract. Several groups of flavonoids, such as flavon-3-ols, flavanones, anthocyanidins, flavonols, flavones and isoflavones, exhibit anti-diabetic actions substantially via their antioxidant activity (Babu et al., 2013). It was reported that flavonoids and flavonoids metabolites were protected against oxidative stress-induced pancreatic  $\beta$ -cell dysfunction and cell death and also restored insulin secretion (Fernandez-Millan et al., 2014; Martin et al., 2014). Additionally, the anti-inflammatory action of flavonoids also contributed to its protective effect against the development of diabetes in an animal model (Fu et al., 2011).

Following the ferric reducing power assay, the PPE extract produced an antioxidant activity with VCEAC and TEAC values of 2.33 and 2.04 g/100 g extract, respectively. The ethanolic extract of *P. palatiferum* was reported to have low reducing power which was not associated with its DPPH scavenging activity (Nguyen and Eun, 2011). The aqueous extract of *P. palatiferum* also exhibited less potent reducing power when compared to trolox (Chayarop et al., 2012). Although the PPE extract exhibited a less potent antioxidative activity when compared to the reference antioxidants in both DPPH radical scavenging assay and ferric reducing power assay, its antioxidant activity was confirmed by two antioxidant assays, which specify different mechanisms of antioxidative action. Treatment with antioxidants has been found to reduce oxidative stress and protect pancreatic  $\beta$ -cells (Kaneto et al., 1999). The antioxidative activity was described in various extracts of medicinal plants traditionally used for the treatment of diabetes, such as *Tinospora crispa*, *Momordica charantia*, and *Pandanus amaryllifolius* (Cavin et al., 1998; Ghasemzadeh and Jaafar, 2013; Sulaiman et al., 2013). Antioxidative activity of the PPE extract is thus likely to partially explain its mechanism against diabetes.

## Conclusion

The PPE extract exerted anti-lipolytic action within a concentration range similar to that which produced DPPH

radical scavenging activity. However, its  $\alpha$ -amylase inhibition was observed only at higher concentrations. Further *in vivo* pharmacodynamics and pharmacokinetics studies are still required to determine the exact mechanism of anti-diabetic action of the PPE extract. Over function of adipocyte lipolysis, postprandial hyperglycemia and oxidative stress are closely associated with the pathogenesis of diabetes and its complications (Bakker et al., 2000; Sakuraba et al., 2002; Karunakaran and Park, 2013). Therefore, anti-lipolytic,  $\alpha$ -amylase inhibitory and anti-oxidative activities of the PPE extract demonstrated here potentially contribute to its anti-diabetic effects.

## ACKNOWLEDGMENTS

This study was funded by Mahasarakham University research grant (Fiscal year 2013) and Faculty of Pharmacy, Mahasarakham University research grant (Fiscal year 2013). We thank Ms. Kasira Navarattanakorn, Ms. Sirapatsorn Kumboonma and Ms. Erin Khaengkhan for their help with the in adipocyte lipolysis study. We would like to express our gratitude to Dr. Tim Cushnie for his kind suggestion on the manuscript.

## Conflict of interest

Authors declare no conflict of interest.

## REFERENCES

- Ali H, Houghton PJ, Soumyanath A (2006). Alpha-amylase inhibitory activity of some Malaysian plants used to treat diabetes; with particular reference to *Phyllanthus amarus*. *J. Ethnopharmacol.* 107:449-455.
- American Diabetes Association (2014). Standards of medical care in diabetes 2014. *Diabetes Care* 37(1):S14-80. doi: 10.2337/dc14-S014.
- Babu PV, Liu D, Gilbert ER (2013). Recent advances in understanding the anti-diabetic actions of dietary flavonoids. *J. Nutr. Biochem.* 24:1777-1789. doi: 10.1016/j.jnutbio.2013.06.003.
- Bakker SJ, Ijzerman RG, Teerlink T, Westerhoff HV, Gans RO, Heine RJ (2000). Cytosolic triglycerides and oxidative stress in central obesity: the missing link between excessive atherosclerosis, endothelial dysfunction, and beta-cell failure? *Atherosclerosis* 148:17-21.
- Bensellam M, Laybutt DR, Jonas JC (2012). The molecular mechanisms of pancreatic  $\beta$ -cell glucotoxicity: recent findings and future research directions. *Mol. Cell. Endocrinol.* 364:1-27. doi: 10.1016/j.mce.2012.08.003.
- Bonina F, Puglia C, Tomaino A, Saija A, Mulinacci N, Romani A, Vincieri FF (2000). *In-vitro* antioxidant and *in-vivo* photoprotective effect of three lyophilized extracts of *Sedum telephium* L. leaves. *J. Pharm. Pharmacol.* 52:1279-1285.
- Buettner R, Parhofer KG, Woenckhaus M, Wrede CE, Kunz-Schughart LA, Schölmerich J, Bollheimer LC (2006). Defining high-fat-diet rat models: metabolic and molecular effects of different fat types. *J. Mol. Endocrinol.* 36:485-501.
- Cavin A, Hostettmann K, Dyatmyko W, Potterat O (1998). Antioxidant and lipophilic constituents of *Tinospora crispa*. *Planta. Med.* 64:393-396.
- Ceriello A (2005). Postprandial hyperglycemia and diabetes

- complications: is it time to treat? *Diabetes* 54:1–7.
- Cernea S, Dobreanu M (2013). Diabetes and beta cell function: from mechanisms to evaluation and clinical implications. *Biochem. Med. (Zagreb)*. 23:266-280.
- Chayarp K, Temsiririrkkul R, Peungvicha P, Wongkrajang Y, Chuakul W, Amnuoyopol S, Ruangwises N (2011). Antidiabetic effects and *in vitro* antioxidant activity of *Pseuderanthemum palatiferum* (Nees) Radlk. ex Lindau leaf aqueous extract. *MU. J. Pharm.* 38:13-22.
- de Sousa E, Zanatta L, Seifriz I, Creczynski-Pasa TB, Pizzolatti MG, Szpoganicz B, Silva FR (2004). Hypoglycemic effect and antioxidant potential of kaempferol-3,7-O-(alpha)-dirhamnoside from *Bauhinia forficata* leaves. *J. Nat. Prod.* 67:829-832.
- Dubois M, Vacher P, Roger B, Huyghe D, Vandewalle B, Kerr-Conte J, Pattou F, Moustaid-Moussa N, Lang J (2007). Glucotoxicity inhibits late steps of insulin exocytosis. *Endocrinology* 148:1605-1614.
- Fernandez-Millan E, Ramos S, Alvarez C, Bravo L, Goya L, Martin MA (2014). Microbial phenolic metabolites improve glucose-stimulated insulin secretion and protect pancreatic beta cells against tert-butyl hydroperoxide-induced toxicity via ERKs and PKC pathways. *Food Chem. Toxicol.* 66:245-253. doi: 10.1016/j.fct.2014.01.044.
- Fu Z, Zhen W, Yuskavage J, Liu D (2011). Epigallocatechin gallate delays the onset of type 1 diabetes in spontaneous non-obese diabetic mice. *Br. J. Nutr.* 105:1218-1225. doi: 10.1017/S0007114510004824.
- Gaidhu MP, Anthony NM, Patel P, Hawke TJ, Ceddia RB (2010). Dysregulation of lipolysis and lipid metabolism in visceral and subcutaneous adipocytes by high-fat diet: role of ATGL, HSL, and AMPK. *Am. J. Physiol. Cell. Physiol.* 298:C961-971. doi: 10.1152/ajpcell.00547.2009.
- Ghasemzadeh A, Jaafar HZ (2013). Profiling of phenolic compounds and their antioxidant and anticancer activities in pandan (*Pandanus amaryllifolius* Roxb.) extracts from different locations of Malaysia. *BMC. Complement. Altern. Med.* 13:341. doi: 10.1186/1472-6882-13-341.
- Giang PM, Bao HV, Son PT (2003). Phytochemical study on *Pseuderanthemum palatiferum* (Nees) Radlk., Acanthaceae. *J. Chem.* 41:115-118.
- Kaneto H, Kajimoto Y, Miyagawa J, Matsuoka T, Fujitani Y, Umayahara Y, Hanafusa T, Matsuzawa Y, Yamasaki Y, Hori M (1999). Beneficial effects of antioxidants in diabetes: possible protection of pancreatic beta-cells against glucose toxicity. *Diabetes* 48:2398-2406.
- Karunakaran U, Park KG (2013). A systematic review of oxidative stress and safety of antioxidants in diabetes: focus on islets and their defense. *Diabetes Metab. J.* 37:106-112. doi: 10.4093/dmj.2013.37.2.106.
- Kumar S, Kumar V, Prakash O (2013). Enzymes inhibition and antidiabetic effect of isolated constituents from *Dillenia indica*. *Biomed. Res. Int.* doi:10.1155/2013/382063.
- Malloy MJ, Kane JP (2012). Agents used in dyslipidemia. In Katzung BG, Masters SB, Trevor AJ (eds) *Basic & Clinical Pharmacology*, McGraw-Hill, New York, pp. 619-634.
- Martin MA, Fernandez-Millan E, Ramos S, Bravo L, Goya L (2014). Cocoa flavonoid epicatechin protects pancreatic beta cell viability and function against oxidative stress. *Mol. Nutr. Food Res.* 58:447-456. doi: 10.1002/mnfr.201300291.
- Matsuo T, Sumida H, Suzuki M (1995). Beef tallow diet decreases beta-adrenergic receptor binding and lipolytic activities in different adipose tissues of rat. *Metabolism* 44:1271-1277.
- Nguyen QV, Eun JB (2011). Antioxidant activity of solvent extracts from Vietnamese medicinal plants. *J. Med. Plants Res.* 5:2798-2811.
- Nkobile N, Houghton PJ, Hussein A, Lall N (2011). Antidiabetic activity of *Terminalia sericea* constituents. *Nat. Prod. Commun.* 6:1585-1588.
- Nolte Kennedy MS (2012). Pancreatic hormones & antidiabetic drugs. In Katzung BG, Masters SB, Trevor AJ (eds) *Basic & Clinical Pharmacology*, McGraw-Hill, New York pp. 743-768.
- Padee P, Nualkaew S, Talubmook C, Sakuljaitrong S (2010). Hypoglycemic effect of a leaf extract of *Pseuderanthemum palatiferum* (Nees) Radlk. in normal and streptozotocin-induced diabetic rats. *J. Ethnopharmacol.* 132:491-496. doi:10.1016/j.jep.2010.07.056.
- Portillo MP, Simón E, García-Calonge MA, Del Barrio AS (1999). Effect of high-fat diet on lipolysis in isolated adipocytes from visceral and subcutaneous WAT. *Eur. J. Nutr.* 38:177-82.
- Rachek LI (2014). Free fatty acids and skeletal muscle insulin resistance. *Prog. Mol. Biol. Transl. Sci.* 121:267-292. doi:10.1016/B978-0-12-800101-1.00008-9.
- Rodbell M (1964). Metabolism of isolated fat cells. I. Effects of hormones on glucose metabolism and lipolysis. *J. Biol. Chem.* 239:375-380.
- Rungprom W, Siripornvisai S, Keawsawal S, Songtrai M (2010).  $\alpha$ -glucosidase inhibitors from medicinal plants for diabetes therapy. *Agric. Sci.* 41:301-304.
- Sakuraba H, Mizukami H, Yagihashi N, Wada R, Hanyu C, Yagihashi S (2002). Reduced beta-cell mass and expression of oxidative stress-related DNA damage in the islet of Japanese type II diabetic patients. *Diabetologia* 45:85-96.
- Shulman GI (2000). Cellular mechanisms of insulin resistance. *J. Clin. Invest.* 106:171-176.
- Srinivasan K, Viswanad B, Asrat L, Kaul CL, Ramarao P (2005). Combination of high-fat diet-fed and low-dose streptozotocin-treated rat: a model for type 2 diabetes and pharmacological screening. *Pharmacol. Res.* 52:313-320.
- Sulaiman SF, Ooi KL, Supriatno (2013). Antioxidant and  $\alpha$ -glucosidase inhibitory activities of cucurbit fruit vegetables and identification of active and major constituents from phenolic-rich extracts of *Lagenaria siceraria* and *Sechium edule*. *J. Agric. Food Chem.* 61:10080-10090. doi: 10.1021/jf4031037.
- Tadera K, Minami Y, Takamatsu K, Matsuoka T (2006). Inhibition of alpha-glucosidase and alpha-amylase by flavonoids. *J. Nutr. Sci. Vitaminol.* 52:149-153.
- Takahama U, Hirota S (2013). Effects of starch on nitrous acid-induced oxidation of kaempferol and inhibition of  $\alpha$ -amylase-catalysed digestion of starch by kaempferol under conditions simulating the stomach and the intestine. *Food Chem.* 141:313-319.
- Taskinen MR (2003). Diabetic dyslipidemia: from basic research to clinical practice. *Diabetologia* 46:733–749.
- Zhai W, Xu C, Ling Y, Liu S, Deng J, Qi Y, Londos C, Xu G (2010). Increased lipolysis in adipose tissues is associated with elevation of systemic free fatty acids and insulin resistance in perilipin null mice. *Horm. Metab. Res.* 42:247-253. doi:10.1055/s-0029-1243599.