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Dipeptidyl Peptidase-IV (DPP-IV) Inhibitory Activity, Antioxidant Property and Phytochemical Composition Studies of Herbal Constituents of Thai Folk Anti-Diabetes Remedy

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Abstract

Type 2 diabetes mellitus (T2DM) patient numbers have dramatically increased by almost 10 times within the past 10 years. Several groups of drugs have been developed to treat T2DM, including dipeptidyl peptidase-IV inhibitor. The Krom Luang Chomphon folk recipe was used as alternative anti-diabetes recipe; however, no scientific data on the DPP-IV inhibitory activities of this recipe has been evaluated. In the present study, 14 selected medicinal herb extracts from this recipe were prepared and investigated for their DPP-IV inhibitory activity, antioxidant property, and phytochemical compositions. The results demonstrated that all extracts exhibited DPP-IV inhibitory activity, but at different levels. The highest inhibitory activities, at 50 μg/mL, were detected in *Lagerstroemia speciose* (L.) Pers. (71.07±0.07 %) and *Terminalia catappa* L. (69.89±0.43 %), while diprotin A (standard) gave 90.07±0.39 % inhibition. All extracts displayed antioxidant activity at varying levels. The lowest IC_{50} in the DPPH assay was found in the ethanolic extract from leaves of *T.catappa* (4.39±0.12 μg/mL), comparable to that of ascorbic acid (IC_{50} = 4.28±0.01μg/mL) and BHT (IC_{50} = 4.82±0.01μg/mL). Phenolic, flavonoid, and anthocyanin compounds were detected in the extracts, alkaloids were detected in 10 extracts, and terpenoids were detected in 11 extracts. Their phytochemical compositions were evaluated for their relationship with DPP-IV inhibitory and antioxidant activities. The results revealed that DPP-IV inhibitory activity was significantly related with phenolic content ($p < 0.05$, $r^2 = 0.560$) while antioxidant activity (DPPH) was related with phenolic content ($p < 0.05$, $r^2 = 0.500$). Therefore, the DPP-IV inhibitory activity and antioxidant activity of each herb extract may vary, depending on the content of terpenoid and phenolic compounds. All selected herbs, especially the leaves of *T. catappa* and *L. speciose*, showed the ability to be used as DPP-IV inhibitors and antioxidants for T2DM treatment. Furthermore, our results are the first reported of DPP-IV inhibitory activity in the 14 herb extracts which are the main ingredients in the Krom Luang Chomphon folk recipe. These findings also support the potential use of this recipe as an alternative treatment for diabetes through a new mechanism.

Keywords: Dipeptidyl peptidase-IV inhibitor, antioxidant, phytochemistry, Krom Luang Chomphon folk recipe, diabetes mellitus

Introduction

Diabetes mellitus (DM) is a metabolic syndrome characterized by hyperglycemia, which may result from defects in insulin secretion, insulin action, or both. The chronic hyperglycemia of diabetes may cause long-term organ damage, dysfunction, or failure, especially in the eyes, kidneys, and nerves, and heart disease, strokes, and serious wounds [1]. The total number of diabetic patients is estimated to rise up to 366 million in 2030 [2]. Nowadays, Type 2 diabetes mellitus (T2DM) patients are the world's fifth leading cause of death [1]. There are a number of causes of T2DM, including insulin resistance, impaired insulin action, and β-cell malfunction resulting in high blood glucose level. Gastrointestinal enzymes such as α-glucosidase, α-amylase and dipeptidyl peptidase-IV (DPP-IV) also play important roles in blood glucose level. DPP-IV enzyme is a new drug target in DM treatment. This enzyme is a membrane bound enzyme involved in the incretin system. DPP-IV inhibitors would help to improve insulin secretion and suppress glucagon release, resulting in lowering blood glucose.

Long term DM is often associated with secondary complications including cerebral ischemia, renal failure, heart disease, high blood pressure, etc. [3].That could be due to an over production of free radicals and a lack of antioxidant processes. Furthermore, in diabetic patients, oxidative stress is always produced, resulting in free radicals which may play an important role in those complications. Moreover, DM patients also have a malfunction of their antioxidant defense system, which gives rise to increased oxidative stress. High levels of free radicals may cause multi-organ damage and many complications in DM patients. Therefore, standard treatment, in combination with antioxidant administration, could help to improve DM treatment effectively.

Traditional antidiabetic remedies can be used as alternatives for the treatment of diabetes, or reinforcements to modern treatment methods. They could also help to reduce the secondary complications of the disease. Currently, a large number of medicinal plants and natural biomolecules have been reported for their antidiabetic effects [4]. Sixty natural products were previously reported to have anti-diabetic activity in many mechanisms, including DPP-IV inhibition [4]. Many researchers are currently searching for DPP-IV inhibitor compounds from natural sources, for new drug development. Methanolic extracts of *Magiferaindica* leaves [5] and *Berberis aristata* barks [6], water and ethanol extracts of *Dodonae aviscosa* (L.) Lacq. aerial parts [7], *Castanospermum australe* seeds [8], and *Pilea microphylla* (L.) whole plants [9] were demonstrated to have DPP-IV inhibitory and antioxidant activities.

Alkaloids (Berberine) from *Berberis* spp. and flavonoid fractions from *P. microphylla* also showed DPP-IV inhibitory activity. They were tested in diabetic rat models, with the results showing that they could be used effectively in blood glucose lowering, as well as positive control of DPP-IV enzyme anti-diabetic drugs (Diprotin A, sitagliptin and vildagliptin).

In this study, 14 herb extracts of thirteen selected plants used in a Thai folk anti-diabetes remedy, namely Krom Luang Chomphon, or Mor Phon's recipe, were prepared and utilized for their DPP-IV inhibitory and antioxidant activities. This folk medicinal recipe has been previously used in Thai traditional medicine to treat diabetes patients effectively. However, no information of the DPP-IV inhibitory activity of these 13 plants has been previously reported. Moreover, their phytochemical compositions, such as phenolic compounds and alkaloid, anthocyanin, and terpenoid constituents, were investigated. The relationship between biological activities and phytochemical contents were also evaluated. The information gained from this study will support the usage of the recipe in the treatment of DM.

Materials and methods

Chemicals

Dipeptidyl peptidase-IV (DPP-IV) enzyme, trichloroacetic acid, folin reagent, and 1,10-phenanthroline solution were purchased from Merck, Germany. Analytical grade gallic acid, sodium carbonate (Na_2CO_3), 2,2-diphenyl-1-picrylhydrazyl (DPPH), sodium acetate trihydrate, butylated hydroxytoluene (BHT), quercetin, gly-pro-*p*-nitroanilide-*p*-toluene sulfonate salt, and ascorbic acid were purchased from Sigma-Aldrich, Switzerland. Sodium chloride was purchased from RCI Labscan Ltd.,

Thailand. Solvents for extraction and analytical processes, including ethanol, methanol, and chloroform, were purchased from Labscan, Thailand. All solvents were of analytical grade and there was no distillation prior to use.

Plant materials

Thirteen plants (**Table 1**), *Abutilon hirtum* Lam., *Acanthus ebracteatus* Vahl., *Diospyros rhodocalyx* Kurz., *Lagerstroemia speciosa* (L.) Pers., *Mimosa pudica* L., *Pandanus amaryllofolius* Roxb., *Phyllanthus amarus* Schumach. & Thonn., *Rhinacanthus nasutus* (L) Kurz., *Senna alata* (L.) Roxb., *Senna siamea* Lam. Irwin & Barneby, *Terminalia catappa* L., *Vitex glabrata* R.Br., and *Zea mays* L. were selected from Krom Luang Chomphon, or Mor Phon's Thai folk anti-diabetes remedy, which have been indicated to treat diabetes. All plants were purchased from Khuan Niang district, Songkhla, and Thai traditional drug stores in Hat Yai, Songkhla,Thailand.

Table 1 List of selected plants from Krom Luang Chomphon recipe which are utilized in this study.

No.	Name	Common name	Thai name	Family	Part	Abbreviations
1	*Abutilon hirtum* Lam.	Florida Keys Indian mallow	Khropchakkrawaan	MALVACEAE	Whole plant	AHW
2	*Acanthus ebracteatus*Vahl.	Sea holly	Ngueakplamo	ACANTHACEAE	Whole plant	AEW
3	*Diospyrosrhodocalyx* Kurz.	Ebony	Ta kona	EBENACEAE	Bark	DRB
4	*Lagerstroemia speciosa* (L.) Pers.	Banaba	Inthaninnam	LYTHRACEAE	Leaves	LSL
5	*Mimosa pudica* L.	Sensitive plant	Maiyarap	FABACEAE	Whole plant	MPW
6	*Pandanus amaryllofolius* Roxb.	Pandanus palm	Toei hom	PANDANACEAE	Leaves	PAL
7	*Phyllanthusamarus* Schumach. & Thonn.	Carry me seed	Luktaibai	EUPHORBIACEAE	Whole plant	PAW
8	*Rhinacanthusnasutus* (L) Kurz.	White crane flower	Tong pan chang	ACANTHACEAE	Leaves	RNL
9	*Senna alata* (L.) Roxb.	Seven golden candle stick	Chumhetthet	LEGUMINASEAE	Leaves	SAL
10	*Senna siamea* Lam. Irwin & Barneby	Cassod tree	Khilek	LEGUMINASEAE	Buds	SSB
					Heartwood	SSH
11	*Terminalia catappa* L.	Tropical almond	Hukwang	COMBRETACEAE	Leaves	TCL
12	*Vitexglabrata* R.Br.	Smooth chastetree	Khainao	VERBENACEAE	Bark	VGB
13	*Zea mays* L.	Corn	Kao pod	POACEAE	Silk	ZMS

Herb extracts preparation process

The plant materials were rinsed thoroughly with tap water, to remove any foreign matter, and dried by a hot air oven at 50 °C for 2 days. The dried plants were then ground with an electric grinder, weighed, and stored in a desiccator at room temperature (25 - 30 °C) protected from light. Each plant powder (30 g) was macerated with 95 % ethanol (150 mL) for 2 days at room temperature. Supernatant above settled material was filtered through a filtering paper (Whatman® No. 1). After that, the same plant powder was re-extracted by the same maceration method with a fresh solvent for another 2 times. Each filtrate was

pooled and evaporated to dryness under reduced pressure at 45 °C by a rotary evaporator, and the resulting herb extracts were kept in a refrigerator at –20 °C, protected from light until use.

Dipeptidyl peptidase-IV (DPP-IV) inhibitory activity testing

DPP-IV inhibitory activity testing was performed according to the report of Al-Masri and colleagues [10] with some modification. Diprotin A was used as a positive standard and diluted to various concentrations by Tris-HCl buffer (50 mM, pH 7.5). Each sample solution was prepared by dilution of the extract with Tris-HCl buffer, to have a final concentration of 50 μg/mL. Diprotin A solution or sample solution (40 μL) was transferred to each well of 96-microplates, followed by the addition of 20 μL of DPP-IV enzyme (0.05 U/mL). After adding the enzyme, the mixture was pre-incubated for 10 min at 37 °C to enhance the binding capacity of the inhibitor. This was followed by the addition of 100 μL of gly-pro-*p*-nitroanilide (GPPN 0.2 mM in Tris-HCl) as a substrate. The incubation was continued at 37 °C for 30 min. The reaction was terminated by the addition of 30 μL of 25 % glacial acetic acid. The absorbance was then measured at 405 nm using a microplate reader (DTX880 Multimode Detector, Beckman Coulter®, Austria). The percentage of inhibition was calculated according to the following equation;

$$\%\text{Inhibition} = \{(A_{control} - A_{sample}) / A_{control}\} \times 100 \quad (1)$$

where $A_{control}$ = absorbance of DPP-IV solution without sample.
A_{sample} = absorbance of DPP-IV react with sample.

The tests were carried out in triplicate for each sample.

Antioxidant activity testing

2,2-Diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging assay

A DPPH scavenging assay was carried out using the method of Brand-Williams [11] with slight modifications. A stock solution of DPPH was prepared by dissolving 24 mg of DPPH in 100 mL methanol and storing it at –20 °C until use. The working solution (24 % w/v) was obtained by mixing 10 mL of the stock solution with 45 mL methanol to obtain a solution which had an absorbance of 1.1 ± 0.02 units at 515 nm using a UV-visible spectrophotometer (Diode array 8452A, Hewlett Packard, USA).

The sample solution (1,000 μg/mL in methanol) was further diluted with methanol to obtain several concentrations in a range of 0.15 - 75 μg/mL. Butylated hydroxytoluene (BHT) and ascorbic acid were used as positive standards. An aliquot (30 μL) of these solutions (samples and positive standards) were allowed to react with 170 µL of the DPPH working solution for 30 min in the dark. The absorbance was then measured at 515 nm using a microplate reader (PowerWaveX, Biotex instruments®, USA). Sample concentrations providing 50 %inhibition (IC_{50}) were calculated from a graph plotted between %inhibitions against sample concentrations. The percentage of inhibition was calculated according to the following equation:

$$\%\text{Inhibition} = \{(A_{control} - A_{sample}) / A_{control}\} \times 100 \quad (2)$$

where $A_{control}$ = absorbance of DPPH solution without sample.
A_{sample} = absorbance of DPPH reaction with sample.

The tests were carried out in triplicate for each sample.

Phytochemical determination

Total phenolic content was determined by the previously reported Folin-Ciocalteu method [12]. The aluminum chloride colorimetric method was used for flavonoid determination [13]. Total anthocyanin content was determined by using spectrophotometrics, according to the pH differential method [14]. Total alkaloid content was determined by using bromocresol green (BCG) as a reagent to form a yellow-colored

product [15]. The total terpenoid content was determined according to the previous reported method [16] with some modification.

Statistical analysis

The experimental data were reported as mean±SD. To compare the results among each other, one-way analysis of variance (one-way ANOVA) and multiple linear regression analysis of variance (stepwise method) were performed, with a 95 % confidence level (p value < 0.05), using SPSS software.

Results and discussion

DPP-IV inhibitory activity

The dipeptidyl peptidase - IV inhibitory activities of the ethanolic extracts from the selected herbs from the folk medicinal recipe were determined by their ability to reduce the DPP-IV enzymatic activity, using diprotin A as the standard reference. The DPP-IV inhibitory activities of the 14 extracts from the 13 selected plants are shown in **Table 2**. Inhibitory activity (%) of all samples at 50 μg/mL was in a range of 16.52 ± 0.11 to 71.07 ± 0.07 %, while diprotin A, at 50 μg/mL, gave 90.07 ± 0.39 % inhibition. Most of the medicinal plants in this list were known to have antidiabetic effects in animal models, such as LSL[17], TCL [18], AHW [19], PAW [20,21], SAL [22], ZMS [23], MPW [24], and PAL [25]. A number of mechanisms have been reported in the hypoglycemic effect of these selected herbs, for example, glucosidase inhibitor [26], amylase inhibitor [21], activate glucose transporter 1 (GLUT1) [19], increase in circulation of insulin level [23], and stimulating or regenerating effects in pancreatic β-cells [18]. However, none of the selected plants have been reported as having DPP-IV inhibitory activities. Our results are the first reported of DPP-IV inhibitory activity in these 14 herbs, which are the ingredients of the Krom Luang Chomphon folk recipe. Two plant extracts that gave high activity in our list were ethanolic extracts of LSL (%Inhibition = 71.07 ± 0.07 % at 50 μg/mL) and TCL (%Inhibition = 69.89 ± 0.43 % at 50 μg/mL). Previously, LSL and TCL were found to have antidiabetic activity both in vivo and in vitro testing. LSL extract has been extensively reviewed [27], where water soluble LSL leaf extracts displayed hypoglycemic effects, both in animal models and human subjects [28]. The antidiabetic activity of the water LSL extract was mentioned to be due to its phytochemical compositions, including corosolic acid, ellagitannins, lagersteroemin, flosin B, and reginin A [29], or a combination thereof. Moreover, the hypoglycemic effect of standardized leaves extract of LSL (Glucesol®) was observed to have significant dose-dependent relationships in a clinical study [28].Liu and colleagues [30,31] reported that the blood-glucose lowering capability of LSL extracts could be due to glucose transport stimulation and adipocyte differentiation inhibition. Moreover, Hou and colleagues [32] proposed that the antidiabetic activity of ethyl acetate extract of LSL could be due to α-glucosidase [32] and α-amylase [32] inhibition. Corosolic acid was found to be the major active compound which played an important role. TCL was also demonstrated to have a hypoglycemic effect in animal models, both in rabbit [33] and in rat models [18]. The result from the rabbit model showed that the aqueous decoction of TCL displayed a dose-dependent hypoglycemic effect; however, the most effective dose was at 40 mg/mL. Not only could the TCL extract reduce hyperglycemia, but also the blood glucose level was brought up to the normal level after the treatment; however, no mechanism was proposed. Ahmed *et al.* [18] showed that TCL aqueous extract exhibited significant hypoglycemic effects in diabetic rat models, without a change in body weight. In addition, Ahmed *et al.* suggested that the blood glucose lowering capacity of TCL extracts could be according to the promotion of β-cells regeneration. Also, TCL ethanolic extract was also shown to have α-glucosidase inhibitory activity, which may be another role in hypoglycemic properties [26]. It was not reported, however, that LSL and TCL extracts have DPP-IV inhibitory activity. Our result is the first report to confirm that the LSL and TCL hypoglycemic activity could be due to their ability to inhibit the DPP-IV enzyme. Two types of incretin hormones are responsible for the glucose regulation process, including glucagon like peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP) [34]. DPP-IV enzyme is a soluble plasma enzyme presented in the capillary of gut mucosa [35], and many organs, e.g., kidney, liver, and intestines [36]. This enzyme degrades GLP-1 and GIP, making them biologically inactive [37]. DPP-IV inhibitors block the enzyme, resulting in prolonging the half-life and

biological activity of both incretin hormones. The latter could promote β-cells regeneration, insulin secretion, and glycogenolysis, so a hypoglycemic effect would be the result [38].

Table 2 Comparative DPP-IV inhibitory activity of the ethanolic extracts of the selected herbs from the anti-diabetes folk medicinal recipe at 50 μg/mL.

Sample	%Inhibition at 50 μg/mL
Diprotin A	90.07 ± 0.39
LSL	71.07 ± 0.07*
TCL	69.89 ± 0.43*
SSB	51.72 ± 0.04*
SSH	49.44 ± 0.43*
PAW	49.11 ± 0.26*
AEW	43.78 ± 0.57*
VGB	38.78 ± 0.43*
SAL	30.20 ± 0.36*
ZMS	30.02 ± 0.05*
RNL	28.97 ± 0.08*
DRB	25.00 ± 0.67*
MPW	23.46 ± 0.03*
PAL	17.54 ± 0.20*
AHW	16.52 ± 0.11*

*Significantly difference when compared with standard Diprotin A

DPPH radical scavenging capacity of the plant extracts

The free radical scavenging activity of the extracts from the 14 ethanolic extracts was also determined by the ability to reduce the DPPH free radicals, using ascorbic acid and BHT as the standard references, and were expressed as IC_{50} value (μg/mL). Ascorbic acid and BHT were found to have IC_{50} values of 4.28 and 4.82 μg/mL, respectively. The DPPH radical scavenging capacity (IC_{50}) of the ethanolic extract samples are summarized in **Table 3**. All extracts could scavenge DPPH radicals; however, they scavenged in different capacities. The antioxidant properties of these herbs have been previously reported. AHW [39], DRB [40], MPW [41], PAL [42], PAW [43], RNL [44], TCL [45], and ZMS [46] were found to have DPPH free radical scavenging activity. The lowest IC_{50} value (the highest DPPH radical scavenging capacity) in this study was found in the TCL ethanolic extract, with an IC_{50} value of 4.39 ± 0.12 μg/mL, and LSL, with an IC_{50} value of 10.25 ± 0.23μg/mL. It is worth noting that TCL ethanolic extract could scavenge DPPH radical as well as ascorbic acid and BHT antioxidants. It is quite common that hyperglycemia in diabetes patients is a major course of oxidative stress. There are a number of pathways involving hyperglycemia, leading to greater free radical production, including mitochondrial respiration, glucose oxidation activation of the polyol pathway, and the formation of advanced glycation and product (AGE) [47,48]. These pathways would promote over production of reactive oxygen species (ROS). Moreover, in diabetes, lower cellular antioxidant capacity is normally observed [49]. Therefore, long-term diabetes is often associated with secondary complications, such as high blood pressure, heart disease, cerebral ischemia, kidney and nervous system diseases, and blindness, which may result from an over production of free radicals. Medicinal plants are known to be rich sources of antioxidants. They may therefore act synergistically with their hypoglycemic properties to exert anti-

diabetic action. The results from this study revealed that 11 extracts showed high radical scavenging capacity; therefore, consumption of these herbs in diabetic patients would help in diabetes treatment. TCL and LSL not only showed high antioxidant properties, but they also had high DPP-IV inhibitory activity. Hence, TCL and LSL should be the medicinal herbs of choice for effective use in T2DM patients.

Table 3 The DPPH radical scavenging activities of the ethanolic herb extracts of plants selected from the anti-diabetes folk medicinal recipe.

Sample	IC_{50} (μg/mL), (n = 3)
Ascorbic acid	4.28 ± 0.01
Butylated hydroxytoluene (BHT)	4.82 ± 0.01
TCL	4.39 ± 0.12
LSL	10.25 ± 0.22
VGB	16.32 ± 0.55
SSH	18.21 ± 0.94*
SSB	40.59 ± 0.95*
AEW	59.44 ± 1.78*
ZMS	62.98 ± 6.10*
PAW	66.82 ± 3.08*
SAL	83.90 ± 12.61*
MPW	96.35 ± 6.19*
DRB	120.81 ± 7.25*
AHW	>150*
PAL	>150*
RNL	>150*

*Significantly difference when compared with standard ascorbic acid and BHT

Phytochemical constituent determinations

Total phenolic, flavonoid, anthocyanin, alkaloid, and terpenoid contents were evaluated. The results are shown in **Table 4**. Every herb contained phenolic, flavonoid, anthocyanin, and terpenoid constituents. RNL contained the highest amount of phenolic compounds, while SAL contained the highest content of flavonoids. The highest anthocyanin content was observed in silks of ZMS. The highest terpenoid content was found in the tuber of VGB. Ten ethanolic herb extracts from the total 14 extracts in Krom Luang Chomphon's recipe contained alkaloid compounds. The highest was observed in the extract from the buds of SSB. These phytochemical screening results are similar to previous reports. Phenolic compounds were found in AHW [50], DRB [40], MPW [51], PAL [42], PAW [43], RNL [52], TCL [45], VGB [53], and ZMS [54]. Flavonoids were also previously observed in DRB [40], MPW [51], LSL [55], SSL [56], SSH [56], TCL [45], and ZMS [54]. Alkaloids were detected similarly in LSL [55], MPW [51], SSL [56], SSH [56], and TCL [57]. Both TCL and LSL, which displayed high DPP-IV inhibitory activity and antioxidants, contain high contents of phenolic compounds and terpenoids and, therefore may play important roles in these activities.

Table 4 Phytochemical compositions of the ethanolic extracts of the selected herbs of anti-diabetes folk medicinal recipe.

Samples	Phenolics[1]	Flavonoids[2]	Terpenoids[3]	Anthocyanins[4]	Alkaloids[5]
AHW	22.54 ± 1.43	61.02 ± 0.27	1,089.85 ± 1.59	9.83 ± 2.34	ND
AEW	54.25 ± 0.85	58.73 ± 0.61	901.94 ± 2.75	17.66 ± 4.03	8.31 ± 0.28
DRB	26.14 ± 1.18	57.99 ± 0.30	969.77 ± 3.18	3.69 ± 1.12	ND
LSL	211.83 ± 2.60	65.39 ± 0.41	2,157.69 ± 1.59	15.64 ± 8.55	5.99 ± 0.22
MPW	42.16 ± 0.71	60.92 ± 0.37	815.78 ± 1.59	19.41 ± 1.28	5.69 ± 0.25
PAL	44.82 ± 2.75	78.14 ± 1.34	1,581.15 ± 2.75	3.71 ± 1.34	4.78 ± 1.01
PAW	44.36 ± 0.92	74.76 ± 1.85	2,522.50 ± 1.59	28.04 ± 6.28	1.13 ± 0.04
RNL	32.57 ± 1.09	69.50 ± 1.03	1,266.75 ± 1.59	10.37 ± 5.01	0.82 ± 0.06
SAL	78.89 ± 1.17	128.86 ± 2.32	2,354.76 ± 3.18	10.68 ± 4.49	2.95 ± 0.25
SSB	150.99 ± 2.10	95.06 ± 0.78	1,879.05 ± 3.18	15.53 ± 6.19	29.25 ± 1.10
SSH	128.62 ± 2.41	43.10 ± 0.43	1,331.83 ± 1.59	8.24 ± 2.76	2.27 ± 0.08
TCL	476.87 ± 5.87	74.91 ± 0.54	1,578.39 ± 2.75	8.12 ± 0.75	5.47 ± 0.44
VGB	237.78 ± 2.47	19.58 ± 0.32	1,955.12 ± 2.75	9.37 ± 6.91	ND
ZMS	61.79 ± 1.24	104.09 ± 0.41	1,045.85 ± 1.59	37.92 ± 5.54	6.11 ± 0.21

Value represents in mean±SD (n = 4).
ND = not detected
[1]The total phenolic content is reported in gallic acid equivalents (μg/g dry mass).
[2]The total flavonoid content is reported in quercetin equivalents (mg/g dry mass).
[3]The total terpenoid content is reported in linalool equivalents (g/g dry mass).
[4]The total anthocyanin content is reported in mg of cyaniding-3-glucoside equivalents (c-3-gE) for 100 g of sample.
[5]The total alkaloid content is reported in atropine equivalents (mg/g dry mass).

Relationship between biological activities and phytochemical contents

The relationship between the DPP-IV inhibitory activities of the selected herbs with their phytochemical contents was established using regression analysis of variance. The results significantly indicated a high relationship only between phenolic content with DPP-IV inhibitory activity ($p < 0.05$) with $r^2 = 0.560$. A number of natural products have previously been found to have DPP-IV inhibitory activities, and most of them contain polyphenols [58], flavonoids, and alkaloids as the active components. Castanospermine, 7-deoxy-6-epi-castanospermine, and australine are major alkaloid compounds found in ethanolic extracts of *C. australe* [8], and they, as well as berberine alkaloids found in *B. aristata* [6] play the major roles in the DPP-IV inhibition of these plants. Moreover, a number of natural products containing flavonoid active components, such as *P. microphylla* (L.) [9], *Urena lobate* [59], and *D. viscose* [7], were also demonstrated to have DPP-IV inhibitory activities. However, the DPP-IV inhibitory activities of the selected herbs in our study did not show a relationship with their alkaloid, flavonoid, terpenoid and anthocyanin contents.

The relationship of DPPH antioxidant activity of the selected herbs with their phytochemical contents also significantly showed a relationship with phenolic content ($p < 0.05$), observed with $r^2 = 0.500$. This revealed that the antioxidant properties of these herb extracts could be due to their phenolic contents. Polyphenols, in particular tannins, phenylpropanoids, flavonoids, etc., are well known natural antioxidants [60]. However, no relationship between their antioxidant activity and other phytochemical

contents was observed. Therefore, any herb that has a high phenolic content should also show increased antioxidant activity.

Conclusions

Fourteen ethanolic herb extracts from 13 medicinal plants used in Krom Luang Chomphon, or Mor Phon's antidiabetic remedy, were found to have DPP-IV inhibitory and antioxidant activities. The result from the DPP-IV inhibitory activity testing found that leaves of LSL gave the highest activity. Our results showed a new anti-diabetic mechanism of the selected herbs, and also supported the use of Mor Phon's antidiabetic remedy as an alternative medicine. The ethanolic extract of TCL leaves showed the best antioxidant activity. Phenolic, flavonoid, and anthocyanin compounds were detected in all herb extracts. Alkaloids and terpenoids were found in some herbs. The screening result found that the DPP-IV inhibitory activity was related with terpenoids and phenolic contents, while the DPPH radical scavenging capacity was related with phenolic content. No relationship between alkaloids, flavonoids, and anthocyanins contents to DPP-IV inhibitory and antioxidant activity was found. The results revealed that the Krom Luang Chomphon recipe could manage diabetic treatment by various mechanisms. Dipeptidyl peptidase -IV inhibitor and antioxidant activity are partly involved in the effect. This information could be used to support the traditional wisdom with scientific results in the development of the recipe for functional and effective pharmacological use in diabetes related disease.

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