Original Article

Exploring Antioxidant and Anti-diabetic Activities, and Chemical Contents of Extracts from Thai Traditional Medicine (Pra-Sa-Ka-Phrao Remedies) and Its Plant Ingredients

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Abstract

Introduction: Pra-Sa-Ka-Phrao complete (PSKPC) remedy is a Thai traditional medicine published in the Thailand National List of Essential Medicines (NLEM). In this research, we have developed a modified version of the remedy, named as Pra-Sa-Ka-Phrao incomplete (PSKPIC), following the FDA Thailand's guidelines for using it as a food supplement. Notably, there is a lack of studies concerning biological activities and chemical constituents of both remedies.

Objectives: This study aimed to investigate and compare the antioxidant and anti-diabetic activities, and chemical contents derived from both remedies and its plant ingredient extracts.

Methods: Extraction was performed by maceration in 95% ethanol and decoction. The antioxidant activity was investigated using a DPPH, ABTS, FRAP, and TBARS assays, while the anti-diabetic (α-amylase, α-glucosidase inhibitory activities) were also evaluated, along with the determination of total phenolic (TPC) and total flavonoid (TFC) contents.

Results: The ethanolic extract of *Zingiber officinale* (ZOE) and water extract of *Ocimum sanctum* (OSW) exhibited the highest antioxidant activity, TPC, and TFC contents. The antioxidant results revealed that the PSKPIC water extract (PSKPICW) showed greater potency than PSKPC water extract in all assays. Additionally, the PSKPICW demonstrated higher TPC and TFC levels compared to the PSKPC remedy. *Glycyrrhiza glabra* (GGE) presented the strongest α-glucosidase inhibitory activity. However, all remedy extracts did not significantly affect anti-diabetic activity.

Conclusions: These results show the efficacy of the PSKPICW remedy, used as food ingredients or food supplements extract, and selected active extracts, such as ZOE and OSW, which supports their use in antioxidant products.

Keywords: Pra-Sa-Ka-Phrao remedy, Antioxidant activity, Anti-diabetic activity, Chemical content, Thai traditional medicine

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Introduction

Oxidative stress is a primary driver of cell and tissue damage that underpins the development of non-communicable diseases (NCDs) by life-style-associated activation.[1] NCDs have been well documented and studied, and some standard vital features have been identified; these include the intracellular presence of oxidative stress due to abnormal production of reactive oxidative species (ROS), inadequate antioxidant defense, and dysregulation of the autophagy pathway, which is responsible for the maintenance of cellular proteostasis and hyperglycemia.[2,3] Moreover, lipid peroxidation is one of the markers for oxidative stress; it also plays a crucial role in necrotic and apoptotic processes.[4]

Pra-Sa-Ka-Phrao (PSKP) remedy is a Thai traditional medicine published in Thailand National List of Essential Medicines (NLEM). It has long been used to treat flatulence and colic pain. It has a spicy taste and contains eight medicinal plants, including *Ocimum sanctum* L., *Citrus hystrix* DC., *Glycyrrhiza glabra* L., *Ferula assafoetida* L., *Piper nigrum* L., *Zingiber officinale* Roscoe, *Piper retrofractum* Vahl and *Allium sativum* L.

Half of the remedy's composition consists of *Ocimum sanctum* and the other half features other plants, with proportions shown in Table 1. In this study, the original remedy was named Pra-Sa-Ka-Phrao complete (PSKPC). Since the Food and Drug Administration of Thailand (FDA) implements certain regulation concerning the use of plants as food supplements, and the latest list issued by the FDA has excluded *Citrus hystrix*, *Ferula assafoetida*, and *Piper retrofractum*.[5,6] Consequently, we prepared the remedy without these three plants and named it Pra-Sa-Ka-Phrao incomplete remedy (PSKPIC) with proportions as shown in Table 1.

In previous studies, all plant ingredients of Pra-Sa-Ka-Phrao remedy showed antioxidant and antidiabetic activities. Notably, *Ocimum sanctum* possessed antidiabetic and anti-oxidant activities.[7] *Citrus hystrix* displayed antioxidant activity,[8] and *Glycyrrhiza glabra* showed activity against hyperglycemia, hyperlipidemia, and associated oxidative stress.[9] Furthermore, *Zingiber officinale* contained antioxidants,[10,11] and *Piper retrofractum* also demonstrated antioxidant activity.[12]

PSKP remedy has the potential for being developed as dietary supplement for the treatment of diabetes mellitus and NCDs patients. However, studies have yet to be conducted on the biological activities and chemical contents of the Pra-Sa-Ka-Phrao remedy. Therefore, this study aimed to investigate and compare antioxidant and antidiabetic activities and chemical contents of complete and incomplete PSKP remedies and its plant ingredient extracts.

Methods

Plant materials and extraction method

The plant ingredients were purchased from different sources in 2019. The identification of plants was carried out by the Herbarium of Southern Center of Thai Medicinal Plants at the Faculty of Pharmaceutical Science, Prince of Songkla University, Songkhla province, Thailand (Table 1). All dried plant materials were cleaned and grinded into coarse powder. The crude powders were extracted by maceration in 95% ethanol at room temperature (RT) for 3 days, then filtered and the process repeated twice. The combined filtrates were evaporated by rotary evaporator at 45 °C. Furthermore, a decoction in distilled water at boiling point for 15 minutes was conducted three times (3×1 L, for each time), filtered and freeze-dried using a lyophilizer. All crude extracts were kept in a freezer (-20 °C) until used.

Table 1 Plant ingredients of Pra-Sa-Ka-Phrao remedies.

Scientific name	Family	Code	Voucher specimen number	Part used	PSKPC* (%w/w)	PSKPIC* (%w/w)
Ocimum sanctum L.	LABIATAE	OS	SKP 095 15 19 01	Leaf	50.00	75.00
Citrus hystrix DC.	RUTACEAE	CH	SKP 166 03 08 01	Peel	22.22	-
Glycyrrhiza glabra L.	LEGUMINOSAE	GG	SKP 072 07 07 01	Root	8.90	13.33
Ferula assafoetida L.	UMBELLIFERAE	FA	SKP 199 06 01 01	Resin	8.90	-
Piper nigrum L.	PIPERACEAE	PN	SKP 146 16 14 01	Fruit	2.22	3.33
Zingiber officinale Roscoe	ZINGIBERACEAE	ZO	SKP 206 26 15 01	Rhizome	2.22	3.33
Piper retrofractum Vahl	PIPERACEAE	PR	SKP 146 16 03 01	Fruit-spike	2.22	-
Allium sativum L.	LILIACEAE	AS	SKP 006 01 19 01	Bulb	2.22	3.33
Sodium chloride	-	-	-	-	1.10	1.68

* PSKPC means Pra-Sa-Ka-Phrao remedy (complete) and PSKPIC means Pra-Sa-Ka-Phrao remedy (incomplete).

Preparation

***In vitro* assay for antioxidant activities**

DPPH radical scavenging assay

The scavenging effect on the DPPH radical was conducted according to Yamasaki et al., 1994.[13] The ethanolic extracts were dissolved in absolute ethanol, and the water extracts were dissolved in sterile water at different concentrations (1, 10, 50, 100 µg/mL). A 100 µL of sample solution was added into 96-well microplates, and 100 µL of DPPH solution was placed into each well. Solution control was absolute ethanol and distilled water, each 100 µL with DPPH 100 µL, and incubated for 30 minutes in the dark at RT. Finally, the absorbance was measured at 520 nm using a microplate reader. The Prism program calculated the EC_{50} values. The positive control was butylated hydroxytoluene (BHT).

$$\% \text{ Inhibition} = \frac{OD_{control} - OD_{sample}}{OD_{control}} \times 100$$

Where $OD_{control}$ was the optical density of solvent without sample solution, OD_{sample} was the optical density of sample solution and the EC_{50} value was calculated by the prism software.

ABTS radical scavenging assay

ABTS radical scavenging was determined according to the modified method of Re et al., 1999.[14] The $ABTS^{\bullet+}$ working solution was added to a microcentrifuge tube (1,000 µL), followed by 10 µL of Standard (final concentration of Trolox 0.1-20 µM, sample 100 µg/mL) or Blank (ultra-pure water). After mixing and incubation in the dark at RT for 6 min, the absorbance of the solution was measured at 734 nm.

The percentage of inhibition was calculated as the follow formular:

$$\% \text{ Inhibition} = \frac{OD_{control} - (OD_{sample} - OD_{negative})}{OD_{control}} \times 100$$

Where $OD_{control}$ was the optical density of solvent without sample solution, OD_{sample} was the optical density of sample solution, $OD_{negative}$ was the optical density of sample solution without $ABTS^{\bullet+}$ solution and the IC_{50} value was calculated by the prism software.

FRAP radical scavenging assay

The FRAP radical scavenging assay was determined by a modified method of Benzie and Szeto, 1999.[15] 20 µL of Standard (The final concentration of Trolox 5-300 µg/mL, ferrous sulphate ($FeSO_4$) 5-800 µg/mL, and sample (100 µg/mL) or Blank (distilled water) were added to 96-well microplates, followed by 180 µL of FRAP reagent (incubated at 37°C for 4 min before use). After incubating at RT for 8 min, the absorbance of the solution was measured at 593 nm, using FRAP working solution as Blank. The reading of relative absorbance should be within the range of 0-2.0; otherwise, the sample should be diluted. The antioxidant potential was determined from a standard curve plotted using Trolox or $FeSO_4 \bullet 7H_2O$ linear regression equation to calculate the FRAP values of the sample.

Assay of thiobarbituric acid reactive substances (TBARS)

TBARS assay was determined by a modified method of Ruberto and Baratta, 2000.[16] 50 µL of sample solution was added into the sample centrifuge tube, and 50 µL of sample solvent (DI/Abs.EtOH) was added into the full reaction mixture (FRM) tube. Then, 25 µL of PBS was added into each tube. After that, 1,250 µL of 2% intralipid was added into each tube (without blank (BLK) sample, PBS 1,250 µL was added). 25 µL of DI was added into BLK FRM and BLK sample, and 25 µL of $FeSO_4 \bullet 7H_2O$ was added into FRM and each sample was incubated at 37 °C for 20 minutes in a water bath. 1,000 µL of 0.6 w/v TBA in 20% w/v TCA was added into each tube and heated in a water bath at 95 °C for 30 min, then the reaction was stopped in a cooled ice bath for around 10 min. 2,500 µL of butanol was added, mixed and centrifuged at 5,000 g 25 °C for 20 minutes. The butanol fraction's upper layer was removed and 200 µL pipetted into the 96-well plate, then optical absorbance was determined at 532 nm. BHT was used as a positive control, and butanol as a Blank.

The percentage of inhibition was calculated using the following equation:

$$\% \text{ Inhibition} = \frac{(FRM - (ST - SA))}{FRM} \times 100$$

Where FRM was the optical density of full reaction mixture, ST was the optical density of sample test mixture, SA was the optical density of sample alone and the IC_{50} value was calculated by the prism software.

***In vitro* assay for anti-diabetic activities**

***In vitro* alpha-amylase inhibitory assay**

According to some modifications of Yuan, et al. (2018),[17] 100 µL sample solution (10 mg/mL), ethanolic extracts were dissolved in dimethyl sulfoxide (DMSO), and water extracts were dissolved in ultra-pure water. Then, it was mixed with 100 µL of a substrate (starch solution 1% w/w) in 20 mM phosphate buffer pH 6.9 containing 6.7 mM sodium chloride and pre-incubate at 37 °C for 10 min in a water bath. 100 µL of alpha-amylase enzyme (1 mg/mL) in buffer pH 6.9 was added and the Blank was added to buffer pH 6.9 and incubated in a water bath at 37 °C for 10 min. The reaction was stopped by adding 200 µL of dinitro salicylic acid (DNSA) reagent and heating 95 °C for 5 minutes in a heated box. Then, it was placed on an ice bath for 5 minutes. 50 µL of solution mixture was removed and placed into a 96-well plate and diluted with 200 µL ultra-pure water, then optical absorbance was determined at 540 nm. Acarbose was used as a positive control.

***In vitro* alpha-glucosidase inhibitory assay**

A slight modification of the method of Wongnawa, et al. (2014) was conducted for inhibitory activity on alpha-glucosidase.[18] 20 µL of sample extract (50 mg/mL), 80 µL of phosphate buffer (pH 6.8), and 50 µL of the substrate 5 mM *p*-nitro-phenyl alpha-D-glucopyranoside (*p*-NPG) in phosphate buffer, a blank phosphate buffer pH 6.8, was added and pre-incubated at 37 °C for 15 min. After pre-incubation, 50 µL of alpha-glucosidase (0.15 unit/mL) was added; blank phosphate buffer pH 6.8 was added and then incubated at 37 °C for 15 min. The reaction was stopped by adding 100 µL of 1 M Na_2CO_3. The release of p-nitrophenol was measured at 405 nm. Acarbose was used as a positive control.

The percentage inhibition of both alpha-amylase and alpha-glucosidase were calculated as the follow formular:

$$\% \text{ Inhibition} = \frac{(OD_{control} - OD_{sample})}{OD_{control}} \times 100$$

Where $OD_{control}$ was the optical density of solvent without sample solution, OD_{sample} was the optical density of sample solution and the IC_{50} value was calculated by the prism software.

Chemical contents

Determination of total phenolic content

The total phenolic content was determined according to modified Folin-Ciocalteu's method.[19] 20 µL of the sample solution was pipetted into a 96-well microplate and then, 100 µL of Folin-Ciocalteu's reagent was added into the well, and 80 µL of a sodium carbonate solution was added in the last step. After that, the 96-well plate was kept at RT for 30 minutes. The absorbance was measured at 765 nm. The total phenolic content was expressed as gallic acid equivalent (GAE) in milligrams per gram of dry material.

Determination of total flavonoid content

The following method, with slight modification of Zhu, et al. (2009), was conducted to determine total flavonoids in extracts.[20] Sample (1 mg/mL) of 500 µL was mixed with 75 µL of sodium nitrite (5% w/v) and 150 µL of $AlCl_3$ (10% w/v) and incubated at RT for 5 min. Then 500 µL of 1 M NaOH solution was added and 275 µL of distilled water placed in a centrifuge tube, incubated for 30 minutes at RT and transferred 200 µL to a 96-well microplate. Finally, the absorbance was measured at 510 nm. Absolute ethanol and distilled water were used as a Blank for the ethanolic and water extracts. The total flavonoid content was expressed as milligram quercetin equivalents (QE)/g dry extract.

Data and Statistical Analysis

All determinations were expressed as the means ± SEM (standard error of mean) of three independent samples in triplicate. The value of EC_{50} and IC_{50} was calculated using GraphPad Prism 8. Linear regression to correlate the total phenolics and total flavonoids was carried out using Microsoft Excel 2019. The differences among the mean values from at least two independent experiments were analyzed with the GraphPad Prism 8, one-way ANOVA. Significant differences were considered statistically significant at the level of p-value < 0.05.

Results

***In vitro* assay for antioxidant activities**

DPPH radical scavenging assay

The results are depicted in Table 2. The PSKPICW remedy showed high antioxidant activity with an EC_{50} value of 18.06 ± 0.36 µg/mL, while the EC_{50} value of the PSKPCW remedy was 32.69 ± 1.59 µg/mL. OSW demonstrated DPPH scavenging activity with an EC_{50} value of 8.93 ± 0.50 µg/mL better than positive control, BHT (EC_{50} = 16.22 ± 1.03 µg/mL). In addition, 95% ethanolic extracts of PSKPICE and PSKPCE remedies showed EC_{50} of 52.88 ± 1.86 and 65.65 ± 1.52 µg/mL, respectively. The ZOE also showed higher antioxidant activity than BHT (EC_{50} of ZOE = 9.56 ± 0.15 µg/mL).

ABTS radical cation scavenging assay

The PSKPICW remedy had the highest scavenging activity at 43.44 ± 2.32 µg/mL when compared with standard Trolox (IC_{50} = 14.68 ± 0.82 µg/mL). In contrast, the PSKPCW remedy showed an IC_{50} value of 73.73 ± 1.38 µg/mL. The OSW showed scavenging activity with IC_{50} values of 32.86 ± 2.72 µg/mL. While the PSKPCE and PSKPICE remedies showed IC_{50} values of 62.83 ± 0.66 and 88.75 ± 1.65 µg/mL, respectively. The ZOE (IC_{50} = 8.98 ± 0.20 µg/mL) showed stronger scavenging activity than Trolox (Table 2).

Ferric reducing / antioxidant power (FRAP) assay

The ethanolic extracts showed FRAP values ranging from 4.06 ± 1.80 to 839.68 ± 10.17 mg Fe^{2+} equivalent/g extract. The PSKPCE and PSKPICE remedies showed high antioxidant activity with FRAP values of 183.17± 1.26 and 145.65 ± 6.55 mg Fe^{2+} equivalent/g extract, respectively. The ZOE showed the highest antioxidant activity (FRAP values = 839.68 ± 10.17 mg Fe^{2+} equivalent/g extract). For the aqueous extracts, FRAP values ranged from 7.84 ± 2.19 to 383.47 ± 13.22 mg Fe^{2+} equivalent/g extract; the PSKPCW and PSKPICW remedies showed antioxidant activity with FRAP values of 172.59 ± 5.72 and 261.54 ± 5.66 mg Fe^{2+} equivalent/g extract, respectively. The OSW showed the highest antioxidant activity of 383.47 ± 13.22 mg Fe^{2+} equivalent/g extract (Table 2).

The trolox equivalent antioxidant capacity (TEAC), the ethanolic extracts showed FRAP values ranging from 0.35 ± 0.02 to 342.17 ± 6.30 mg Trolox equivalent/g extract, and water extracts showed TEAC values ranging from 2.26 ± 0.67 to 147.63 ± 4.85 mg trolox equivalent/g extract (Table 2).

Determination of lipid peroxidation on thiobarbituric acid reactive substances (TBARS) assay

The results are presented in Table 2. The PSKPCE and PSKPICE remedies showed IC_{50} of 1.02 ± 0.02 and 4.99 ± 0.69 µg/mL, respectively, followed by the OSE showed higher lipid peroxidation inhibitory activity than BHT as a positive control (IC_{50} = 2.33 ± 0.31 µg/mL) while IC_{50} value of BHT as 3.36 ± 0.09 µg/mL. The PSKPCW remedy showed high antioxidant activity with an IC_{50} value of 1.18 ± 0.15 µg/mL, while the IC_{50} value of the PSKPICW remedy was 8.23 ± 0.52 µg/mL. The OSW, with IC_{50} values of 1.04 ± 0.01 µg/mL, showed higher antioxidant activity than BHT.

Determination of *In vitro* assay for anti-diabetic activities

***In vitro* alpha-amylase and alpha-glucosidase inhibitory assay**

The results of the alpha-amylase and alpha-glucosidase inhibitory activity inhibitory activity are showed in Table 3. The finding revealed that all sample solutions at a concentration of 1,000 µg/mL demonstrated no significant effect on alpha-amylase activity (IC_{50} > 1,000 µg/mL) when compared to the positive control, acarbose (IC_{50} = 39.19 ± 0.38 µg/mL), for both the ethanolic and water extracts. Regarding alpha-glucosidase inhibition, GGE and ASE exhibited stronger activity with IC_{50} values of 39.37 ± 1.55 µg/mL and 109.46 ± 4.84 µg/mL, respectively, better than the positive control, acarbose (IC_{50} = 215.75 ± 1.40 µg/mL). CHE and CHW showed moderate activity with IC_{50} values of 599.39 ± 13.26 and 2,579.93 ± 48.71 µg/mL, respectively.

Determination of chemical contents

Determination of total phenolic content by using Folin-Ciocalteau's reagent

The ethanolic extracts range from 37.03 ± 2.87 to 178.20 ± 1.86 mg GAE/g extract, the PSKPCE and PSKPICE remedies showed a total phenolic content of 59.37 ± 0.37 and 57.02 ± 0.35 mg GAE/g extract, and the ZOE showed the highest total phenolic contents of 178.20 ± 1.86 mg GAE/g extract. The water extracts range from 3.33 ± 1.16 to 115.64 ± 2.60 mg GAE/g extract, the PSKPCW and PSKPICW remedies showed a total phenolic content of 55.79 ± 1.23 and 83.19 ± 1.67 mg GAE/g extract, respectively, and OSW showed the highest total phenolic contents of 115.64 ± 2.60 mg GAE/g extract (Table 2).

Determination of total flavonoid content by using aluminum chloride ($AlCl_3$) colorimetric method

The ethanolic extracts range from 96.13 ± 3.83 to 816.90 ± 4.35 mg QE/g extract, PSKPCE and PSKPICE remedies showed a total flavonoid content of 202.58 ± 1.22 and 192.79 ± 2.06 mg QE/g extract, respectively. The ZOE showed the highest total flavonoid contents of 816.90 ± 4.35 mg QE/g extract. The water extracts range from 85.14 ± 4.83 to 470.58 ± 3.60 mg QE/g extract, PSKPCW and PSKPICW remedies showed a total flavonoid content of 225.40 ± 1.72 and 334.77 ± 4.93 mg QE/g extract, respectively. The OSW showed the highest total flavonoid contents of 470.58 ± 3.60 mg QE/g extract (Table 2).

Table 2 Antioxidant activities (DPPH, ABTS, FRAP and TBARS assay) and chemical contents (total phenolic and flavonoid contents) of PSKP remedies and its plant ingredient extracts (mean ± SEM, n = 3).

Botanical name (Thai name)	CODE	DPPH	ABTS	FRAP		TBARS	TPC	TFC
		EC_{50} (µg/mL)	IC_{50} (µg/mL)	mg Fe^{2+} eq./g[1]	mg Trolox eq./g[2]	IC_{50} (µg/mL)	mg GAE/g[3]	mg QE/g[4]
Ocimum sanctum	OSE	31.21 ± 2.38*	98.47 ± 0.90*	165.43 ± 4.63	62.37 ± 1.95	2.33 ± 0.31	53.60 ± 1.15	215.97 ± 3.23
(Ka-Phrao-Daeng)	OSW	8.93 ± 0.50	32.86 ± 2.72*	383.47 ± 13.22	147.63 ± 4.85	1.04 ± 0.01	115.64 ± 2.60	470.58 ± 3.60
Citrus hystrix	CHE	>100*	85.56 ± 0.62*	126.15 ± 6.84	47.27 ± 0.70	14.29 ± 1.69*	48.98 ± 0.50	242.77 ± 2.02
(Ma-Krut)	CHW	>100*	>100*	83.05 ± 4.52	30.10 ± 0.62	15.35 ± 1.99*	42.06 ± 0.58	121.69 ± 3.74
Glycyrrhiza glabra	GGE	58.75 ± 3.00*	43.10 ± 2.00*	154.18 ± 7.28	57.10 ± 4.36	7.07 ± 1.11	61.62 ± 2.40	234.48 ± 4.96
(Cha-Em-Thet)	GGW	>100*	>100*	13.11 ± 2.07	3.18 ± 0.26	33.27 ± 2.33*	14.46 ± 1.16	103.78 ± 4.75
Ferula assafoetida	FAE	>100*	>100*	4.06 ± 1.80	0.35 ± 0.02	2.51 ± 0.24	42.08 ± 1.41	96.13 ± 3.83
(Ma-Ha-Hing)	FAW	>100*	>100*	7.84 ± 2.19	2.26 ± 0.67	1.10 ± 0.04	3.33 ± 1.16	105.77 ± 0.66
Piper nigrum	PNE	94.96 ± 1.97*	>100*	185.69 ± 5.92	71.48 ± 4.27	>100*	68.49 ± 0.78	167.93 ± 1.96
(Phrik-Thai-Lon)	PNW	>100*	>100*	15.08 ± 2.80	3.76 ± 0.13	>100*	11.25± 0.94	100.29 ± 4.52
Zingiber officinale	ZOE	9.56 ± 0.15	8.98 ± 0.20	839.68 ± 10.17	342.17 ± 6.30	5.17 ± 0.50	178.20 ± 1.86	816.90 ± 4.35
(Khing)	ZOW	66.63 ± 1.00*	91.41 ± 0.16*	88.61 ± 5.29	32.28 ± 0.15	11.68 ± 1.71*	33.32 ± 0.63	143.04 ± 4.41
Piper retrofractum	PRE	>100*	>100*	87.12 ± 9.81	38.87 ± 4.83	>100*	37.03 ± 2.87	225.12 ± 2.55
(Di-Pli)	PRW	89.39 ± 1.97*	>100*	62.90± 4.54	22.04 ± 1.09	>100*	30.76 ± 0.68	114.14 ± 4.83
Allium sativum	ASE	>100*	>100*	60.33 ± 0.78	21.23 ± 1.33	15.28 ± 2.42*	41.97 ± 3.00	105.74 ± 3.24
(Kra-Thiam)	ASW	>100*	>100*	21.93 ± 3.56	6.62 ± 0.21	4.80 ± 1.43	13.84 ± 1.17	85.14 ± 4.83
Pra-Sa-Ka-Phrao	PSKPCE	65.65 ± 1.52*#	62.83 ± 0.66*#	183.17 ± 1.26	70.60 ± 4.19	1.02 ± 0.02	59.37 ± 0.37	202.58 ± 1.22
remedy (Complete)	PSKPCW	32.69 ± 1.59*#	73.73 ± 1.38*#	172.59 ± 5.72#	65.98 ± 2.49#	1.18 ± 0.15	55.79 ± 1.23#	225.40 ± 1.72#
Pra-Sa-Ka-Phrao	PSKPICE	52.88 ± 1.86*#	88.75 ± 1.65*#	145.65± 6.55	56.85 ± 4.77	4.99 ± 0.69	57.02 ± 0.35	192.79 ± 2.06
remedy (Incomplete)	PSKPICW	18.06 ± 0.36#	43.44 ± 2.32*#	261.54 ± 5.66#	104.32 ± 1.62#	8.23 ± 0.52	83.19 ± 1.67#	334.77 ± 4.93#
Positive control	BHT	16.22 ± 1.03	NT	NT	NT	3.36 ± 0.09	NT	NT
	Trolox	NT	14.68 ± 0.82	NT	NT	NT	NT	NT

Note; 1 mean (y = 0.004x+0.01299, R2 = 0.9997), 2 mean (y = 0.0094x+0.01588, R2 = 0.9998), 3 mean (y = 0.0045x+0.0146, R2 = 0.9992), 4 mean (y = 0.0009x-0.0189, R2 = 0.9997). (NT) means not tested. E means ethanolic extract and W means water extract. BHT and Trolox as positive controls (* Significant difference: $p < 0.05$ vs BHT and Trolox; #: $p < 0.05$ vs complete and incomplete remedies). Data were analyzed by using one-way ANOVA.

Table 3 IC_{50} of alpha-amylase and alpha-glucosidase inhibitory activities of PSKP remedies and its plant ingredient extracts (n = 3).

Botanical name (Thai name)	CODE	IC_{50} (µg/mL; mean ± SEM)	
		Alpha-amylase inhibitory activity	Alpha-glucosidase inhibitory activity
Ocimum sanctum	OSE	>1,000*	>1,000*
(Ka-Phrao-Daeng)	OSW	>1,000*	>500*
Citrus hystrix	CHE	>1,000*	599.39 ± 13.26*
(Ma-Krut)	CHW	>1,000*	2,579.93 ± 48.71*
Glycyrrhiza glabra	GGE	>1,000*	39.37 ± 1.55*
(Cha-Em-Thet)	GGW	>1,000*	>3,000*
Ferula assafoetida	FAE	>1,000*	>3,000*
(Ma-Ha-Hing)	FAW	>1,000*	>3,000*
Piper nigrum	PNE	>1,000*	>1,500*
(Phrik-Thai-Lon)	PNW	>1,000*	>3,000*
Zingiber officinale	ZOE	>1,000*	>1,500*
(Khing)	ZOW	>1,000*	>3,000*
Piper retrofractum	PRE	>1,000*	>1,500*
(Di-Pli)	PRW	>1,000*	>3,000*
Allium sativum	ASE	>1,000*	109.46 ± 4.84*
(Kra-Thiam)	ASW	>1,000*	>3,000*
Pra-Sa-Ka-Phrao remedy	PSKPCE	>1,000*	>1,000*
(Complete)	PSKPCW	>1,000*	>1,000*
Pra-Sa-Ka-Phrao remedy	PSKPICE	>1,000*	>1,000*
(Incomplete)	PSKPICW	>1,000*	>1,000*
Acarbose		39.19 ± 0.38	215.75 ± 1.40

Note; E means Ethanolic extract and W means Water extract. Acarbose as positive control (* Significant difference: $p < 0.05$ vs Acarbose; #: $p < 0.05$ vs complete and incomplete remedies). Data were analyzed by using one-way ANOVA.

Discussion

PSKP remedy, a Thai traditional medicine included in the NLEM of Thailand for anti-flatulent and carminative properties in children. This study found that PSKP remedy exhibited the highest activity on anti-oxidant activity, however, it showed no effectiveness against alpha-amylase and alpha-glucosidase. Interestingly, both PSKP remedies and its plant ingredients demonstrated a potent effect in inhibiting lipid peroxidation through the inhibition of TBARS formation, except for PN and PR. The PSKPC extracts also showed a superior inhibitory effect compared to PSKPIC extracts, suggesting that the plant ingredients in the complete remedy, particularly CH and FA, also played an essential role in this activity. The inhibitory effect on TBARS relate to lipid peroxidation and malondialdehyde (MDA). TBARS are formed as a by-product of lipid peroxidation and MDA is one of several end products formed by the decomposition of lipid peroxidation products, serving as a marker of oxidative stress.[21] Therefore, inhibition of TBARS formation may result from the reduction of lipid peroxidation, which could lead to a decrease in MDA level. This finding represents the first research, indicating that PSKP and its plant ingredients exhibited significant potential as lipid peroxidation inhibitors. Further studies should be investigated to explore the under-

lying mechanisms and conduct *in vivo* studies for a more comprehensive understanding.

Regarding the radical scavenging activities, both PSKP extracts exhibited moderate scavenging activities against both DPPH and $ABTS^{\bullet+}$ radicals, except for PSKPICW, which showed strong scavenging activity on DPPH radical. OSW and ZOE also exhibited potent scavenging activities. The aqueous extract of OS exhibited higher activity than the ethanol extract, and the aqueous extract of PSKP also showed similar results. This indicated that DPPH and $ABTS^{\bullet+}$ scavenging activities of PSKP extracts relate to the OS. Our radical scavenging results of OS related to previous studies, which showed potent DPPH and $ABTS^{\bullet+}$ scavenging activities with EC_{50} not more than 20 µg/mL.[22] For ZO rhizome, a previous study of Ali et al., 2018 showed potent DPPH scavenging activity with IC^{50} value of 8.29 ± 1.73 µg/mL,[23] as well as ABTS•+, ZO presented a strong activity with an IC_{50} value of 0.81 µg/mL.[24]

Additionally, we found that the FRAP and TEAC values showed a similar trend to the scavenging activity. PSKPICW showed the highest FRAP and TEAC, while OSW and ZOE demonstrated higher values than other plant extracts. Our results were consistent with previous *in vitro* studies conducted in 95% ethanol extract of red holy basil (OS) presented higher antioxidant activity than white holy basil for both TEAC and FRAP values.[25] In addition, the FRAP assay of the rhizomes of ZO displayed a potent antioxidant capacity expressed as trolox equivalents.[26]

Both PSKP remedies lack inhibitory activity on alpha-amylase and alpha-glucosidase, as well as their plants ingredients, except CH, GG and AS. The ethanolic extract of GG and AS demonstrated stronger alpha-glucosidase inhibitory activity than the positive control, acarbose, whereas CHE showed moderate activity. Previous research has demonstrated that both GG methanolic and aqueous extracts inhibited enzyme alpha-amylase and alpha-glucosidase activities.[27,28] Interestingly, AS displayed significant results in rat everted intestinal sac experiments, showing the increasing of glucose uptake and reduction in all observed parameters. In addition, treatment with aged garlic extract positively reversed the diabetic changes in the targeted parameters to levels significantly lower than those measured in the control diabetic group.[29,30]

With regard to TPC and TFC, most of the PSKP extracts showed comparable TPC, except for the aqueous PSKPIC extract, which presented the highest TPC. Moreover, the aqueous extracts of PSKPC and PSKPIC showed higher flavonoid contents compared to the ethanolic extracts. Among the plant ingredients, ZOE demonstrated the highest TPC and TFC, followed by OSW. Interestingly, ZOE showed potent activity against DPPH, ABTS and TBARS, whereas, OSW showed better inhibitory effect on TBARS but lesser effect on DPPH and ABTS. These results suggested that the compounds in OSW specifically affected inhibitory activity on TBARS, while ZOE excelled in ABTS. However, ZOE still retained potent activity on TBARS with IC_{50} less than 10 µg/mL. When considering PSKP, the extracts showed potent activity on TBARS, highlighting the significance of OS in the remedies. In addition, although the ethanolic extract of CH, GG and PR showed comparable TPC and TFC to both the aqueous and ethanol extracts of PSKPC, they showed lesser effects on TBARS. These results indicated that the phenolic and flavonoid compounds in these plants did not specifically inhibit the lipid peroxidation.

In conclusion, this study investigated the biological activities of the PSKP remedy for the first time. We also modified the remedy according to the FDA plant list for use as a food supplement, assessing a comparison to the original formula. Our findings indicated that both original (complete; PSKPC) and modified (incomplete; PSKPIC) remedies exhibited potent inhibitory effect on TBARS formation, but the original remedy showed superior activity compared to the modified one. Therefore, the effect of PSKP remedy on insight into the mechanism of lipid peroxidation should be investigated. Detailed phytochemistry analysis should be conducted to identify compounds serving as markers,as well as to develop products for oxidative stress reduction.

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