Original Article

Quality Control, Antidiabetic, and Anti-inflammatory Effects, as Measured by Alpha-Amylase and Alpha-Glucosidase Activities, of a Weight-Loss Remedy from Worayokasan Scripture

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Abstract

Introduction: Diabetes increases the risk of free radical generation and health complications. Obesity is a major risk factor for developing Type 2 Diabetes (T2DM). The Weight-Loss (WL) remedy from Worayokasan scripture, consists of *Cyperus rotundus* L. (CR), *Terminalia chebula* Retz. (TCb), and *Tinospora crispa* (L.) Miers ex Hook.f. & Thomson (TCp). The WL remedy is used to reduce obesity, nourish the body, and act as a tonic. However, there is currently limited information on this natural remedy's potential anti-diabetic and anti-inflammatory effects.

Objectives: The objectives of this study were to evaluate the anti-diabetic, anti-inflammatory properties and quality control of the WL remedy and its plant ingredients.

Methods: The quality control was conducted using the Thai Herbal Pharmacopoeia (THP) method. The potential anti-diabetic effects were evaluated through the inhibition of α-amylase and α-glucosidase enzymes, and the potential anti-inflammatory effects were measured by assessing the reduction of nitric oxide (NO) production in the WL remedy and its plant constituents.

Results: The WL remedy passed the quality control guidelines set by the THP. TCpW demonstrated the most potent α-glucosidase inhibitory activity, with an IC_{50} of 201.28 µg/mL, surpassing acarbose. WLH, WLW, and WLE showed moderate α-glucosidase inhibitory activity. However, all samples exhibited low α-amylase inhibitory activity. The CRE extract exhibited the strongest anti-inflammatory activity by inhibiting NO production with an IC_{50} value of 31.93 µg/mL.

Conclusions: The results suggest that the WL remedy possessed anti-diabetic and weight-loss characteristics, and has the potential to be developed into a dietary supplement for individuals with T2DM.

Keywords: Weight-Loss remedy, Alpha-Amylase, Alpha-Glucosidase, Type 2 diabetes

Volume 2023, Issue 2, Page 118-128

https://asianmedjam.com

Received: 5 March 2023 **Revised: 26 April 2023** **Accepted: 2 June 2023**

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Introduction

Obesity and type 2 diabetes mellitus are major public health concerns worldwide, with overweight or obesity being the most significant predictor of Diabetes mellitus type 2 (T2DM).[1,2] T2DM is an endocrine and metabolic disorder characterized by chronically elevated blood glucose, which is expected to affect 783 million people by 2045.[3] Over 90% of all diabetes globally is type 2, and chronic hyperglycemia is linked to several complications, including cardiovascular disease, renal failure, neuropathy, and eye damage.[4-6] Antioxidants are crucial for protecting the body from damage caused by reactive oxygen species (ROS).[7] Therefore, substances that scavenge free radicals may help treat disorders such as diabetes, liver cirrhosis, atherosclerosis, and cancer.

To treat diabetes, one way is to reduce post-prandial glycemia by inhibiting enzymes responsible for carbohydrate breakdown, such as α-amylase and α-glucosidase.[8] However, synthetic drugs have limited effectiveness and severe side effects such as flatulence, stomach discomfort, and diarrhea.[9-11] As a result, researchers are working to develop new bioactive with high inhibitory potential and fewer side effects to prevent and treat T2DM.[12] Plants and plant-derived metabolites with α-glucosidase and α-amylase inhibitory properties, such as alkaloids, phenolic acids, flavonoids, terpenoids, anthocyanins, and glycosides, have been widely studied for their antidiabetic effects.[13,14] Additionally, natural polyphenols can activate antioxidant enzymes, remove unbound radicals, reduce α-tocopherol radicals, and retard oxidase enzymes.[15]

The book of Thai traditional medicine, Worayokasan scripture, contains a Weight-Loss remedy made from three readily available herbs: Cyperus rotundus L. (rhizomes), Terminalia chebula Retz. (fruit), and Tinospora crispa (L.) Miers ex Hook.f. & Thomson (stem). C. *rotundus* has been utilized for carminatives, which treat chronic diseases of the digestive tract. Indications of T. *chebula* include appetite enhancement, digestive support, liver stimulant, stomachic, gastrointestinal prokinetic agent, and mild laxative. T. *crispa* has been utilized for the treatment of internal inflammations, the reduction of thirst, the stimulation of appetite, and the maintenance of good health.[16-18] The WL remedy is used for reducing obesity, nourishing the body, and serving as a tonic. There is a possibility that the WL remedy may help reduce blood glucose levels since weight loss has been related to improvements in glycemic control, insulin sensitivity, and lipid profile.[19,20]

This study aimed to evaluate the potential hypoglycemic effect by α-amylase and α-glucosidase inhibition, and the anti-inflammatory effect by production inhibition of nitric oxide production (by LPS-activated RAW 264.7 cell line) of the WL remedy and its ingredients. Before testing these bioactivities for the safety and efficacy of herbal remedies, quality control is necessary, and standard tests such as moisture content, total ash, acid-insoluble ash, and extractive values were performed.[21] The ingredients of the WL remedy are widely used in traditional medicine in various countries, including Thailand, but there are no reports this remedy on their anti-diabetic or anti-inflammatory activities

Materials and Methods

Plant Materials and Extractions

The rhizomes of *Cyperus rotundus* Linn. (CR), fruits of *Terminalia chebula* Retz. (TCb), and stems of *Tinospora crispa* (L.) Miers ex Hook.f. & Thomson. (TCp) were obtained from different provinces in Thailand. The herbarium of the Southern Center of Thailand Medical Plants, Faculty of Pharmaceutical Sciences, Prince of Songkla University, Thailand, identified and verified the specimen voucher (Table 1). The plant materials were dried at 50°C, coarsely powdered, and extracted using two methods; decoction with water and maceration with 95% ethanol. The Weight-Loss remedy, comprising an equal percentage of CR, TCb, and TCp, was extracted using three methods: decoction, maceration, and digestion by boiling with 0.1N HCl (pH = 2) to simulate the gastric condition. The digested product was extracted with chloroform and then dried. The formula below was used to determine the percent yield. All extracts were stored at -20°C until use.

Table 1 Herbal ingredients in Weight Loss remedy from Wor-Ra-Yo-Ka-Sarn Scripture

Scientific Name	Family	Part of Use	Voucher Specimen Number[(a)]	Code
Cyperus rotundus L.	CYPERACEAE	Rhizome	SKP 060 03 18 01	CR
Terminalia chebula Retz.	COMBRETACEAE	Fruit	SKP 0459 20 03 01	TCb
Tinospora crispa (L.) Miers ex Hook.f. & Thomson	MENISPERMACEAE	Stem (wine)	SKP 114 20 03 01	TCp

[(a)]Voucher specimen number were shown at Prince of Songkla University Herbarium (PSU)

Chemical and Reagents

Dulbecco's Modified Eagle medium (DMEM), fetal bovine serum (FBS), penicillin-streptomycin (P/S), trypan blue stain 0.4%, and trypsin-EDTA from Gibco®, USA. Lipopolysaccharide from *E. coli* (LPS), phosphoric acid 85%, N-(1-Naphthy) ethylene-diamine dihydrochloride, sulfanilamide, thiazolyl blue tetrazolium bromide (MTT), α-glucosidase (Saccharomyces cerevisiae), potassium persulfate, bovine serum albumin (BSA), p-nitrophenyl α-D-glucopyranoside (p-NPG), and α-amylase from porcine pancreas type VI-B were purchased from Sigma-Aldrich®, USA. Sodium hydroxide was purchased from Univar®, Australia. Sodium bicarbonate was purchased from BHD®, England. Phosphate-buffered saline (PBS) was purchased from Amresco®, USA. Sodium potassium tartrate, disodium hydrogen phosphase hepatahydeate, 3,5-Dinitrosalicylic acid, sodium carbonate, sodium chloride, and acarbose were purchased from Merck®, Germany.

Quality control of the remedy and its plant ingredients.

The quality control method for the herbal material includes four tests: loss on drying, total ash, acid-insoluble ash, and extractive value. Herbs are chosen at random for testing three times in each test (n=3). The standard values for quality control were followed as outlined in the Thai Herbal Pharmacopoeia (THP).[21]

Loss on drying

Loss on drying involves weighing 2 grams of the dried plant powder and spreading it onto a dish. The sample is then dried in an electronic moisture analyzer at 105°C until a constant weight is reached. The percentage of drying loss is calculated.

$$\text{Loss on drying (\%)} = \frac{\text{Initial weight (g) - Final weight (g)}}{\text{Initial weight (g)}} \times 100$$

Total ash content

For total ash content, a clean and pre-weighed crucible is baked for five hours at 105°C until its weight is stabilized. Then, two grams of dried plant powder is poured in the crucible and ignited in a muffle furnace at 450°C for 9 and 5 hours. After cooling in a desiccator, the crucible is weighed again and repeated until the weight stabilizes. The percentage of total ash is calculated by comparing the weight before and after burning, where total ash is the residue left after combustion and consists of physiological and non-physiological ash.

$$\text{Total ash (\%)} = \frac{\text{Stable weight after burning sample (g)}}{\text{Stable weight of beginning sample (g)}} \times 100$$

Acid insoluble ash

Total ash is used to calculate acid-insoluble ash. Then, add 25 mL of 10% hydrochloric acid (HCl) to a crucible with all the ash. Heat the mixture for 5 minutes. Collect the insoluble debris on Whatman ashless filter paper No. 42 and rinse it with distilled water until the filtrate is neutral (pH = 7). Dry the ashless filter paper and then burn it at 450°C in the original crucible. After drying the residue, weigh the crucible until the weight becomes constant. Calculate total acid-insoluble ash like total ash.

Extractive value

To determine the ethanol-soluble extractive value, take 5 grams of dried material and macerate it with 100 ml of 95% ethanol in a closed Erlenmeyer flask for 24 hours. Shake the flask for 6 hours, then

leave it at room temperature for 18 hours. Filter the sample quickly to prevent ethanol loss. Then, take 20 ml of the filtrate and evaporate it in a tarred, flat-bottomed, shallow dish at 105°C until the weight stabilizes.

Instead of 95% ethanol, use chloroform and water (2.5:97.5% v/v) to get the water-soluble extractive value.

Inhibitory effect on α-amylase activity

The assay followed Lordan.[22] Briefly, 100 µL of extract and α-amylase from porcine pancreases (0.5 mg/mL) in 0.02 M sodium phosphate buffer (pH 6.9 with 0.006 M sodium chloride) were combined and incubated at 37 °C for 10 minutes. Next, 100 µL of 1% w/v starch solution was added to each microcentrifuge tube and mixed at timed intervals. After 10 minutes at 37 °C, 200 µL DNS reagent was added to determine the enzyme's reducing sugar (96 mM 3,5-dinitrosalicylic acid, 3.5 M sodium potassium tartrate in 2 M NaOH). Then, the tubes were heated at 90-100 °C for 5 min. After that, 50 µL of the reaction mixtures were transferred to 96-well microplates and diluted with 200 µL ultrapure water. Finally, the plate was measured by a microplate reader at 405 nm. The inhibition percentage was calculated using the following equation, and Prism program was used to estimate IC_{50} (n = 3).

$$\text{The percentage of inhibition} = \left[\frac{(\textit{Mean of ODcontrol-Mean of ODsample})}{\textit{Mean of ODcontrol}}\right] \times 100$$

Inhibitory effect on α-glucosidase activity

To investigate the inhibition of α-glucosidase, we modified established methods.[23,24] Briefly, ethanolic extracts were dissolved in DMSO and aqueous extracts in distilled water at 10 mg/mL concentrations. Then, 20 µL of the sample (in phosphate buffer, pH 6.8), 80 µL of 100 mM phosphate buffer, and 50 µL of the α-glucosidase enzyme (0.2 U/mL in phosphate buffer) were mixed in 96-well microplates. The plate was incubated at 37°C for 15 min. Next, 50 µL of a substrate (5 mM p-NPG) was added and incubated at 37°C for 15 minutes. The reaction was stopped by adding 100 µL of a 1 M Na_2CO_3 solution. The p-nitrophenol absorbance was measured at 405 nm. The IC_{50} was measured by Prism program (n=3).

Anti-inflammatory: inhibiting nitric oxide production

We used mouse macrophage leukemia-like cells (RAW 264.7) grown in DMEM with 10% fetal bovine serum and 1% penicillin-streptomycin to test samples for anti-inflammatory effects. We measured nitric oxide production inhibition using a modified Anuthakoengkun and Itharat method.[25]

In brief, RAW 264.7 cells were seeded in 96-well plates at a density of 1×10^5 cells/well and incubated them at 37°C with 5% CO_2 and 95% humidity for 24 hours. After that, the medium was replaced with a fresh medium containing 10 ng/mL of LPS and different concentrations of the sample and then incubated the cells for another 24 hours. To evaluate the nitric oxide production, we measured the nitrite in the supernatant using the Griess reagent. For this, we transferred 100 µL of the supernatant from each well to a 96-well plate and mixed it with 100 µL of Griess reagent.

To measure cell viability after sample treatment, the MTT colorimetric technique, ensuring that cytotoxicity was less than 30%. To do this, we added 10 µL of MTT solution (5 mg/mL in PBS) to each well and incubated it for 2 hours. Then, we added 100 µL of isopropanol with 0.04 M HCl to dissolve the formazan crystals. Finally, we measured the absorbance of the microplate at 570 nm.

Using GraphPad Prism software (CA, USA), we calculated the inhibition percentage and IC_{50} value of the sample.

$$\text{The percentage of inhibition} = \left[\frac{(\textit{Mean of ODcontrol-Mean of ODsample})}{\textit{Mean of ODcontrol}}\right] \times 100$$

Note: Mean of ODcontrol = Mean of $ODcontrol_{(+LPS)}$ - Mean of $ODcontrol_{(-LPS)}$
Mean of ODSample = Mean of $ODsample_{(+LPS)}$ - Mean of $ODsample_{(-LPS)}$

Statistical analysis

All tests were performed in triplicate, and values were reported as means ± SEM. The statistical difference between the samples and the positive control was compared using a one-way ANOVA followed by Dunnett's multiple comparison tests (*P*-value < 0.05).

Results

Quality control

The Weight-Loss remedy (WL) includes three plant ingredients, each with specific scientific and family names, voucher specimen numbers, and parts used. These details are listed in Table 1. The quality control of these ingredients was assessed through requirements of THP, and the results are presented in Tables 2.

Loss on drying

The percentage of loss on drying, which indicates the amount of moisture lost during the drying process, was between 6.3% and 8.2% for all samples. WL had the highest percentage of loss on drying at 8.2%. However, all the samples met the standard values set by THP 2021. The results of the loss on drying test are presented in Table 2.

Total ash content

The total ash content, which measures the inorganic residue left after combustion, was highest for *T. crispa* (8.56 ± 0.17%), exceeding the THP 2021 standard. Nevertheless, the total ash content of WL was within the standard value of 5.36 ± 0.21%, as presented in Table 2.

Acid insoluble ash

The acid-insoluble ash content, which represents the amount of inorganic matter that remains after boiling in acid, ranged from 0.11% to 0.98% (w/w) for all samples. The WL remedy had an acid-insoluble ash content of 0.26 ± 0.04% (w/w), which met the THP 2021 standard. Table 2 shows the results of the acid-insoluble ash test for all samples.

Extractive value

The extractive value, which measures the amount of soluble matter extracted from the plant material using solvents, was highest for *T. chebula,* with ethanol-soluble and water-soluble values of 28.25 ± 0.46% and 37.50 ± 0.83%, respectively. In comparison, WL had ethanol-soluble and water-soluble extractive values of 18.33 ± 0.14% and 33.59 ± 0.66%, respectively. The water-soluble extractive value was generally higher than the ethanol-soluble extractive value for all samples, as shown in Table 2.

Extraction yield

Table 3 shows that the ethanolic extract of the WL remedy had a slightly higher extraction yield percentage than the water extract, with values of 17.36% and 14.46% (w/w), respectively. However, in general, water extracts yielded higher percentages than ethanolic extracts for the plant samples. *T. chebula* produced high extraction yields for both ethanolic and aqueous extracts (14.08% and 14.33%, respectively), while the hydrolyzed extract of the WL remedy had the lowest yield (0.26%).

Table 2 Quality control of the plants of the WL remedy and its ingredients.

Sample	%Loss on drying (Mean ±SEM)	Std.	%Ash contents (Mean ± SEM)				%Extractive value (Mean ± SEM)			
			Total ash	Std.	Acid insoluble ash	Std.	Water-soluble	Std.	Ethanol-soluble	Std.
WL	8.20 ± 0.59	≤ 10	5.36 ± 0.21	≤ 10	0.26 ± 0.04	≤ 2	22.45 ± 1.28	-	12.64 ± 0.61	-
C. rotundus	7.03 ± 1.11	≤ 8	4.09 ± 0.10	≤ 5	0.98 ± 0.03	≤ 2	9.94 ± 0.30	> 11	1.84 ± 0.07	> 4
T. chebula	6.51 ± 0.31	≤ 11	3.27 ± 0.28	≤ 3.5	0.11 ± 0.05	≤ 0.6	37.50 ± 0.83	> 28	28.25 ± 0.46	> 20
T. crispa	6.30 ± 0.41	≤ 11	8.56 ± 0.17	≤ 7	0.17 ± 0.03	≤ 0.5	18.00 ± 0.18	> 10	4.04 ± 0.17	> 5

Note: WL = Weight-Loss remedy, Std. = Standard value

Table 3 Hypoglycemic Activities of Weight-Loss Remedy and its Plant Ingredients.

Sample	Extract	%Yield	IC_{50} value of Anti-diabetic Activities (μg/mL, Mean ± SEM)	
			α-Amylase inhibition	α-Glucosidase inhibition
Weight Loss	WLE	17.36	> 1000*	894.32±0.95*
	WLW	14.46	> 1000*	680.07±1.24*
	WLH	0.26	> 1000*	540.04±12.84*
C. rotundus	CRE	3.25	> 1000*	> 1000*
	CRW	14.68	> 1000*	> 1000*
T. chebula	TCBE	14.08	> 1000*	> 1000*
	TCBW	14.34	> 1000*	407.26±4.40*
T. crispa	TCPE	4.32	> 1000*	392.19±2.29*
	TCPW	13.85	> 1000*	201.28±2.64*
Positive control	Acarbose		11.04±1.08	219.94±2.54

Note: *significantly different ($p < 0.05$) compared with the positive control (acarbose).

α-amylase and α-glucosidase inhibition

Table 3 displays the results of α-amylase and α-glucosidase inhibitions. All plant samples showed weak activity as α-amylase enzyme inhibitors (IC_{50} > 1000 µg/mL). *T. crispa* water extract (TCpW) was the most effective for α-glucosidase inhibition, with IC_{50} values of 201.28 ± 2.64 µg/mL, more effective than the positive control, acarbose (IC_{50} = 219.94 ± 2.54 g/mL). At a concentration of 500 µg/mL, Figure 1 showed that TCpW had a higher percentage of inhibition than acarbose (71.49 ± 0.68% vs. 67.08 ± 0.98%). Three WL remedy extracts moderately inhibited the α-glucosidase enzyme (IC_{50} values ranging from 540.04 - 894.32 µg/mL), with WLH being more potent than WLW and WLE, respectively.

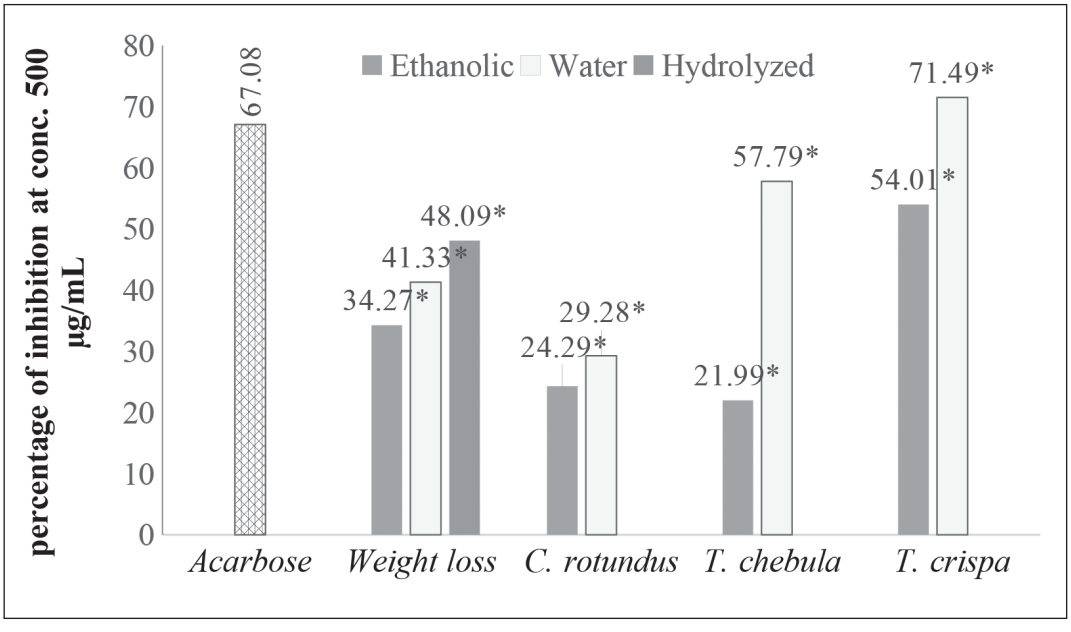

Figure 1 Percentage of Inhibition α-glucosidase at 500 µg/mL *significantly different ($p < 0.05$) compared with the positive control (acarbose).

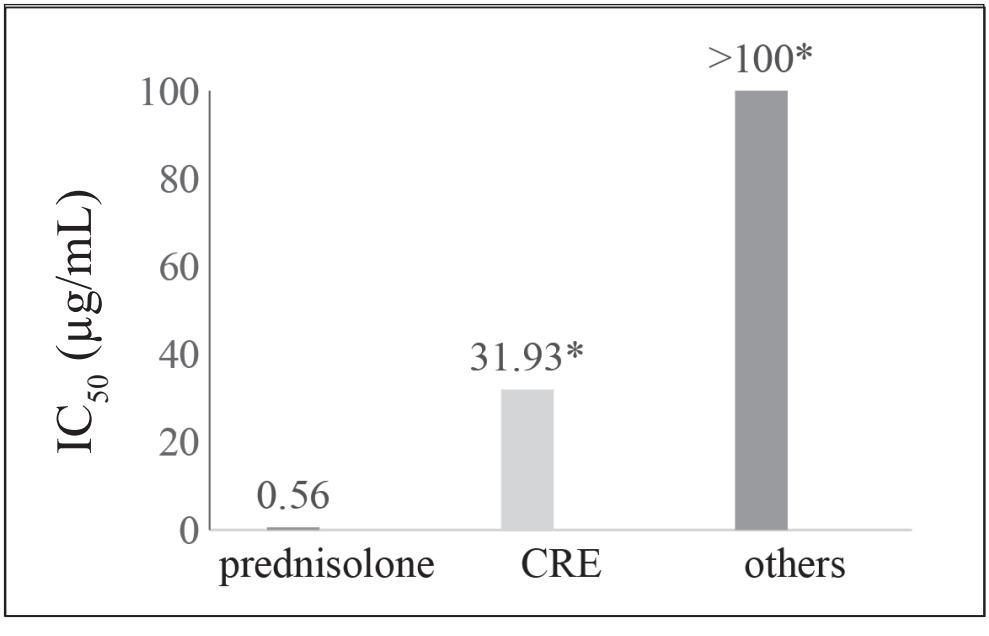

Figure 2 The IC_{50} on various extracts of inhibition on NO production, *significantly different ($p < 0.05$) compared with the positive control (prednisolone).

Anti-inflammatory: inhibiting nitric oxide (NO) production

MTT assays indicated that all extracts were not cytotoxic at concentrations of 100 µg/mL, except for CRE and prednisolone, which were used at 50 µg/mL. The anti-inflammatory activity against LPS-induced NO production by RAW 264.7 cells was evaluated, and the results are presented in Table 4 and Figure 2. None of the three extracts of the WL remedy significantly reduced NO production (IC_{50} > 100 µg/mL). At 100 µg/mL, the hydrolyzed extract of the WL remedy (WLH) showed a higher inhibition percentage than both the WLW) and WLE. However, CRE exhibited high inhibitory effects on NO production with an IC_{50} value of 31.93±1.18 µg/mL, although it was less potent than the positive control. MTT assays indicated that all extracts were not cytotoxic at concentrations of 100 µg/mL, except for CRE and prednisolone, which were used at 50 µg/mL.

Table 4 Extraction Yield and Percentage Inhibition (at 50 µg/mL concentration) of LPS-Induced NO Production from RAW 264.7 Cells by Weight-Loss Remedy and its Plant Ingredients (Cell viability of Plant Extracts) (Mean±SEM) (n=3).

Sample	Extraction	Code	%Inhibition NO Production		IC_{50} ± SEM (µg/mL)
			Conc. 50 µg/mL (%cell viability)	Conc. 100 µg/mL (%cell viability)	
Weight Loss remedy	95%EtOH	WLE	0.28±0.69 (111.70±1.01)	11.27±0.53 (100.50±0.54)	>100*
	Water	WLW	2.58±1.51 (120.38±3.20)	1.54±2.87 (120.40±1.45)	>100*
	Hydrolyzed	WLH	19.51±0.05 (93.42±0.36)	47.93±1.34 (80.00±4.82)	>100*
C. rotundus	95%EtOH	CRE	76.45±0.67 (83.95±3.13)	90.82±3.73 (62.11±3.97)	31.93±1.18*
	Water	CRW	9.45±7.06 (97.26±3.62)	13.94±9.48 (100.01±6.12)	>100*
T. chebula	95%EtOH	TCbE	4.46±3.95 (100.67±4.60)	28.02±5.79 (100.45±0.85)	>100*
	Water	TCbW	16.10±0.96 (103.74±4.77)	17.41±2.10 (105.15±5.78)	>100*
T. crispa	95%EtOH	TCpE	3.97±2.23 (110.15±0.30)	19.49±0.25 (102.83±2.87)	>100*
	Water	TCpW	-1.68±0.32 (78.52±0.97)	-1.49±1.63 (81.01±0.50)	>100*
prednisolone	Positive control	-	91.80±5.13 (76.56±0.82)	- (< 70)	0.56±0.20

Note: * *P*-value < 0.05 vs. positive control (Acarbose)

Discussion

Research on anti-obesity and anti-diabetic drugs is ongoing globally due to the increasing prevalence of obesity and associated comorbidities. This study demonstrates that the Weight-Loss (WL) remedy, containing ethanolic, water, and hydrolyzed extracts, can inhibit α-amylase and α-glucosidase activities in vitro. Almost all of the investigated plants met the quality control requirements outlined in the Thai Herbal Pharmacopoeia guideline. However, certain values exceeded the standard, such as the total ash content of *T. crispa,* which exceeded the requirement. Upon testing the acid-insoluble ash, it met the requirement, indicating that the contaminated material was likely gravel and silica or other inorganic compounds. The ex-

tractive values of *C. rotundus* and *T. crispa* were lower than the standard, possibly because the herbs were harvested in a different season or stored for an extended period. Nevertheless, the WL remedy met all quality-control standards, even though some plant ingredients were out of standard. The combination of these herbs as a WL remedy was found to be effective in meeting the standard guidelines of the Thai Herbal Pharmacopoeia. The WL remedy exhibited strong α-glucosidase inhibitor activity, while all plant samples showed poor α-amylase inhibitor activity. WLW extract was more effective than WLE extract in inhibiting α-glucosidase corresponding to *T. crispa* (TCp) and *T. chebula* (TCb) water extracts were also more effective than ethanol extracts in inhibiting α-glucosidase activity. TCp stems have been used traditionally as a folk medicine in Thailand, Malaysia, and Indonesia for anti-diabetic purposes, consistent with previous findings.[18] In an in vivo study, diabetic rats treated with TCp water extract had reduced fasting blood glucose levels and increased serum insulin levels.[26] Borapetosides A and C, isolated from TCp, were found to improve peripheral glucose uptake and insulin sensitivity. However, several clinical studies indicate that TCp may induce hepatotoxicity.[27]

Water extract of *T. chebula* (TCb) showed good α-glucosidase inhibition. According to previous study, TCb was rich in phenolic and flavonoid content. The phenolic compounds in TCbW can effectively suppress free radicals, making the WL remedy a potentially effective antioxidant. It was also found that ethanolic and water extracts of the WL remedy had a high phenolic content and potent antioxidation. In DPPH and ABTS assays, the water extract of TCb and WL remedy demonstrated exceptionally high antioxidant activity.[28] *T. chebula* had a high yield percentage, resulting in an excellent antioxidant WL remedy. *T. chebula* extracts also lowered blood glucose levels in normal and alloxan-diabetic mice when administered orally.[29] In addition, TCb extract protected the liver of male rats against diazinon-induced hepatotoxicity.[30]

The ethanol extract of *C. rotundus* (CRE) was the only extract that effectively inhibited nitric oxide release for anti-inflammatory efficacy. In contrast, CRE was found to have low α-glucosidase inhibitory efficacy in this investigation. However, a previous study showed that a 70% ethanol extract of CR rhizomes inhibited α-glucosidase better than acarbose and phytochemicals isolated from a methanol extract of CR rhizomes, such as Flavan-3-ol and three stilbene dimers (Scirpusin A&B and Cassigarol E), were found to have a higher inhibitory action against α-glucosidase and α-amylase than acarbose.[31] In alloxan-induced hyperglycemic rats, oral treatment of 200 and 500 mg/kg of 70% ethanol extract of CR rhizomes effectively decreased blood glucose.[32,33]. Piceatannol and dimeric stilbenoids (CRps) isolated from CR reduced diet-induced obesity in mice without side effects, and a pilot clinical trial indicated a reduction in body weight, BMI, and serum lipid profile in CRps-treated patients with no side effects.[34]

The acid hydrolyzed water extract of the WL remedy (WLH) was found to inhibit α-glucosidase more effectively than the water extract (WLW). Similar results were reported by Temrangsee, where acid-hydrolyzed extracts of 14 samples inhibited α-glucosidase more effectively than water extracts.[35] When taken orally, the WL remedy may be exposed to the acidic conditions of the stomach. Therefore, acid hydrolysis in the stomach may improve the WL remedy's inhibition of α-glucosidase. These results suggest that the WL remedy effectively inhibits α-glucosidase and can reduce glucose absorption into the bloodstream, leading to a decrease in expected caloric intake and weight loss.[36] Concerning the relationship between antioxidants and diabetes, a previous study has demonstrated that antioxidants reduce oxidative stress in experimental diabetes and diabetic complications. According to a previous study, the WL remedy has excellent antioxidant activity. Therefore, it may help protect blood vessels, reduce inflammation, and prevent subsequent obesity.[28,37] This finding supports its traditional use in Thai medicine, as described in the Worayokasan scripture. Combining herbs as a remedy has also been found to have various other biological activities, including anti-diabetic effects. Further animal and clinical studies should be conducted to evaluate the WL remedy's anti-diabetic and other biological activities, including potential liver, kidney, or overall body toxicity. I don’t understand why this last sentence is included, it does not follow.

Acknowledgments

The authors would like to thank Thammasat University's Faculty of Medicine and Center of Excellence in Applied Thai Traditional Medicine Research (CEATMR) for their financial, laboratory assistance of this study and Dr. Davies is a Bualuang ASEAN Chair Professor.

Conflicts of interest. The authors declare no conflicts of interest.

References

1. GBD 2015 Obesity Collaborators, Afshin A, Forouzanfar MH, et al. Health Effects of Overweight and Obesity in 195 Countries over 25 Years. *N Engl J Med*. 2017;377(1):13-27.
2. Maggio CA, Pi-Sunyer FX. Obesity and type 2 diabetes. *Endocrinol Metab Clin North Am.* 2003;32(4):805-viii.
3. IDF Diabetes Atlas 2021 | IDF Diabetes Atlas. IDF Diabetes Atlas 2021 | IDF Diabetes Atlas.
4. Alrefai H, Allababidi H, Levy S, Levy J. The Endocrine System in Diabetes Mellitus. *Endocrine.* 2002;18(2):105-120.
5. Ur Rehman N, Halim SA, Al-Azri M, et al. Triterpenic Acids as Non-Competitive α-Glucosidase Inhibitors from Boswellia elongata with Structure-Activity Relationship: In Vitro and In Silico Studies. *Biomolecules.* 2020;10(5):751.
6. Tripathi BK, Srivastava AK. Diabetes mellitus: complications and therapeutics. *Med Sci Monit.* 2006;12(7):RA130-RA147.
7. Sasidharan I, Sundaresan A, Nisha VM, Kirishna MS, Raghu KG, Jayamurthy P. Inhibitory effect of Terminalia chebulaRetz. fruit extracts on digestive enzyme related to diabetes and oxidative stress. *Journal of Enzyme Inhibition and Medicinal Chemistry.* 2012;27(4):578-586.
8. Souza PM, Sales PM, Simeoni LA, Silva EC, Silveira D, Magalhães Pde O. Inhibitory activity of α-amylase and α-glucosidase by plant extracts from the Brazilian cerrado. *Planta Med.* 2012;78(4):393-399.
9. Chiasson JL, Josse RG, Gomis R, Hanefeld M, Karasik A, Laakso M. Acarbose for prevention of type 2 diabetes mellitus: the STOP-NIDDM randomised trial. *The Lancet.* 2002;359(9323):2072-2077.
10. Younus M, Hasan MM ul, Ahmad K, et al. α-Glucosidase Inhibitory, Anti-Oxidant, and Anti-Hyperglycemic Effects of Euphorbia nivulia–Ham. in STZ-Induced Diabetic Rats. *Dose-Response.* 2020;18(3):15593258 2093942.
11. Harrison DE, Strong R, Alavez S, et al. Acarbose improves health and lifespan in aging HET3 mice. *Aging Cell.* 2019;18(2):e12898.
12. Dirir AM, Daou M, Yousef AF, Yousef LF. A review of alpha-glucosidase inhibitors from plants as potential candidates for the treatment of type-2 diabetes. *Phytochemistry Reviews.* 2021;21(4):1049-1079.
13. Ardalani H, Hejazi Amiri F, Hadipanah A, Kongstad KT. Potential antidiabetic phytochemicals in plant roots: a review of in vivo studies. *J Diabetes Metab Disord.* 2021;20(2):1837-1854.
14. Kumar V, Prakash O, Kumar S, Narwal S. α-glucosidase inhibitors from plants: A natural approach to treat diabetes. *Pharmacognosy Reviews.* 2011;5(9):19.
15. Aruoma OI, Landes B, Ramful-Baboolall D, et al. Functional benefits of citrus fruits in the management of diabetes. *Preventive Medicine.* 2012;54:S12-S16.
16. Ayuraved-Wittayarai Foundation. Thai traditional medicine textbook (Paet-Saat- Song-Kror), 1st edition. *Ministry of Public Health.* 1998;453-486.
17. Chaiyawathanananthn, P. Thai Traditional Medical Remedies in Worayokasarn Scripture for Treating Obesity. Asian Medical Journal and Alternative Medicine. 2022;22(3):173–175.
18. Ahmad W, Jantan I, Bukhari SNA. *Tinospora crispa* (L.) Hook. f. & Thomson: A Review of Its Ethnobotanical, Phytochemical, and Pharmacological Aspects. *Frontiers in Pharmacology.* 2016;7.
19. Markovic TP, Campbell LV, Balasubramanian S, et al. Beneficial Effect on Average Lipid Levels From Energy Restriction and Fat Loss in Obese Individuals With or Without Type 2 Diabetes. *Diabetes Care.* 1998;21(5):695-700.

20. Markovic TP, Jenkins AB, Campbell LV, Furler SM, Kraegen EW, Chisholm DJ. The Determinants of Glycemic Responses to Diet Restriction and Weight Loss in Obesity and NIDDM. *Diabetes Care.* 1998;21(5):687-694.
21. Department of Medical Sciences, Ministry of Public Health. Thai Herbal Pharmacopoeia 2021 Supplement 2022. (Available from:https://bdn.go.th/thp/ebook/qQIcZatkpR9gC3q0GT5gMJq0qT5co3uw).
22. Lordan S, Smyth TJ, Soler-Vila A, Stanton C, Ross RP. The α-amylase and α-glucosidase inhibitory effects of Irish seaweed extracts. *Food Chemistry.* 2013;141(3):2170-2176.
23. Kumar JA, Tiwari AK, Ali AZ, et al. New antihyperglycemic, alpha-glucosidase inhibitory, and cytotoxic derivatives of benzimidazoles. *J Enzyme Inhib Med Chem.* 2010;25(1):80-86.
24. Gowri PM, Tiwari AK, Ali AZ, Rao JM. Inhibition of α-glucosidase and amylase by bartogenic acid isolated from *Barringtonia racemosa* Roxb. seeds. *Phytotherapy Research.* 2007;21(8):796-799.
25. Anuthakoengkun A, Itharat A. Inhibitory effect on nitric oxide production and free radical scavenging activity of Thai medicinal plants in osteoarthritic knee treatment. *J Med Assoc Thai.* 2014;97 Suppl 8:S116-S124.
26. Noor H, Hammonds P, Sutton R, Ashcroft SJH. The hypoglycaemic and insulinotropic activity of *Tinospora crispa:* studies with human and rat islets and HIT-T15 B cells. *Diabetologia.* 1989;32(6):354-359.
27. Thomas A, Rajesh EK, Kumar DS. The Significance of Tinospora crispain Treatment of Diabetes Mellitus. *Phytotherapy Research.* 2016;30(3):357-366.
28. Boonyuenyong K, Houngiam K, Chaiyawatthanananthn P. Anti-oxidation Activities of a Thai Remedy in Worayokasan Scripture. *Asian Medical Journal and Alternative Medicine.* 2022;22
29. Sabu MC, Kuttan R. Anti-diabetic activity of medicinal plants and its relationship with their antioxidant property. *Journal of Ethnopharmacology.* 2002;81(2):155-160.
30. Ahmadi-Naji R, Heidarian E, Ghatreh-Samani K. Evaluation of the effects of the hydroalcoholic extract of *Terminalia chebula fruits* on diazinon-induced liver toxicity and oxidative stress in rats. *Avicenna J Phytomed.* 2017;7(5):454-466.
31. Tran HH, Nguyen MC, Le HT, et al. Inhibitors of α-glucosidase and α-amylase from Cyperus rotundus. *Pharm Biol.* 2014;52(1):74-77.
32. Raut NA, Gaikwad NJ. Antidiabetic activity of hydro-ethanolic extract of Cyperus rotundus in alloxan induced diabetes in rats. *Fitoterapia.* 2006;77(7-8):585-588.
33. Elbashir SMI, Devkota HP, Wada M, et al. Free radical scavenging, α-glucosidase inhibitory and lipase inhibitory activities of eighteen Sudanese medicinal plants. *BMC Complementary and Alternative Medicine.* 2018;18(1).
34. Majeed M, Nagabhushanam K, Bhat B, et al. The Anti-Obesity Potential of *Cyperus rotundus* Extract Containing Piceatannol, Scirpusin A and Scirpusin B from Rhizomes: Preclinical and Clinical Evaluations. *Diabetes Metab Syndr Obes*. 2022;15:369-382.
35. Temrangsee P, Itharat A, Sattaponpan C, Pipatrattanaseree W. Inhibitory Effect on Alpha-glucosidase Activity of Benjakul, Soros Benjakul and Their Plant Components. *Thammasat Medical Journal.* 2019;Vol. 19 (No. 4): 645-653.
36. Ćorković I, Gašo-Sokač D, Pichler A, Šimunović J, Kopjar M. Dietary Polyphenols as Natural Inhibitors of α-Amylase and α-Glucosidase. *Life.* 2022;12(11):1692.
37. Bajaj S, Khan A. Antioxidants and diabetes. Indian J Endocrinol Metab. 2012;16(Suppl 2):S267-S271.