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Original Research Article

Effects of Plant Part Substitution in a Thai Traditional Recipe on α-Glucosidase Inhibition

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ABSTRACT

A Thai polyherbal antipyretic remedy, TPDM6315, consists of herbal constituents that possess anti-diabetic properties. It contains 15 plants, of which the roots of 3 crude drugs, *Solanum indicum* (R-*Si*), *Solanum trilobatum* (R-*St*), and *Gymnopetalum integrifolium* (R-*Gi*), are insufficiently supplied in the herbal market, while their stems or aerial parts (S-*Si*, S-*St*, and S-*Gi*, respectively), are available. This study aimed to determine the anti-diabetic properties of the recipe extract and evaluate the possible substitution of those crude drugs. The chemical properties and the *in vitro* α-glucosidase inhibition were investigated in the single herbal ingredient and the recipe extracts. The results indicated that TPDM6315 extracts inhibited α-glucosidase as a mixed-type inhibitor. The high-performance liquid chromatography (HPLC) profiles between the root and stem of those herbs were different but highly similar to the recipe extracts. The substituted recipe (S-Et, containing S-*Si*, S-*St*, and S-*Gi*) exhibited higher potency on α-glucosidase inhibition than the original recipe (R-Et, containing R-*Si*, R-*St*, and R-*Gi*) (P<0.01). This study revealed substitution in TPDM6315 was possible for α-glucosidase inhibitory properties since it only partially affected the overall chemical profile while raising the α-glucosidase inhibitory activity of the recipe extracts.

***Keywords*:** anti-diabetes, alpha-glucosidase inhibition, Thai traditional medicine, herbal substitution

Introduction

A Thai traditional herbal formula, TPDM6315, contains 15 herbs and has been recorded in a textbook of Thai traditional medicines. It has been used to relieve disorders related to inflammation and fever.[1] The herbs in this recipe are *Phyllanthus emblica* L., *Terminalia bellirica* (Gaertn.) Roxb., *Terminalia chebula* Retz., *Gymnopetalum chinensis* (Lour.) Merr., *Dracaena loureiroi* Gagnep., *Santalum spicatum* L., *Tinospora crispa* (L.) Miers ex Hook. f. & Thomson, *Picrorhiza kurrooa* Royle ex Benth., *Cyperus rotundus* L., *Digitaria ciliaris* (Retz.) Koeler, *Angelica dahurica* (Fisch. Ex Hoffm.) Benth. & Hook. f. ex Franch. and Sav., *Zingiber officinale* Roscoe., *Gymnopetalum integrifolium* Kurz, *Solanum indicum* L., and *Solanum trilobatum* L., in which at least 10 of them have been reported for their anti-inflammatory and anti-diabetic properties.[1]

The original TPDM6315 is composed of the roots of *S. indicum*, *S. trilobatum*, and *G. integrifolium*, which are not generally available in the herbal drugstore, while the whole plant, stem, or aerial parts of those crude drugs are easier to find. The lack of those raw materials leads to the difficulty of the commercial production of this herbal formula. It is known that particular plant parts might contain different chemical constituents, resulting in distinct bioactivities. Hence, before changing the plant part used in the recipe, it is necessary to evaluate the pharmacological activities and the chemical components.[2,3]

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α -Glucosidase is an intestinal enzyme that hydrolyzes dietary complex carbohydrates into glucose. The inhibition of this enzyme activity causes less glucose absorption, which reduces postprandial plasma glucose levels and results in a hypoglycemic effect.[4] TPDM6315 contains herbs of potent α -glucosidase inhibition, such as *P. emblica*, *T. chebula*, and *T. bellirica*, which contain bioactive substances e.g. gallic acid, ellagic acid, chebulinic acid, and chebulagic,[5–8] and *C. rotundus* [9] in addition.

To date, there has been no evidence to support the effects of the substitution parts of *G. integrifolium*, *S. indicum*, and *S. trilobatum* in the TPDM6315 recipe on the chemical patterns or biological activities. This study aimed to consider the possibility of using the stem or the whole plant of those three herbs instead of their root parts. The chemical profile and the α-glucosidase inhibitory activity between the extracts from the conventional formula (R-recipe) and the substitution formula (S-recipe) were compared. The kinetics of α-glucosidase inhibition of the recipe extracts were also described.

Materials and Methods

Chemicals

All general solvents and chemicals were AR grade. Solvents for HPLC were HPLC grade. Folin-Ciocalteu reagent, trifluoroacetic acid (TFA), 2,2-diphenyl-1-picrylhydrazyl (DPPH), α-glucosidase (*Saccharomyces cerevisiae*), gallic acid, *p*-nitrophenyl α-D-glucopyranoside (PNPG), and acarbose were purchased from Sigma-Aldrich (St. Louis, MO). Ellagic acid, chebulagic acid, chebulinic acid, and 6-gingerol were obtained from Biopurify Phytochemicals (Chengdu, China). Phellopterin was supported by Associate Prof. Dr. Chavi Yenjai, Khon Kaen University, Thailand.

Plant materials

All crude drugs were purchased from the herbal drugstores in Khon Kaen province, Thailand, in June 2019. Roots of G. *integrifolium* (R-*Gi*), S. *indicum* (R-*Si*), and S. *trilobatum* (R-*St*) were collected from the

open field in Lam Plai Mat district, Burirum province, Thailand. They were identified by Associate Prof. Dr. Somsak Nualkaew and deposited as specimens at the Faculty of Pharmaceutical Sciences, Khon Kaen University, Khon Kaen province, Thailand. The herbarium numbers of those specimens for this set of formula were TP01-TP15 for *Z. officinale, G. integrifolium, S. indicum, S. trilobatum, G. chinense, T. bellirica, T. chebula, P. emblica, C. rotundus, T. crispa, D. ciliaris, S. spicatum, D. loureiroi, A. dahurica, and P. kurrooa,* respectively. They were washed and dried in the hot air oven at 45 ˚C and kept in a cool, dry place in an airtight container until used.

Extraction

TPDM6315 consisting of 15 herbs, was prepared into 2 recipes. Each recipe was composed of the same herbs except for *G. integrifolium* (*Gi*), *S. indicum* (*Si*), and *S. trilobatum* (*St*). The conventional recipe (R-recipe) contained root parts (R-*Gi*, R-*Si*, and R-*St*, respectively), while the substitute recipe (S-recipe) included the stem or aerial part (S-*Gi*, S-*Si*, and S-*St*, respectively).

The herbal mixture of each recipe was ground, sieved through 60 mesh, and macerated in 95% EtOH in a ratio of 1:5 (powdered drug 300 g: EtOH 1500 mL) for three rounds of 3 days. The filtrate was collected, evaporated by a rotary evaporator at 50 ˚C and freeze-dried to obtain the ethanol extracts of R-recipe, **R-Et** (% yield 11.8), and S-recipe, **S-Et** (% yield 11.6).

All the single herbs in this study were macerated with 95% EtOH using the same procedures.

HPLC chromatogram of the extracts

The HPLC was performed using the Agilent InfinityLab LC Series 1220 Infinity II LC System. The 30 mg/mL extract of 20 µL in MeOH was loaded into the RP-18 column (Synergi C-18, 250 x 4.6 mm, 4 µm, Phenomenex, USA). The mobile phase, a gradient of solvent A: 0.05% TFA in acetonitrile; and solvent B: 0.05% TFA in water, was performed as follows: 0-10 min: 90%B; 10-20 min: 82%B; 20-30 min: 80%B; 30-35 min: 73%B; 35-40 min: 70%B; 40-45 min: 60%B; 45-50 min: 50%B; 50-60 min: 0%B; and 60-70 min: 100%B. The flow rate was 0.8 mL/min, and the UV detector was set at a wavelength of 254 nm. The peaks were identified by comparing the retention time and by spiking the standards such as gallic acid, chebulagic acid, ellagic acid, chebulinic acid, 6-gingerol, and phellopterin.

Determination of total phenolic content

The assay was performed by the Folin-Ciocalteu method in a 96-well plate.[10] The reaction mixture consisted of sample 20 µL, 10% Folin-Ciocalteu's reagent 100µL, and 7% Na_2CO_3 80 µL, then incubated for 30 min and protected from light. The absorbance was measured at 760 nm by a microplate reader. The total phenolic content was calculated from the standard graph of gallic acid, y = 0.006x + 0.0554, R^2 = 0.996, and expressed as mg gallic acid equivalent (GAE) per gram of extract.

DPPH radical scavenging assay

The reactions were assayed in a 96-well plate by the method of Timotius *et al*,[11] with slight modification. The extracts were diluted in ethanol to various concentrations. Ascorbic acid was used as a positive control. Sample 100 µL was added to 0.2 mM DPPH 100 µL, incubated in the darkness for 30 min at room temperature, and measured absorbance at 517 nm by a microplate reader (Ensight, Promega, USA). The percentage of DPPH radical scavenging was calculated using the following formula: % Radical scavenging = $[(A_{control}-A_{sample})/A_{control}]$ x 100, where $A_{control}$ was the absorbance of DPPH and A_{sample} was the absorbance of the samples with DPPH. The results were shown as IC_{50} (µg/mL).

α-Glucosidase inhibition assay

The assay was performed in a 96-well plate, according to Simamora *et al*,[12] with a slightly modified protocol. The reaction mixture of 250 µL containing 50 µL of sample in 0.1 M sodium phosphate buffer pH 6.8, 0.5 unit/mL *α*-glucosidase 15 µL, and 0.1 M sodium phosphate buffer (pH 6.8) 20 µL, were mixed and incubated for 10 min at 37ºC. After that, 15 µL of 5 mM PNPG was added and incubated for 30 min at 37ºC. The reaction was stopped by adding 1 M Na_2CO_3 150 µL and measured for the absorbance at 405 nm by using a microplate reader. The following equation was used to calculate the percentage of *α*-glucosidase inhibition: % inhibition = $[(A_{control}- A_{sample})/A_{control}]$ x 100, where $A_{control}$ was the absorbance of the reaction between PNPG and *α*-glucosidase, and A_{sample} was the absorbance of the reaction between PNPG and *α*-glucosidase in the presence of the sample. The IC_{50} (µg/mL) was compared with the positive control acarbose. The enzyme velocity (*V*) was the changing of absorbance per min (ΔA/min). The kinetics of enzyme inhibition was considered by the Lineweaver-Burk plots of 1/*V* (min/ΔA) (Y-axis) vs. 1/[PNPG] (mM^{-1}) (X-axis) using various concentrations of PNPG in the presence of the recipe extracts concentrations of IC_{50}, 1-fold IC_{50}, and 2-fold IC_{50} for R-Et, and IC_{50}, 0.5-fold IC_{50}, and 2-fold IC_{50} for S-Et.

The secondary plot from the Lineweaver-Burk was performed to indicate the type of inhibition. The *K*i was obtained from the graph between *K*m/*V*max vs. the concentration of the recipe extract, and the *K*i′ was achieved by plotting between 1/*V*max and the concentration of the recipe extract.[13]

Statistical analysis

The results were expressed as the mean ± standard deviation (SD) of triplicate. The statistical analysis was performed by a one-way variance analysis (ANOVA) using SPSS (Version 23, IBM), followed by Duncan's test for multiple comparisons. *P* value less than 0.05 ($P<0.05$) was considered significant.

Results and Discussion

TPDM2315 is a traditional Thai medicine consisting with 15 herbs of anti-fever, anti-inflammation, and antidiabetic properties. It can potentially be a commercial product for blood glucose lowering via the *α*-glucosidase inhibition property. Due to the lack of *G. integrifolium*, *S. indicum*, and *S. trilobatum* roots supply in the herbal drugstore, we evaluated the possibility of using the alternative part of these herbs, which could be sufficient for the manufacture on an industrial scale. The consideration was based on the chemical and the *in vitro* bioactivities information.

Crude drugs morphology

The appearance of root parts of R-*Gi*, R-*Si*, and R-*St* were different from the stems of its corresponding herbs that are available in the herbal drugstore, as shown in Figure 1. This led to a simple identification of both herbal parts. The crude drugs from an herbal drugstore of *G. integrifolium* (S-*Gi*) contained stem, twig, leaves, and root; the crude drug of *S. indicum* (S-*Si*) was the stem part, and that of *S. trilobatum* (S-*St*) consisted of fruit, stem, twig, and leaves. R-*Gi* was solely the tap root, whereas R-*Si* and R-*St* consisted of the branch and the tap roots.

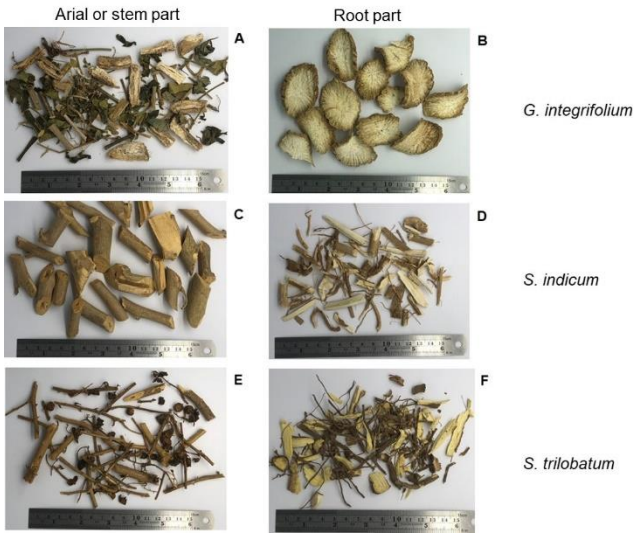

Figure 1: Crude drugs of *G. integrifolium* (whole plant, A), *S. indicum* (stem, C), and *S. trilobatum* (aerial part, E), which have been available in the herbal drugstore, and their root parts (B, D, F, respectively).

Total phenolic contents (TPC)

TPC of all individual herbs varied from 10.00 to 1,312.71 mg GAE/g extract. *P. emblica* fruit contained the highest phenolic content, while *G. integrifolium* root (R-*Gi*) had the lowest. The substituted herbs S-*Gi*, S-*Si*, and S-*St* contained higher phenolic contents than their roots R-*Gi*, R-*Si*, and R-*St*, which significantly distributed a higher TPC of the recipe extracts S-Et than that of R-Et (P<0.05) (Table 1).

HPLC chromatogram of G. integrifolium, S. indicum, and S. trilobatum and the recipe extracts

The effect of substitutions on the α-glucosidase inhibition might result from the different chemical profiles between different parts of *S. indicum*, *S. trilobatum*, and *G. integrifolium*. Therefore, the HPLC chromatograms were compared.

There were different HPLC patterns between the roots and the substituted parts, as shown in Figure 2. Ellagic acid was found in the S-*Gi*, S-*Si*, and S-*St* but not in the roots (R-*Gi*, R-*Si*, and R-*St*). Chebulinic acid appeared in S-*St* but could not be detected in its root (R-*St*). These findings corresponded to the higher total phenolic content of the substituted parts than their roots (Table 1).

The HPLC chromatogram of the recipe extract R-Et was highly similar to that of S-Et, as shown in Figure 3. The biomarker peaks of gallic acid, chebulagic acid, ellagic acid, chebulinic acid, and phellopterin were presented in both R-Et (Figure 3A) and S-Et (Figure 3B). Two of the major HPLC peaks that could be identified were gallic acid and ellagic acid. As indicated by the peak height and the area under the peak, R-Et contained a lower amount of ellagic acid than S-Et. This result supported the presence of ellagic acid in S-*Gi*, S-*Si*, and S-*St* of the S-recipe, and the absence of this compound in R-*Gi*, R-*Si*, and R-*St* of the R-recipe (Figure 2).

All markers used in this study are contained in the herbal ingredients of TPDM6315. Gallic acid, chebulagic acid, ellagic acid, and chebulinic acid were the principal bioactive substances in *T. chebula*, *T. bellirica* and *P. emblica* fruits.[14–18]

Table 1. Total phenolic contents and DPPH radical scavenging activity of herbal ingredients and formula extracts

	Part	**TPC (mg GAE/g extract)**	**Antioxidant (DPPH assay)* IC_{50} (µg/mL)**	**α-glucosidase inhibition** IC_{50} (µg/mL)**
Single herb ethanol extracts				
Phyllanthus emblica L.	Fruit	1312.71 ± 4.07	14.09 ± 0.08	0.66 ± 0.01
Terminalia bellirica (Gaertn.) Roxb.	Fruit	399.47 ± 4.81	15.01 ± 0.22	0.05 ± 0.00
Terminalia chebula Retz.	Fruit	202.18 ± 2.04	17.98 ± 0.39	0.12 ± 0.06
Gymnopetalum chinensis (Lour.) Merr.	Fruit	27.63 ± 0.46	>500	6.39 ± 0.05
Dracaena loureiroi Gagnep.	Stem	253.73 ± 7.86	37.71 ± 0.49	21.42 ± 0.30
Santalum spicatum L.	Stem	165.07 ± 6.11	56.23 ± 1.91	26.88 ± 0.33
Tinospora crispa (L.) Miers ex Hook. f. & Thomson	Stem	55.53 ± 3.18	101.03 ± 3.05	7.17 ± 0.44
Picrorhiza kurrooa Royle ex Benth.	Root	79.31 ± 2.41	70.54 ± 0.06	74.24 ± 6.55
Cyperus rotundus L.	Rhizome	187.73 ± 7.21	40.02 ± 1.11	18.57 ± 0.22
Digitaria ciliaris (Retz.) Koeler	Rhizome	43.27 ± 0.50	317.60 ± 7.91	6.21 ± 0.04
Angelica dahurica (Fisch. Ex Hoffm.) Benth. & Hook. f. ex Franch. & Sav.	Rhizome	177.96 ± 2.34	340.15 ± 13.64	331.99 ± 2.42
Zingiber officinale Roscoe.	Rhizome	187.51 ± 7.19	60.56 ± 1.84	189.30 ± 2.12
Gymnopetalum integrifolium Kurz	Root (R-*Gi*)	10.00 ± 0.71	>500	151.22 ± 0.90
	Whole plant (S-*Gi*)	27.49 ± 1.55	239.86 ± 8.03	126.16 ± 0.97
	P-value $^{\$}$	<0.01	<0.01	<0.01
Solanum indicum L.	Root (R-*Si*)	26.91 ± 1.07	248.82 ± 9.69	28.86 ± 1.59
	Stem (S-*Si*)	58.21 ± 1.02	119.59 ± 5.27	13.68 ± 0.37
	P-value $^{\$}$	<0.01	<0.01	<0.01
Solanum trilobatum L.	Root (R-*St*)	31.21 ± 0.69	118.42 ± 5.08	54.04 ± 0.78
	Aerial part (S-*St*)	36.21 ± 0.35	97.39 ± 4.40	33.85 ± 1.27
	P-value$^{\$}$	<0.01	<0.01	<0.01
Recipe ethanol extracts				
R-Et		106.58 ± 4.07	38.96 ± 1.55	72.31 ± 1.22
S-Et		167.96 ± 0.38	36.00 ± 8.41	35.58 ± 3.36
	P-value$^{\&}$	<0.01	<0.01	<0.01

*IC_{50} of ascorbic acid = 9.01 ± 0.26 µg/mL; **IC_{50} of acarbose = 177.78 ± 4.44 µg/mL

$^{\$}P$<0.01, t-test between root part (R) and the substituted (S) of TPC, antioxidant (DPPH assay) or α-glucosidase inhibition, n = 3

$^{\&}P$<0.01, t-test between R-Et and S-Et of TPC, antioxidant (DPPH assay) or α-glucosidase inhibition, n = 3

Table 2. The kinetic parameters from the Lineweaver–Burk and the secondary plots

Kinetic parameters	R-Et (µg/mL)			S-Et (µg/mL)		
	0	**72.3**	**144.6**	**0**	**17.8**	**71.2**
*K*m (mM)	7.93	5.34	7.23	7.93	7.18	6.59
*V*max (ΔA/min)	0.47	0.28	0.20	0.47	0.25	0.06
*K*i (mM)	105.52 (R^2 = 0.843)			6.70 (R^2 = 0.980)		
*K*i′ (mM)	105.34 (R^2 = 0.999)			4.83 (R^2 = 0.981)		
Inhibition type	Mixed-type			Mixed-type		
	Non-competitive = competitive			Non-competitive > competitive		

*K*i =dissociation constant of the binding of the inhibitor to the free enzyme
*K*i′ = dissociation constant of the binding of the inhibitor to the enzyme-substrate complex

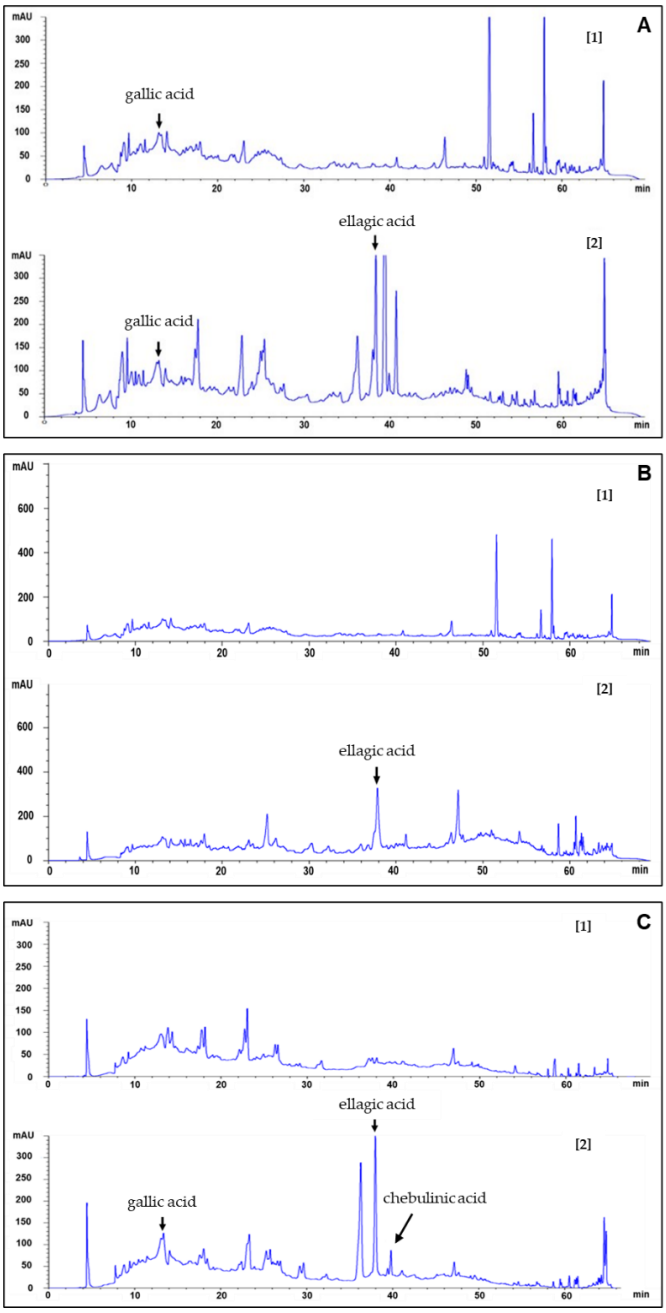

Figure 2: HPLC chromatogram of the ethanol extracts of the root part (R) and the substituted part (S). (A: *G. integrifolium* (root [1] and whole plant [2]); B: *S. indicum* (root [1] and stem [2]); C: *S. trilobatum* (root [1] and aerial part [2]).

Phellopterin is a furanocoumarin from *A. dahurica*,[19,20] and could be detected in a comparable amount from both R-Et and S-Et (Figure 3). 6-Gingerol is an abundant component in *Z. officinale* [21] but could not be detected in both R-Et and S-Et. It might be lost from the drying process of ginger[22] by the supplier. Besides some bioactive compounds contained in TPDM6315 were known, many of them still have not been identified. Those are, i.e., borapetol, and borapetosides from *T. crispa*,[23,24]; picroside I from *P. kurroa*,[25] and others. The purification of the chemical constituents of each herb should be the next step of the TPDM6315 chemical characterization.

DPPH radical scavenging activity

The preliminary screening of antioxidation of all herbal constituents and the formula extracts was performed by DPPH radical scavenging assay. The single herb showed antioxidant effects ranging from mild (*G. chinense*) to potent (*P. emblica, T. bellirica,* and *T. chebula*). The roots of *S. indicum, S. trilobatum,* and *G. integrifolium* exhibited less potency than their substituted parts. Both R-Et and S-Et were comparable potent antioxidants with slightly different IC_{50} values (25.98 – 37.21 µg/mL) (Table 1). These results revealed that the substitution of plant parts in TPDM6315 did not affect the antioxidant activity of the recipe extracts.

α-glucosidase inhibition in vitro

Most of the single herbs possessed strong α-glucosidase inhibition effects. Three of the most potent herbs were *P. emblica, T. bellirica*, and *T. chebula* (IC_{50} 0.66, 0.05, and 0.12 µg/mL, respectively) (Table 1). All substituted parts S-*Gi*, S-*Si*, and S-*St* exhibited more potency than their roots R-*Gi*, R-*Si*, and R-*St*, which corresponded to the formula extracts that S-Et exhibited lower IC_{50} than that of R-Et (P<0.05) as shown in Table 1 (the IC_{50} of R- and S-Et were 72.31 and 35.58 µg/mL, respectively). These results suggested that the more intense α-glucosidase inhibition activity of S-Et might come from the substituted herbs.

The antioxidant and α-glucosidase inhibitory properties of TPDM6315 extracts were paralleled to those of its bioactive compounds. Gallic acid is a potent antioxidant,[26] while chebulagic acid and chebulinic acid are potent α-glucosidase inhibitors.[6,8] Gallic acid 25 µM inhibits α-glucosidase 43.9 ± 0.7%,[5] and the IC_{50} of ellagic acid, chebulagic acid, and chebulinic acid are 53.1, 39.2, and 35.8 µg/mL, respectively.[6] Regarding S-Et that acted as a stronger α-glucosidase inhibitor than R-Et, it also contained higher levels of ellagic acid, chebulagic acid, and chebulinic acid than R-Et. Hence, those compounds might involve this activity of TPDM6315 extract. It was found that the strong antioxidant came from single herbs containing high total phenolic contents, especially *T. chebula*, *T. bellirica*, and *P. emblica*. However, there was no relationship between total phenolic contents and the α-glucosidase inhibitory property.

The kinetics of α-glucosidase inhibition of R- and S-Et were determined. The Michalis-Menten plot of the reaction (Figure 4) indicated the reduction of α-glucosidase velocities in the presence of the recipe extracts, R- and S-Et, in a dose-dependent manner. At a concentration of 144.6 µg/mL of R-Et, the velocity of the enzyme was less than R-Et 72.3 µg/mL. S-Et 71.2 µg/mL showed lower enzyme velocity than S-Et 17.8 µg/mL. Therefore, it was clear that S-Et was a more potent inhibitor than R-Et.

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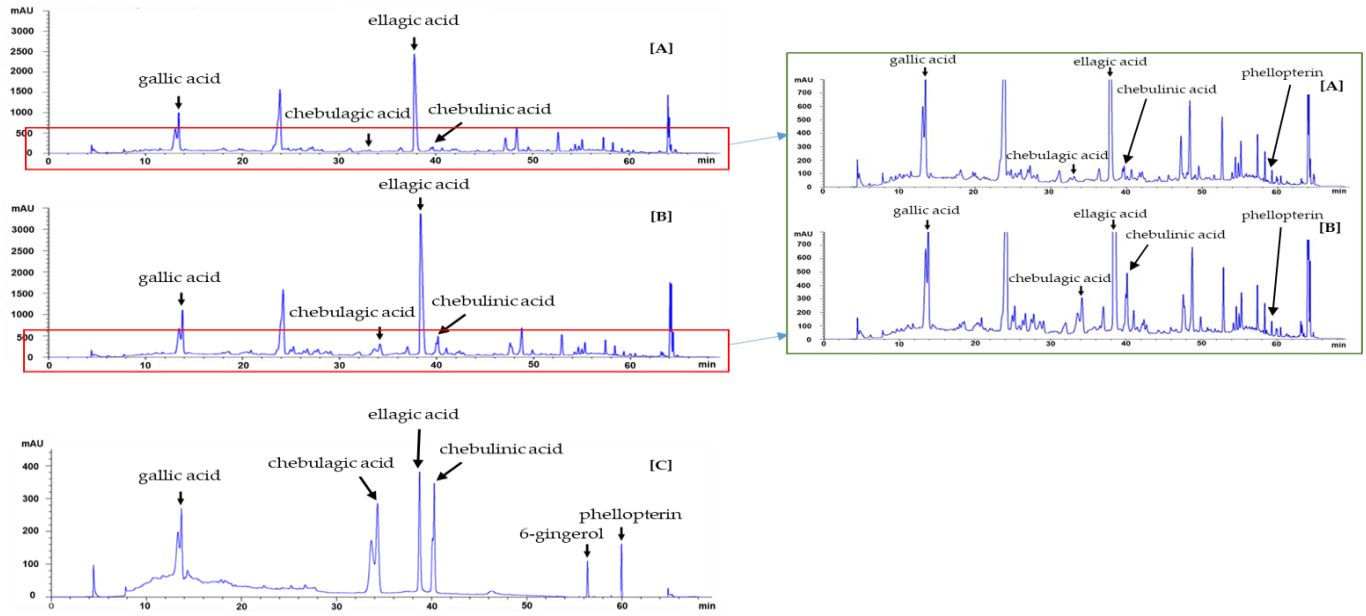

Figure 3: Comparative HPLC profile of the ethanol extracts of TPDM6315. A: the conventional recipe (R-Et); B: the recipe with herbal substitution (S-Et); C: standard mixture consisting of gallic acid; chebulagic acid; ellagic acid; chebulinic acid; 6-gingerol; and phellopterin. The inserted frame showed the peaks of R-Et (A) and S-Et (B) at the 0-700 mAU region.

The Lineweaver–Burk plots of R-Et and S-Et that produced more than one position of the line intersections suggested that they might be mixed-type inhibitors (Figure 5A and 5B). Thus, the secondary plot was performed to determine the types of enzyme inhibition of the extracts. The *Ki*/*Ki*′ of R-Et and S-Et were 1 and 1.3, respectively, revealing that R-Et inhibited enzyme as competitive inhibitor equal to non-competitive inhibitor. At the same time, S-Et inhibited the enzyme function as non-competitive rather than competitive inhibition for 1.3-fold (Table 2).

The kinetic studies of the recipe extracts supported the α-glucosidase activities of the recipe. The mixed-type action of R-Et and S-Et possibly resulted from the activities of many substances belonging to 15 herbs in TPDM6315 that provided various modes of inhibition. For example, ellagic acid, and chebulagic acid, the constituents of these extracts are mixed-type and non-competitive inhibitors, respectively.[8,27] Several plant extracts exhibit mixed-type inhibition, such as propolis,[28] *Salvia mirzayanii*, *Zataria multiflora*, and *Otostegia persica*.[29] Mixed-type inhibition might result in an increased *K*m value when the inhibitor favors binding to the free enzyme (E) or a decreased *K*m value from the tendency of the inhibitors to bind the enzyme-substrate complex (ES).[28] From our studies, both R-Et and S-Et decreased *V*max dose-dependently. The *K*m value of R-Et increased and decreased in higher concentrations, demonstrating the binding functions of R-Et to E and ES. For S-Et, both *K*m value and *V*max decreased as their concentration increased.

Furthermore, *K*i and *K*i′ values represent the dissociation constants of inhibitor to bind free enzymes and ES, respectively. Those values of S-Et were smaller than R-Et, indicating a better binding affinity to the free enzyme, or ES. The *Ki*/*Ki*′ suggested either the inhibitor favors binding to the free enzyme or ES.[13] It was found that, in R-Et, *K*i was equal to *K*i′, which meant it bound to a free enzyme comparable to ES or acted as a competitive inhibitor equally to a non-competitive inhibitor. In S-Et, the *K*i was 1.3 times higher than *K*i′, suggesting that it was non-competitive rather than competitive.

There have been no previous reports for α-glucosidase inhibition activities of *S. indicum*, *S. trilobatum*, or *G. integrifolium*. This study first demonstrated that R-*Si*, S-*Si*, R-*St*, and S-*St* possessed potent α-glucosidase inhibitory effects, while R-*Gi* and S-*Gi* exhibited moderate potency (Table 1). Although the roots of *G. integrifolium*, *S. indicum*, and *S. trilobatum* contained different chemical constituents than their substitutes, S-*Gi*, S-*Si*, and S-*St*, those three herbs accounted for only 15.8% of the recipe. Moreover, the major bioactive peaks, the antioxidant, and the α-glucosidase inhibition activities of TPDM6315 extracts seemed to be dominated by *T. chebula*, *T. bellirica*, and *P. emblica*. Therefore, this led to the similarity of the chemical profile and antioxidant activity between R-Et and S-Et. On the other hand, the potentiated α-glucosidase inhibitory effect of S-Et might be from the substituted S-*Gi*, S-*Si*, and S-*St* that led to the elevation of ellagic acid contents. Our study suggested that substituting plant parts affected the potency of biological activity through the different types and amounts of chemical constituents.

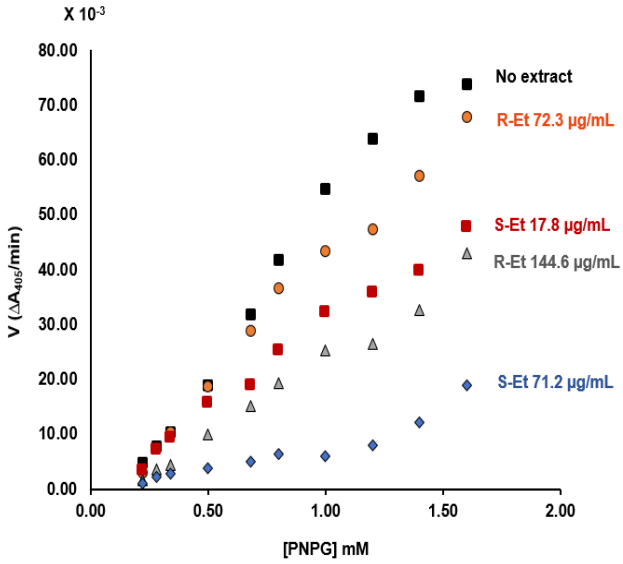

Figure 4: Michaelis-Menten plot between the reaction velocity (*V*) of α-glucosidase to PNPG (0.22-1.6 mM) in the presence of the recipe extracts R-Et and S-Et. (Black square: without recipe extract; orange circle: R-Et 72.3 µg/mL (IC_{50} of R-Et); gray triangle: R-Et 144.6 µg/mL (2-fold IC_{50} of R-Et); red square: S-Et 17.8 µg/mL (0.5-fold IC_{50} of S-Et); blue rectangular: S-Et 71.2 µg/mL (2-fold IC_{50} of S-Et).

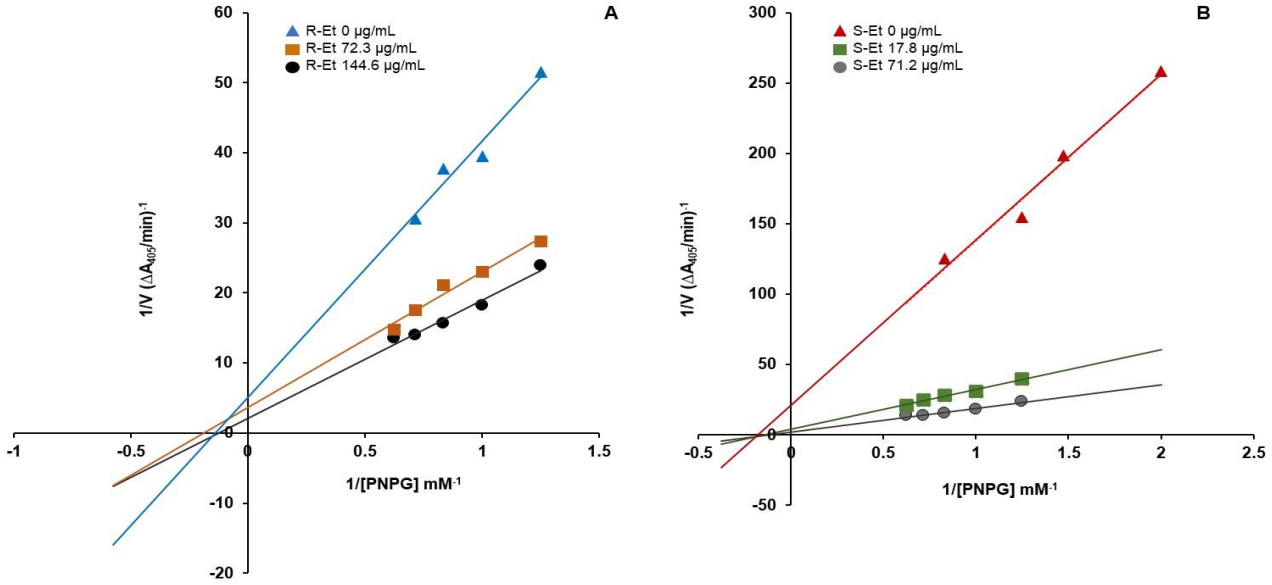

Figure 5: Lineweaver-Burk plots of R-Et and S-Et on the *α*-glucosidase activity. A: the concentrations of the conventional recipe, R-Et were 0 (black dot), 72.3 (orange square), and 144.6 µg/mL (blue triangle); B: the concentrations of the recipe with herbal substitution, S-Et were 0 (gray dot), 17.8 (green square), and 71.2 µg/mL (red triangle).

Conclusion

TPDM6315 acted as a mixed-type *α*-glucosidase inhibitor. The substitution of plant parts of *G. integrifolium*, *S. indicum*, and *S. trilobatum* in TPDM6315 resulted in a mild change in the overall chemical pattern of the recipe extracts. The significantly elevated activities were obtained with unchanged type of enzyme inhibition. It could be concluded that the substitution of those plant parts is possible for the *α*-glucosidase inhibitory effects. The other mechanisms of activities of R-Et and S-Et would be further investigated.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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