Original Article

In Vitro α-Amylase, α-Glucosidase Inhibition, and Anti-Oxidant Activities of Plant Components in Ya-Hom Teppajid

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Abstract

Introduction: Diabetes is characterized by high blood sugar levels or hyperglycemia causing the increase of free radicals which increase the risk of various diseases. Ya-Hom Teppajid which consists of 45 plants and their properties had shown anti-diabetic activities in some herbs and the rest of the herbs have not been studied for these activities.

Methods: The ethanolic extract of plant components in Ya-Hom Teppajid were to investigate α-amylase, α-glucosidase inhibitory, and antioxidant activities (DPPH, and TBARS), including total phenolic (TPC) and total flavonoid (TFC).

Results: Twenty-four plants showed antioxidant activities, while fifteen plants exhibited enzyme inhibition of diabetes mechanisms. The ethanolic extract of *N. lotus* had stronger enzyme inhibitory activities and antioxidant activities than other plant components, although less than the positive standard.

Conclusions: Our result can support the efficacy of Ya-Hom-Teppajid for a diabetic. Interestingly, the whole flower of *N. lotus* was suggested as a better part due to its stronger antioxidant activity by free radicals scavenging property. These herbs can be consumed as food ingredients or food supplements and their consumption should be beneficial to diabetic patients.

Keywords: Ya-Hom Teppajid, α-amylase, α-glucosidase, Diabetes mellitus, Antioxidant

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Introduction

Diabetes is caused by abnormalities of insulin function, also known as insulin resistance directly affects the rise in blood sugar levels. When there is a large amount of sugar accumulated and for a long time, the internal organs will deteriorate.[1] There are serious complications that cause premature death, according to the International Diabetes Federation (IDF) there were 425 million people worldwide with diabetes in 2017, and it was estimated that there will be as many as 629 million people by 2045. It was found that 90-95% of all diabetes patients were typed 2 diabetic patients.[2] This type of diabetes, in addition to abnormal pancreatic disorders causing insufficient insulin secretion or insulin resistance also related to environmental factors such as obesity, overweight, lack of exercise, stress, etc.[3] According to western medicine, diabetes is an incurable chronic disease, however, controlling the amount of blood sugar level will reduce the risk of violent incidences from this disease.

The important function of the α-amylase enzyme from saliva and pancreas is in the digestion of carbohydrates.[4] The role of catalytic hydrolysis of α-1,4-glucan linkages is breaking down carbohydrates into oligosaccharides and monosaccharides. The α-glucosidase is a prominent enzyme found in microvillar membranes of the small intestine which catalyzes the digestion of disaccharides to give glucose which can be absorbed into the blood.[5] The amount of elevated blood sugar is directly related to the enzymes,[6] leading to hyperglycemia which directly affects the increase of free radicals in the body. When the metabolic process of glucose is higher than normal, the enzyme NADH reductase is stimulated to produce reactive oxygen species (ROS) free radicals which oxidize biomolecules in the body.[7] For this reason, the physicians treat diabetic patients with oral medications that have a mechanism of action as α-amylase and α-glucosidase inhibitors but lifelong medication can inevitably create side effects. Therefore, choosing proper herbs that have the same activities to replace or reduce the use of synthetic drugs can increase patients' quality of life.

Ya-Hom Theppajid remedy is traditional medicine in the National Essential Drugs list,[8] indicating its safety and effectiveness but there are cautions when using it in conjunction with anticoagulant and antiplatelet drugs. Elderly people take this medicine continuously because it helps to improve their health. The indications for this Thai traditional medicine are dizziness, palpitations and as a single remedy for maintaining heart function.[9] The remedy consists of forty-five herbs, previous studies had shown anti-diabetic activity in some herbs, the ethanolic extract from *Nymphaea* spp. showed strong antioxidant activity and anti-diabetic activity via in vitro inhibition of α-amylase and α-glucosidase activities[10] and in an animal model.[11] An aqueous extract of *Cuminum cyminum* gave a significant reduction in blood glucose level in alloxan-diabetic rats.[12] Chromatographic fraction of *Myristica fragrans* aril showed good α-glucosidase inhibitory activities.[13] The active compound from *Aquilaria crassna* leaves, mangiferin also showed anti-diabetic activity.[14] *Carum carvi, Cinnamomum verum,* and *Citrus sinensis* also exerted significant hypoglycemic effects in diabetes-induced rats.[15,16,17] The rest of herbs have not been studied for these activities. The aims of this study were to test the plant components of Ya-hom Teppajid for the inhibitory activities on α-amylase and α-glucosidase enzymes and their antioxidant property through DPPH and TBARS tests.

Methods

Chemicals and Reagents

The chemicals and reagents were of analytical grades. A-amylase (Sigma-Aldrich, USA), Soluble starch and 3,5-dinitrosalicylic acid (DNS) (Sigma-Aldrich, USA), α-glucosidase (Saccharomyces cerevisiae), and Acarbose (Sigma-Aldrich, USA), p-nitrophenyl α-D-glucopyrano-side (p-NPG) (Sigma-Aldrich, Switzerland), 2,2-diphenyl-1-picrylhydrazyl (DPPH) (Fluka, USA), Butylated hydroxytoluene (BHT) (Fluka, Germany), Thiobarbituric acid (TBA) (Sigma-Aldrich, USA), Folin-Ciocalteu's reagent (Fluka, USA), Gallic acid (Sigma-Aldrich, USA), Sodium nitrite (NaNO2) and Aluminum chloride (AlCl3) (Sigma, USA).

Preparation of Crude Extract

Forty-five plant materials were bought from different sources. The identification of plants was done by the herbarium of the Southern Center of Thai Medicinal Plants at the Faculty of Pharma-

ceutical Sciences, Prince of Songkla University, Songkhla, Thailand (Table 1). Each plant material was macerated in 95% ethanol for 3 days, then filtered and repeated the process twice. The combined filtrates were dried using a rotary evaporator. The percentage yield of the extract was recorded. All dried extracts were stored at -20°C until use.

Table 1 Component plants in Teppajid remedy

Botanical name	Family	Voucher number	Thai name	Part used
Alyxia reinwardtii Blume	Apocynaceae	SKP013011801	Cha-lude	bark
Amomum krervanh Pierre ex Gagnep.	Zingiberaceae	SKP206012001	Krawan	fruit
Anethum graveolens L.	Umbelliferae	SKP199010701	Tian ta tak-ka-tan	fruit
Angelica dahurica Benth.	Umbelliferae	SKP199010401	Kote sor	root
Angelica sinensis (Oliv.) Diels.	Umbelliferae	SKP199011901	Kote chiang	root
Aquilaria crassna Pierre ex Lecomte.	Thymeleaceae	SKP193010301	Krit sana	wood
Artemisia annua L.	Compositae	SKP051010101	Kote chula lampa	aerial part
Atractylodes lancea (Thunb.) DC.	Compositae	SKP051011201	Kote kamao	rhizome
Carum carvi L.	Apiaceae	SKP199030301	Tien ta kob	seed
Cinnamomum verum J.Presl.	Lauraceae	SKP096032201	Op choei thet	bark
Citrus aurantifolia (Christm.) Swingle.	Rutaceae	SKP166030101	Ma-now	Peel
Citrus hystrix DC.	Rutaceae	SKP166030801	Ma-krut	Peel
Citrus ichangensis Swingle.	Rutaceae	SKP166030901	Som ma ngua	Peel
Citrus maxima Merr.	Rutaceae	SKP166031301	Som-O	Peel
Citrus medica Linn. Var. Linetta.	Rutaceae	SKP166031301	Som-sa	Peel
Citrus reticulata Blanco.	Rutaceae	SKP166031801	Som kiew waan	Peel
Citrus sinensis Osbeck.	Rutaceae	SKP166031901	Som jeen	Peel
Cuminum cyminum L.	Umbelliferae	SKP199030301	Tian khao	fruit
Dracaena lourieri Gagnep.	Dracaenaceae	SKP005041201	Chan daeng	stem
Euphorbia antiquorum Linn	Euphorbiaceae	SKP071050101	Kra lumphak	stem
Foeniculum vulgare Miller subsp. var. vulgare.	Apiaceae	SKP199062201	Tian khao plueak	fruit
Jasminum Sambac (L.) Aiton.	Oleaceae	SKP129101901	Mali	flower
Kaempferia galanga L.	Zingiberaceae	SKP206110701	Proh hom	rhizome
Lagerstroemia calyculata Kurz.	Sapotaceae	SKP171130501	Khon dok	wood
Lepidium sativum Linn.	Cruciferae	SKP160141901	Tian dang	seed
Ligusticum sinense Oliv. cv. Chuanxiong Hort.	Umbelliferae	SKP199121901	Kote hua bua	rhizome
Mammea siamensis Kosterm.	Guttiferae	SKP083131901	Sarapi	flower
Mesua ferrea L.	Guttiferae	SKP083130601	Bunnak	flower
Mimusops elengi L.	Sapotaceae	SKP171130501	Pi-gul	flower
Myristica fragrans Houtt.	Myristicaceae	SKP121130601	Nutmeg	seed
Myristica fragrans Houtt.	Myristicaceae	SKP121130601	Mace	aril
Nardostachys grandiflora DC.	Valerianaceae	SKP201140701	Kote cha damang si	Root
Nelumbo nucifera Gaertn.	Nelumbonaceae	SKP125141401	Bua luang	pollen
Nigella sativa L.	Ranunculaceae	SKP160141901	Tian dam	Seed
Nymphaea lotus L.var.pubescens Hook.f. & Th.	Nymphaeaceae	SKP127141601	Bua khom	flower
Nymphaea stellata Wild.	Nymphaeaceae	SKP127141401	Bua phuean	flower
Picrorrhiza kurroa Benth.	Scrophulariaceae	SKP177161101	Kote kan prao	Root
Pimpinella anisum L.	Umbelliferae	SKP199160101	Tian sat-ta-but	fruit
Plantago ovata Forssk.	Plantaginaceae	SKP147161501	Tian kled hoi	seed
Saussurea lappa Clarke.	Asteraceae	SKP219011201	Kote kra-dook	Root
Syzygium aromaticum (L.) Merr.& L.M.Perry.	Myrtaceae	SKP123190101	Kan plu	flower
Tarenna hoaensis Pitard.	Rubiaceae	SKP165200801	Chan kha	stem
Terminalia chebula Retz. var chebula	Combretaceae	SKP045920301	Kote pung pla	gall
Trachyspermum ammi (L.) Sprague.	Apiaceae	SKP199200101	Tian yao wa pa ni	seed
Vetiveria zizanioides (L.) Nash ex Small.	Poaceae	SKP08122 2601	Fag hom	root

Biological Activity Testing

Inhibitory Effect on α-Amylase Activity

The α-amylase inhibitory activity was determined according to a standard method with slight modification.[18] Briefly, five concentrations (50, 100, 200, 500, 1000, and 2000 μg/mL) of the test sample were prepared. A 40 μL of the test sample in DMSO/water, 40 μL phosphate buffer (100 mM, pH 6.9 containing 6.7 mM NaCl), and 25μL of α-amylase (6.5 Unit/mL) were mixed, and pre-incubated at 37°C for 10 minutes. Then 100 μL of 1% soluble starch was added as a substrate and incubated at 37°C for 10 minutes. A 200 μL of DNS color reagent was added and boiled at 90°C for 5 minutes. After cooling on ice for 10 minutes, the content of each tube was diluted with water, then measured the absorbance at 540 nm. The result was expressed as percentage inhibition, which was calculated using the formula: $[(Ab_{scontrol} - Abs_{sample})/Abs_{control}]$ x 100, Abs = Absorbance or Optical density (OD). The IC_{50} values were calculated with the Prism program.

Inhibitory Effect on α-Glucosidase Activity

The α-glucosidase inhibitory activity was modified from an established method.[19] In brief, five concentrations (50, 100, 200, 500, 1000, and 2000 μg/mL) of the test sample and acarbose were prepared. A 20 μL of the test sample in DMSO/water, 80 μL of 100 mM phosphate buffer (pH 6.8), and 50 μL of the substrate (5mM p-nitrophenyl α-D-glucopyranoside in phosphate buffer) were added to 96-well plate and pre-incubated at 37°C for 5 minutes. Then a 50 μL of α-glucosidase (0.25 Unit/mL in phosphate buffer) was added and incubated at 37°C for 15 minutes. The reaction was stopped by adding 100 μL of 1M sodium carbonate solution (Na_2CO_3). The release of p-nitrophenol was measured at 405 nm by a microplate reader (Biotek, USA). All tests were run in triplicate. The percentage of enzyme inhibition was calculated by the following formula: $[(Abs_{control} - Abs_{sample})/Abs_{control}]$ x 100, Abs is Absorbance or Optical density (OD). The IC_{50} values were calculated with the Prism program.

DPPH Radical Scavenging Assay

The antioxidant activity was determined by the scavenging effect on DPPH radical.[20] Tested sample was dissolved in absolute ethanol or distilled water (1, 10, 50, 100 μg/mL). A 100 μL of the extract and 100 μL of 6 x 10^{-5} M DPPH (in absolute ethanol) were transferred into a 96-well plate and incubated for 30 minutes in the dark at room temperature, the absorbance was measured at 520 nm. BHT was used as a positive control. The percentage of DPPH activity scavenging was calculated by the following formula: $[(Abs_{control} - Abs_{sample})/Abs_{control}]$ x 100, Abs is Absorbance or Optical density (OD). The EC_{50} values were calculated with the Prism program.

Lipid Peroxidation (TBARS) Assay

Lipid peroxidation by inspection of thiobarbituric acid reactive substances (TBARS) was determined according to an established method with slight modification.[21] The amount of Malondialdehyde (MDA) released from the reaction was obtained by homogenizing the pig brain with 200 μL of 10 mM (Tris-HCl), 100 μL of sample extract, and 40 μL of 100 μM Fe^{2+}/EDTA. The mixture was incubated at 37ºC for 1 hour. The color reaction was developed by adding 200 μL of 8.1% sodium dodecyl sulfate, 500 μL of 2.8% trichloroacetic acid in distilled water, and 0.6% thiobarbituric acid (TBA) in 0.1M NaOH. Then incubated at 100ºC for 1 hour. After cooling down for 10 minutes, the absorbance was measured at 532 nm. The tests were repeated in triplicate. The percentage of enzyme inhibition was calculated by the following formula: $[(Abs_{control} - Abs_{sample})/Abs_{control}]$ x 100, Abs is Absorbance or Optical density (OD). The IC_{50} values were calculated with the Prism program.

Determination of Total Phenolic Content

Total phenolic contents (TPC) were determined by the modified Folin-Ciocalteu method.[22] A 20 μL of the tested sample was mixed with 100 μL of Folin-Ciocalteu's reagent, and 80 μL of sodium carbonate in a 96-well plate. The plate was well mixed and allowed to stand for 30 minutes to develop color. The absorbance value of the sample was measured at 765 nm. Total phenolic content was calculated from the calibration curve of gallic acid as mg gallic acid equivalent (GAE) per g. All measurements were performed in triplicate.

Determination of Total Flavonoid Content

Total flavonoid content (TFC) was determined according to the established method with slight modification.[23] Briefly, 500 µL of the tested sample was added to 75 µL of 5% $NaNO_2$ and 150 µL of 10% $AlCl_3$. After 5 minutes of standing at room temperature, the reaction mixture was treated with 0.5 mL of 1M NaOH, then measured the absorbance values at 510 nm. Total flavonoid content was calculated from the calibration curve of quercetin as mg quercetin equivalent (QE) per g. All measurements were performed in triplicate.

Statistical Analysis

All experiments were presented as mean ± standard error of the mean (SEM) in triplicate. One-way ANOVA followed by Dunnett's test was used to compare sample groups and positive control (P-value < .05). The Pearson correlation coefficient (r) between TPC and TFC in the extracts and antioxidant properties. Statistical analysis was calculated with the Prism Software program.

Results

Plant Material Extractions

The percentage yield of 95% ethanolic extract (% w/w yield) of 45 plants showed was shown in Table 3 values between 1.28% to 32.68%. *T. chebula* gave the highest yield and *A. krervanh* gave the lowest yield.

Inhibitory Effect on α-Amylase Activity

The result of α-amylase inhibiting activity of the 95% ethanolic extract was shown in Table 2, three plants showed strong inhibitory activity i.e. *N. lotus, A. crassna,* and *C. cyminum* with IC_{50} values of 988.53 ± 8.36, 1,022.40 ± 9.65, and 1,029.79 ± 9.87 µg/mL, respectively, while that of acarbose was 7.59 ± 0.83 µg/mL.

Inhibitory Effect on α-Glucosidase Activity

The results of α-glucosidase inhibiting activity by the 95% ethanolic extract showed that *M. fragrans* (Nutmeg), *N. Lotus,* and *C. cyminum* were most active with IC_{50} values of 528.67 ± 11.68, 542.00 ± 13.58 and 611.67 ± 8.45 µg/mL, respectively, while that of acarbose was 198.05 ± 16.79 µg/mL. The other extract which showed no inhibitory effect or IC_{50} more than 2,000 µg/mL were not shown (Table 2). The plants that inhibited both α-amylase and α-glucosidase activities were *A. crassna, C. cyminum, M. ferrea, N. lotus, N. stellata,* and *T. chebula.*

DPPH Radical Scavenging Assay

The results of the DPPH scavenging activity of the 95% ethanolic extract of 45 plants were shown in Table 3. *T. chebula, N. lotus, N. stellata,* and *S. aromaticum* showed stronger radical scavenging activities than BHT (positive control) with EC_{50} values of 2.35 ± 0.51, 3.85 ± 0.23, 5.84 ± 0.34, and 7.46 ± 0.85 µg/mL, respectively. From statistical analysis, five plants that showed similar results with BHT were *M. fragrans* (Mace), *M. fragrans* (Nutmeg), *M. ferrea, T. hoaensis* and *E. antiquorum* (EC_{50} values in the range 12.78 - 20.60 µg/mL, P > .05). Those extracts with moderate activities were *D. lourieri, N. grandiflora, M. siamensis, M. elengi, L. sativum, C. verum, P. kurroa, C. sinensis, A. krervanh, L. sinense, A. dahurica, T. ammi, P. ovata, A. sinensis,* and *C. cyminum* (EC_{50} values in the range 22.11 - 96.32 µg/mL, P < .05). There were 21 plants without DPPH scavenging activity (EC_{50} > 100 µg/mL).

Lipid Peroxidation (TBARS) Assay

The results of anti-lipid peroxidation activities by TBARS method of 95% ethanolic extract of 45 plants were shown in Table 3, *N. lotus, T. chebula,* and *M. fragrans* (Mace) showed good antioxidant activity with IC_{50} values of 279.83 ± 5.06, 326.74 ± 8.39, and 470.66 ± 9.16 µg/mL respectively. Those extracts with moderate activities were *F. vulgare, T. ammi, A. sinensis, D. lourieri, N. stellata, M. ferrea, M. fragrans* (Nutmeg), *C. cyminum, M. siamensis,* and *L. sativum* (IC_{50} values in the range 501.89 - 1597.52 µg/mL). There were 32 plants that showed weak activities by the TBARS method (IC_{50} > 2,000 µg/mL).

Determination of Total Phenolic and Total Flavonoid Content

The results of TPC were from 6.80 to 566.17 mg GAE/g dry extract, calculated from the standard Gallic acid graph (y = 0.006x + 0.0233, R^2 = 0.9996). TFC were found between 7.58 to 371.30 mg QE/g dry extract as calculated from

the standard graph of quercetin (y = 0.0003x - 0.0035, R^2 = 0.9994) (Table 3). Those extracts that showed the higher TPC and TFC values were *N. lotus* (283.93 ± 1.12 and 360.83 ± 5.10), *S. aromaticum* (241.78 ± 0.01 and 97.04 ± 4.24), *N. stellata* (136.55 ± 1.15 and 230.44 ± 12.16), *T. chebula* (127.44 ± 4.57 and 230.44 ± 12.16), *D. lourieri* (125.85 ± 1.65 and 245.00 ± 2.94), *M. ferrea* (114.18 ± 0.80 and 125.56 ± 1.00), *T. hoaensis* (113.07 ± 2.30 and 105.52 ± 2.81), and *M. siamensis* (65.81 ± 2.44 and 185.65 ± 2.25).

Discussion

From all of the results, plant components in Ya-Hom Teppajid demonstrate to support a relative with antidiabetic and antioxidant activities. Most plant extracts with antioxidant activities also demonstrated antidiabetic activity and α-amylase and α-glucosidase inhibition activities. In addition, those herbs that have α-glucosidase are also effective against α-amylase. Moreover, the correlation analysis of the inhibition of α-glucosidase activity and antioxidant activity revealed a positive correlation with anti-α-glucosidase activity (DPPH r = 0.6289, TBARS r = 0.6131) but not with anti-α-amylase activity. This result was in accord with the previous study.[10]

Our results were unanimous with previous studies on *Nymphaea* spp.[10] which explained the positive result in an animal model.[11] The aqueous extract of *C. cyminum* also lower blood sugar significantly in alloxan diabetic rats,[12] this could be explained by its anti-α-glucosidase and anti-α-amylase activities in this study (Table 2). This result corresponds with a prior study on antioxidant activity of *N. lotus* petal ethanolic extract by DPPH, FRAP and cell membrane stabilization by 2,2 '-Azobis (2-Amidinopropane) Dihydrochloride (AAPH) induced hemolysis.[24] The IC_{50} of petal 95% ethanolic extract was 8.96 ± 0.78 µg/mL which was higher than our extract from the whole flower (IC_{50} 3.85 ± 0.23 µg/mL). This demonstrated that the whole flower of *N. lotus* should be used.

Table 2 IC_{50} of α-amylase and α-glucosidase inhibition of 95% ethanolic extracts

Plant species	α-amylase inhibition (IC_{50} µg/mL ± SEM)	α-glucosidase inhibition (IC_{50} µg/mL ± SEM)
Aquilaria crassna	1,022.40 ± 9.65	1,348.01 ± 8.10
Atractylodes lancea	1,917.42 ± 20.14	> 2,000
Cinnamomum verum	> 2,000	1,940.74 ± 22.67
Cuminum cyminum	1,029.79 ± 9.87	611.69 ± 14.64
Euphorbia antiquorum	> 2,000	918.05 ± 15.75
Foeniculum vulgare	> 2,000	1,680.43 ± 8.29
Mesua ferrea	1,568.41 ± 11.00	1,227.51 ± 9.24
Mimusops elengi	1,429.70 ± 9.86	> 2,000
Myristica fragrans (Nutmeg)	> 2,000	516.74 ± 1.71
Nelumbo nucifera	> 2,000	1,825.13 ± 6.68
Nymphaea lotus	988.53 ± 8.36	528.92 ± 2.93
Nymphaea stellata	1,587.93 ± 30.46	667.75 ± 10.59
Pimpinella anisum	> 2,000	1,489.01 ± 7.38
Terminalia chebula	1,914.96 ± 15.03	1,148.48 ± 9.38
Vetiveria zizanioides	1,806.31 ± 20.71	> 2,000
Acarbose	7.59 ± 0.83	198.05 ± 16.79

Table 3 Extraction yields, total phenolic and flavonoid contents, DPPH radical scavenging activity, TBARS of 95% ethanolic extracts

Plant species	**Yield (%W/W)**	**TPC (mg gallic acid eq./g)**	**TFC (mg quercetin eq./g)**	**DPPH (EC_{50} μg/mL ± SEM)**	**TBARS (IC_{50} μg/mL ± SEM)**
Alyxia reinwardtii	9.40	36.01 ± 2.17	7.58 ± 0.41	> 100	> 2,000
Amomum krervanh	1.28	31.00 ± 1.80	18.02 ± 1.83	71.93 ± 3.40	> 2,000
Anethum graveolens	2.81	13.41 ± 0.10	19.63 ± 0.30	> 100	> 2,000
Angelica dahurica	3.19	42.58 ± 2.24	15.95 ± 0.87	75.76 ± 5.62	> 2,000
Angelica sinensis	5.18	43.82 ± 2.18	89.07 ± 11.90	89.78 ± 7.67	598.11 ± 4.95
Aquilaria crassna	4.32	11.00 ± 1.26	67.15 ± 6.74	> 100	> 2,000
Artemisia annua	5.78	20.88 ± 0.90	12.19 ± 0.25	> 100	> 2,000
Atractylodes lancea	7.93	37.34 ± 0.06	53.29 ± 7.19	> 100	> 2,000
Carum carvi	3.71	6.80 ± 0.37	11.93 ± 0.60	> 100	> 2,000
Cinnamomum verum	9.75	14.44 ± 0.46	23.53 ± 1.85	65.16 ± 1.12	> 2,000
Citrus aurantifolia	6.90	37.80 ± 1.64	86.85 ± 2.18	> 100	> 2,000
Citrus hystrix	10.71	40.20 ± 1.21	76.39 ± 7.28	> 100	> 2,000
Citrus ichangensis	7.24	34.31 ± 2.00	86.67 ± 2.78	> 100	> 2,000
Citrus maxima	12.62	53.88 ± 1.80	13.90 ± 2.89	> 100	> 2,000
Citrus medica	8.58	45.79 ± 0.64	77.87 ± 2.36	> 100	> 2,000
Citrus reticulata	5.48	39.84 ± 0.82	92.96 ± 8.40	> 100	> 2,000
Citrus sinensis	16.93	56.06 ± 3.18	88.69 ± 4.66	67.75 ± 6.87	> 2,000
Cuminum cyminum	3.75	23.60 ± 0.44	72.11 ± 11.27	96.32 ± 4.48	1,271.67 ± 8.98
Dracaena lourieri	8.95	125.85 ± 1.65	245.00 ± 2.94	22.11 ± 2.82	602.21 ± 2.63
Euphorbia antiquorum	23.79	52.61 ± 0.65	70.41 ± 6.51	20.60 ± 0.75	> 2,000
Foeniculum vulgare	5.62	11.80 ± 0.10	42.64 ± 0.62	> 100	501.89 ± 8.63
Jasminum Sambac	27.13	28.68 ± 0.43	93.52 ± 4.32	> 100	> 2,000
Kaempferia galanga	4.08	10.11 ± 2.15	57.41 ± 1.12	> 100	> 2,000
Lagerstroemia calyculata	8.61	14.50 ± 1.77	65.94 ± 1.09	> 100	> 2,000
Lepidium sativum	2.15	41.01 ± 1.21	25.53 ± 0.38	63.16 ± 9.12	1,597.52 ± 7.81

Table 3 Extraction yields, total phenolic and flavonoid contents, DPPH radical scavenging activity, TBARS of 95% ethanolic extracts (Cont.)

Plant species	**Yield (%W/W)**	**TPC (mg gallic acid eq./g)**	**TFC (mg quercetin eq./g)**	**DPPH (EC_{50} μg/mL ± SEM)**	**TBARS (IC_{50} μg/mL ± SEM)**
Ligusticum sinense	6.87	33.47 ± 1.13	12.56 ± 2.49	74.01 ± 4.89	> 2,000
Mammea siamensis	20.52	65.81 ± 2.44	185.65 ± 2.25	46.11 ± 4.48	1,548.93 ± 13.43
Mesua ferrea	20.43	114.18 ± 0.80	125.56 ± 1.00	15.09 ± 1.92	676.89 ± 2.79
Mimusops elengi	15.24	27.87 ± 1.41	15.25 ± 6.19	54.67 ± 1.40	> 2,000
Myristica fragrans (Nutmeg)	12.65	65.62 ± 1.54	17.67 ± 1.60	14.49 ± 3.35	880.49 ± 7.57
Myristica fragrans (Mace)	19.45	81.33 ± 1.17	13.86 ± 1.12	12.78 ± 1.83	470.66 ± 9.16
Nardostachys grandiflora	4.85	64.51 ± 3.56	46.57 ± 2.07	25.73 ± 2.79	> 2,000
Nelumbo nucifera	13.96	30.69 ± 3.35	37.13 ± 5.01	> 100	> 2,000
Nigella sativa	10.09	7.58 ± 0.58	16.72 ± 0.63	> 100	> 2,000
Nymphaea lotus	14.30	283.93 ± 1.12	360.83 ± 5.10	3.85 ± 0.23	279.83 ± 5.06
Nymphaea stellata	8.83	136.55 ± 1.15	150.56 ± 6.74	5.84 ± 0.34	672.23 ± 11.23
Picrorrhiza kurroa	19.65	65.03 ± 1.35	42.71 ± 6.20	65.54 ± 4.69	> 2,000
Pimpinella anisum	9.02	7.08 ± 1.01	72.63 ± 3.90	> 100	> 2,000
Plantago ovata	2.82	19.68 ± 0.32	21.36 ± 0.53	84.39 ± 2.16	> 2,000
Saussurea lappa	12.48	20.78 ± 0.87	13.72 ± 1.57	> 100	> 2,000
Syzygium aromaticum	15.75	241.78 ± 0.01	97.04 ± 4.24	7.46 ± 0.85	> 2,000
Tarenna hoaensis	2.93	113.07 ± 2.30	105.52 ± 2.81	15.82 ± 2.99	> 2,000
Terminalia chebula	32.68	127.44 ± 4.57	230.44 ± 12.16	2.35 ± 0.51	326.74 ± 8.39
Trachyspermum ammi	6.13	77.28 ± 1.84	15.20 ± 0.70	78.57 ± 6.15	518.21 ± 1.40
Vetiveria zizanioides	8.64	21.61 ± 0.74	55.94 ± 3.39	> 100	> 2,000
BHT	-	-	-	11.34 ± 0.98	-
Propyl gallate	-	-	-	-	0.520 ± 0.34

Additionally, we found a positive correlation between antioxidants and antidiabetic activities. *N. lotus* and *C. cyminum* showed good activities of α-glucosidase activity and DPPH with an IC_{50}, the EC_{50} value of 542.00 ± 13.58, 3.85 ± 0.23 µg/mL, and 611.67 ± 8.45, 96.32 ± 4.48 µg/mL respectively. Both *N. lotus* and *C. cyminum* showed a good inhibitory effect in both activities. Our results were inconsistent with previous in vivo studies conducted in alloxan diabetic mice fed with ethanolic extract of *N. lotus* and aqueous extract of *C. cyminum* which resulted in a significant decrease in blood sugar.[25,26]

The Pearson statistical correlation analysis direction of the linear relationship between TPC, TFC, and antioxidant activity revealed a strong positive correlation between DPPH assay and TPC (r = 0.7676, $P < .001$) and moderate positive correlation with TFC (r = 0.516, $P < .001$). The TBARS assay showed moderate positive correlation with TPC (r = 0.5124, $P < .001$) and TFC (r = 0.5189, $P < .001$). According to statistical analysis, it can be concluded that antioxidant activity had a statistically significant relationship with TPC and TFC. Both of these contents in all 45 herbs contributed to their antioxidant activities (Figure 1).

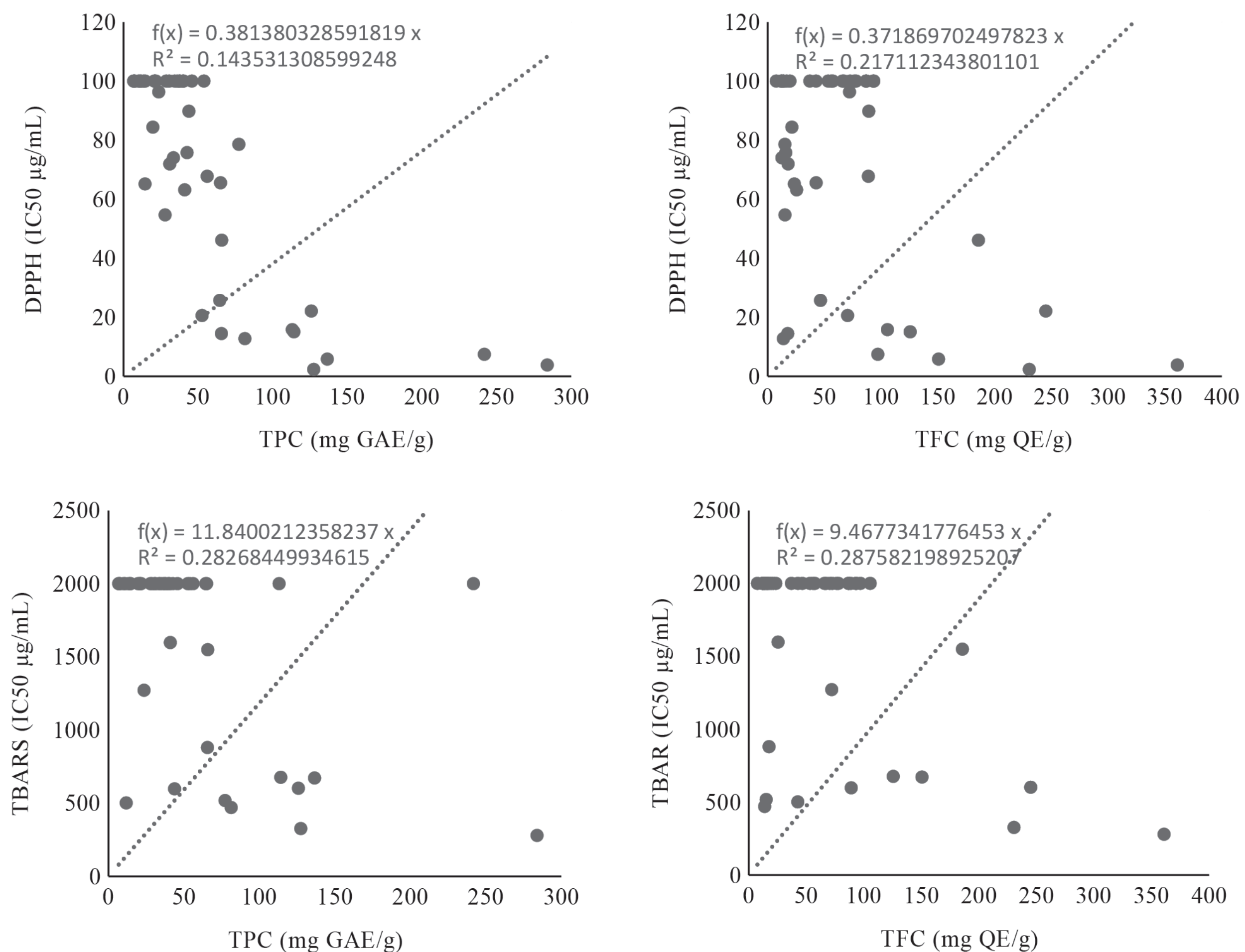

Figure 1 The Pearson statistical correlation scatters plot of the linear relationship between (A) DPPH and TPC, (B) DPPH and TFC, (C) TBARS and TPC, and (D) TBARS and TFC.

Lastly, the most beneficial plants were *N. lotus* (flower), *C. cyminum* (seed), and *M. fragrans* (nutmeg) due to their antioxidant activities along with anti-α-glucosidase and anti-α-amylase activities. *A. crassna* showed anti-α-glucosidase and anti-α-amylase activities without any antioxidant activity and should receive further investigation on antidiabetic activity. Other plants that showed antidiabetic potential were *N. stellata* and *T. chebula* which had strong antioxidant activities against free radical and lipid oxidation as well as anti-α-glucosidase and anti-α-amylase activities. The whole flower of *Nymphaea* spp. (*N. lotus* and *N. stellata*) should be used instead of petals due to its higher antioxidant property which may reflect on its protective activity of cell membrane from free radicals. Our results suggested that these herbs can be consumed as food or food supplements in diabetic patients.

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Conflicts of interest. The authors declare that they have no conflict of interests.

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