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RESEARCH ARTICLE

Clinical and Biologic Efficacy of Thai Herbal Blood Glucose-Lowering Formulation for the Treatment of Borderline Diabetes: A Pilot Double-Blind Randomized Controlled Trial

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Abstract

A traditional Thai herbal composition consisting of *Caryota mitis*, *Lagerstroemia speciosa*, *Piper sarmentosum*, *Pluchea indica*, and *Zea mays* has been used for the treatment of Type 2 diabetes. Nevertheless, there is an absence of scientific data to validate the therapeutic advantages. This research aimed to investigate the biological and clinical efficacy of a Thai herbal formulation in reducing blood glucose levels in patients with borderline diabetes. The chemical characteristics, *in vitro* antioxidant activity, α-glucosidase and α-amylase inhibition, and cytotoxicity assays were investigated in the aqueous and ethanol extracts of Thai herbal formulation. The clinical efficacy and safety of Thai herbal formulation for lowering blood glucose levels were evaluated in patients with borderline diabetes over a 12-week duration. The main chemical constituents of the Thai herbal formulation, resulting in exhibit antioxidant and glucose-lowering properties, include quinic acid, gallic acid, chlorogenic acid, and compounds. The aqueous and ethanol extracts of Thai herbal formulation had no inhibitory effect on either cell line including L929 cells and Vero cells. In the clinical investigation, Thai herbal formulation significantly reduced FBS and HbA1c levels ($p < 0.05$) compared with the control group after a period of 12 weeks, demonstrating its efficacy in managing diabetes. The findings indicated that the Thai herbal formulation had promise for managing diabetes. However, further inquiry is required to elucidate the mechanisms behind these results.

Keywords: Thai herbal formulation, Thai herbal medicine, Fasting blood glucose, Hemoglobin A1c, Diabetes, Antioxidant activity, α-glucosidase inhibition, α-amylase inhibition

Introduction

Prediabetes is a precursor to type 2 diabetes, representing an intermediate stage with elevated blood glucose levels between normal glucose tolerance and type 2 diabetes. This condition is also associated with nephropathy, small fiber neuropathy, diabetic retinopathy, and an increased risk of macrovascular disease [1,2]. The World Health Organization (WHO) characterizes prediabetes as a condition of intermediate hyperglycemia, based on 2 criteria: Impaired fasting glucose (IFG), indicated by fasting plasma glucose (FPG) levels of 6.1 - 6.9 mmol/L (110 to 125 mg/dL), and impaired glucose tolerance (IGT), indicated by a 2-hour plasma glucose level of 7.8 - 11.0 mmol/L (140 - 200 mg/dL) following a 75 g oral glucose load, or a combination of both as assessed by a 2-hour oral glucose tolerance test (OGTT) [3]. The American Diabetes

Association (ADA) establishes the same threshold for impaired glucose tolerance (IGT) at 140 - 200 mg/dL but defines a lower threshold for impaired fasting glucose (IFG) at 100 - 125 mg/dL. Additionally, this includes hemoglobin A1c (HbA1c) criteria, identifying prediabetes as a level within 5.7% and 6.4%. These classifications identify persons along the hyperglycemia continuum who, although not meeting current diagnostic criteria for diabetes, are at a higher risk of developing diabetes [4].

Traditional medicine has been extensively utilized in both developed and developing countries to alleviate or control the occurrence and increase in diabetes mellitus [5-7]. Traditional Thai medicine categorizes diabetes, termed "sweet urine," as a result of an imbalance among the 4 elements, resulting in irregular urination [8,9]. Dr. Chit Boonlueang, a traditional Thai herbalist from Wang Wiset District, Trang Province, has treated approximately 1,032 diabetic patients, mostly those with type 2 diabetes, according to patient records since 1992. Thai herbal formulation including *Caryota mitis* [10,11], *Lagerstroemia speciosa* [12,13], *Piper sarmentosum* [14,15], *Pluchea indica* [16,17], and *Zea mays* [18,19] could lower blood sugar, nourish the body, prevent hypoglycemia, stimulate pancreatic insulin production, restore liver function, and treat urinary problem to promote appropriate blood circulation and vitality, consequently controlling elemental balance. This herbal formulation has effectively treated diabetes without any adverse effects; nevertheless, there is a lack of scientific evidence to support the therapy. Therefore, the present study aimed to assess the biological and clinical efficacy of Thai herbal formulation for lowering blood glucose levels in patients with borderline diabetes.

Materials and methods

Preparation and extraction of Thai herbal formulation

Five medicinal plants (**Table 1**), named *Pluchea indica*, *Lagerstroemia speciosa*, *Piper sarmentosum* (leaves), *Zea mays* (pistils), and *Caryota mitis* (rhizome), were purchased at a Triburi herbal store in Songkla, Thailand. All medicinal plants were sanitized, dehydrated in an oven at 60 °C for 18 h, cut into small fragments, mashed into a fine powder, and sieved. All medicinal plants were gathered in a desiccator. The physico-chemical parameters of the herbs, involving foreign matter, loss on drying, total ash, acid-insoluble ash, and extractive values (water-soluble extractive, ethanol-soluble extractive, 50% ethanol-soluble extractive, and 70% ethanol-soluble extractive), were evaluated in triplicate following the methodologies identified in the Thai Herbal Pharmacopoeia and the Ayurvedic Pharmacopoeia of India [20,21].

The powder samples (200 g) were made in bottles. All the plants were blended for the formulation. Each plant and formulation were immersed in 95% ethanol or distilled water and shaken in an incubator shaker at 70 rpm and 50 °C for 48 hours, repeated thrice. After that, the infusion was filtered using Whatman No.1 paper. The filtrate was concentrated using a rotary evaporator at 45 °C under reduced pressure for ethanol extracts or freeze-dried for aqueous extracts.38 All extracts were preserved in desiccators at 4 °C for future use. The percentage yield of the extract was calculated using the dry weight of the extract (a) and the weight of the initial dry material (b) according to the formula: Percentage yield (%) equals (a/b)×100.

Table 1 Thai herbal formulation for diabetes.

Scientific name (Family name)	Part used	Ethnomedical use [8,9]	Pharmacological activity	Phytochemicals	Ratio (%)
Caryota mitis Lour. (Arecaceae)	root	diabetes mellitus, detoxifies the liver and lungs, pulmonary plague, cardiac abnormalities, splenic disorders, and liver recovery	antioxidant, antimicrobial [10]	cyclopentanone, 1,8-nonadien-4-ol, phenol, acide carbolique, 2-methoxy-4-vinylphenol, pentadecanoic acid, pyrogallol 1,3-dimethyl ether, phthalic acid, 11-octadecenoic acid, decylenic alcohol, pyroglutamic acid, (2e,6e)-farnesol, 1,5-heptadiene, stearic acid, cyclopentaneundecanoic acid, 3-butenoic acid, palmitic acid, oleic acid, hendecynoic acid [11]	1
Lagerstroemia speciosa Pers. (Lythraceae)	leave	diabetes mellitus, urination, kidney stones, hypertension and fever	anti-diabetes, hypoglycemic, α-	ursolic acid, corosolic acid, asiatic acid, alphitolic acid, ellagic acid, ellagic acid,	1

Scientific name (Family name)	Part used	Ethnomedical use [8,9]	Pharmacological activity	Phytochemicals	Ratio (%)
			amylase inhibitor, antioxidant [12]	3'-o-methyl-3, 4-methylenedioxy ellagic acid, 3, 4, 3'tri-o-methyl flavellagic acid, 3, 4, 3', 4'-tetra-o-methyl flavellagic acid, 3',4'-di -o- methyl-3, 4-methylenedioxy flavellagic acid6,7-dihydroxy coumarin, (+)-dihydrodehydrodiconiferyl alcohol 9'-o-sulfate, quercetin, caffeic acid , kaempferol, quercetin, maslinic acid [13]	
Piper sarmentosum Roxb. (Piperaceae)	leave	diabetes mellitus, appetizing, cough, flatulence and tonic	anti-diabetic, hypoglycemic, antioxidant [14]	α-ylangene, α-copaene, α-cadinene, β-cadinene, δ-cadinene, γ-cadinene, cadinol, α-cadinol, δ-cadinol, t-muurolol, cadinadiene, β-eudesmol, bicyclogermacrene, germacrene d, bicyclogermacreneβ-caryophyllene, caryophyllene oxid, trans-caryophyllene, α-humulene, (e)-nerolidol, cis-caryophyllene, α-farnesene, e,z-farnesol, guaiol, methyl eugenol, 3,4,5-trimethoxycinnamic acid, eugenol, cinnamyl alcohol, methyl 3-phenylpropionate [15]	1
Pluchea indica (L.) Less. (Asteraceae)	leave	hemorrhoids, tuberculosis, kidney stones, urination, sweating, rejuvenate, and fever	antihyperglycemic, α-glucosidase inhibition, antioxidant [16]	3-o-caffeoylquinic acid, 4-o-caffeoylquinic acid, 5-o-caffeoylquinic acid, 3,4-o-dicaffeoylquinic acid, 3,5-o-dicaffeoylquinic acid, and 4,5-o-dicaffeoylquinic acid 3,4-dihydroxybenzaldehyde 3,4-dihydroxybenzoic acid esculetin, kaempferol, luteolin, quercetin [17]	1
Zea mays Linn. (Poaceae)	pistil	atresia, liver disorders, hepatitis, jaundice, nephritis, edema, gallbladder inflammation and hypertension	antidiabetic, hypolipidemic, anti-oxidative, anti-inflammatory [18]	maysin, chlorogenic acid, chrysoeriol-6-c-β-boivinopyranosyl-7-o-β-glucopyranoside, chrysoeriol, luteolin, 2,2-diphenyl-1-picryl-hydrazyl, 6-c-β-fucopyranoside, 2"-o-β-l-rhamnosyl-6-c-3"-deoxyglucosyl-3'-methoxyluteolin, chrysoeriol-6-c-β-boivinopyranosyl-7-o-β-glucopyranoside, chrysoeriol [19]	1

Analysis of chemical contents of Thai herbal formulation using Liquid Chromatography-Quadrupole Time-of-Flight Mass Spectrometry (LC-QTOF MS)

The extract of the Thai herbal formulation was analyzed utilizing a liquid chromatograph-quadrupole time-of-flight mass spectrometry (LC-QTOF MS) instrument (1290 Infinity II LC-6545 Quadrupole-TOF, Agilent Technologies, USA), employing a Zorbax Eclipse Plus C18 Rapid Resolution HD column (150 mm length × 2.1 mm inner diameter, particle size 1.8 μm) [22]. In summary, the mobile phase included 0.1% formic acid in water (v/v, A) and methanol (B). The programmed gradient was delineated as follows: 10% B from 0 to 5 min, 10% - 30% B from 5 to 10 min, 30% - 85% B from 10 to 15 min, 85% - 100% B from 15 to 20 min, 100% B from 20 to 23 min, and a return to 10% B from 23 to 30 min, at a flow rate of 0.2 mL/min. A post-run duration of 5 min was recorded, using an injection volume of 2 μL and sustaining a column temperature of 40 °C. Dual electrospray ionization (ESI) source operating in both negative and positive ionization modes. The extracts were analyzed with auto MS/MS mode within the m/z range of 100 to 1,200. The Mass Hunter Workstation software (version B 08.00 Qualitative Analysis Workflows and Qualitative Analysis Navigator, Agilent Technologies, USA) was used for data analysis. The MassHunter METLIN PCD (Personal Compound Database) and PCDL (Personal Compound Database and Library) were used to identify the bioactive compounds.

Determination of total phenolic and flavonoid content of Thai herbal formulation

The total phenolic content in plant extracts was examined based on previous research with minor modifications [23]. Briefly, an aliquot of 100 µL from each sample was oxidized using 500 µL of 0.2 N Folin-Ciocalteu's reagent and then neutralized with the addition of 400 µL of 7.5% Na_2CO_3. Absorbance was recorded at 765 nm after a 30-minute mixing period at ambient temperature. Gallic acid served as the reference standard. The results were expressed as milligrams of gallic acid equivalents (GAE) per gram of extract.

The total flavonoid content in plant extracts was determined based on a previous study with minor modifications [24]. Briefly, the extracts were combined with 100 µL of 5% aluminum chloride (w/v), followed by the addition of 400 µL of 2.5% $NaNO_3$ after 5 min, and then 500 µL of 5% $AlCl_3$. The mixture was set to stand at room temperature for 10 min. The solution was combined with 2,000 µL of distilled water, and the absorbance was recorded at 415 nm using a UV-visible spectrophotometer. Quercetin used as the reference standard. The total flavonoid content was determined in milligrams of quercetin equivalents (QE) per gram of plant extract.

Evaluation of total antioxidant capacity of Thai herbal formulation

The total antioxidant capacity of Thai herbal formulation was assessed by 3 assays: The DPPH organic synthetic radical, the ABTS radical cation, and the FRAP methodology.

DPPH radical-scavenging assay

The free radical scavenging activity was assessed according to a previous study with minor modifications [25]. Briefly, the DPPH was solubilized in ethanol to achieve a concentration of 0.039 mg/mL. The plant extract was diluted with distilled water at varied concentrations to generate the sample solution. The extract solution (100 µL) was combined with 0.1 mmol/L DPPH ethanolic solution (900 µL). Subsequent to 30 minutes, the reaction was maintained in darkness at ambient temperature. The absorbance of the solution was then measured at 515 nm. Ascorbic acid served as the positive standard, and the percentage inhibition of DPPH in the test samples was determined using the formula: DPPH inhibition (%) = [(Ac – As)/Ac]×100. Ac indicates the absorbance of the control, while As represents the absorbance of the sample solution.

ABTS radical-scavenging assay

ABTS radical-scavenging assay was measured according to a previous study with minor modifications [26]. Briefly, the ABTS+ solution (900 µL) was combined with the extract (100 µL) and stirred well. The combination was maintained at ambient temperature for 6 min. The absorbance was immediately measured at 734 nm. Ascorbic acid served as a positive standard. The percentage inhibition of $ABTS^{+\cdot}$ in the test samples was calculated using the formula: ABTS inhibition (%) = [(Ac − As)/Ac]×100. Ac represents the absorbance of the control, while As indicates the absorbance of the sample solution.

Ferric reducing antioxidant power (FRAP) assay

FRAP assay was determined according to a previous study with minor modifications [27]. Briefly, the Frap reagent was made by combining 300 mM acetate buffer, 10 mL TPTZ in 40 mM HCl, and 20 mM $FeCl_3 \cdot 6H_2O$ in a ratio of 10:1:1 at 37 °C. The FRAP reagent (900 µL) was mixed with the diluted plant extract (100 µL) and well stirred. After a 5-minute incubation of the solution at room temperature, the absorbance was assessed at 593 nm. The concentration was ascertained using quercetin as a standard. The results were expressed as milligrams of quercetin equivalents (GAE) per gram of extract.

Assessment of Thai herbal formulation's antidiabetic activity mechanism of action via enzyme inhibition α-glucosidase inhibitory activity

The inhibitory effect of Thai herbal formulation extract on α-glucosidase was conducted as previously described [26,28]. Briefly, 30 µL from each sample prepared in 3% dimethyl sulfoxide was combined with 20 µL of sodium phosphate buffer (pH 6.7) containing 0.1 U/mL of α-glucosidase and incubated at 37 °C for 10 min. Then, 40 µL of pNPG solution (1 mM) were included into the mixture and incubated at 37 °C for 30 min. Subsequently, 0.1 M Na_2CO_3 was introduced, and the absorbance was measured using a spectrophotometer at 405 nm. Acarbose served as the positive control.

The α-glucosidase inhibitory activity was determined as a percentage of inhibition, measured using the following equation: Inhibition (%) = [((AC − ACb) − (AS − ASb))/(AC − ACb)]×100. Ac indicates the absorbance of the control including the enzyme; Acb represents the absorbance of the control blank (buffer devoid of enzyme); As and Asb are the absorbance of the sample (enzyme with inhibitor) and the sample blank (inhibitor without enzyme), respectively.

α-amylase inhibitory activity

The inhibitory effect of the Thai herbal formulation extract against α-amylase was assessed as previously described [26,28]. Briefly, 100 µL of Thai herbal extract dissolved in dimethyl sulfoxide (3%) was combined with 100 µL of α-amylase solution (0.1 U/mL) in phosphate buffer (pH 6.9) and incubated at 37 °C for 30 min. Subsequent to pre-incubation, 100 µL of a 0.25% starch solution in phosphate buffer (pH 6.9) was introduced into each tube. The reaction was conducted at 37 °C for 30 min. The reaction was ended by the addition of 200 µL of the DNS reagent. The test tubes were then incubated in a boiling water bath for 5 min and then cooled to room temperature. Absorbance was measured at 540 nm with a spectrophotometer. Acarbose served as the positive control.

The α-amylase inhibitory activity was determined as a percentage of inhibition, measured using the following equation: Inhibition (%) = [((AC − ACb) − (AS − ASb))/(AC − ACb)]×100. Ac represents the absorbance of the control including the enzyme; Acb indicates the absorbance of the control blank (buffer devoid of enzyme); As and Asb are the absorbance of the sample (enzyme with inhibitor) and the sample blank (inhibitor without enzyme), respectively.

Evaluation of cytotoxic activity of Thai herbal formulation

The cytotoxic effect was assessed using the MTT assay with standard cell lines: L929 cells (mouse fibroblast cell line) and Vero cells (African green monkey kidney cell line) [29,30]. L929 cells were grown in Dulbecco's Modified Eagle Medium (DMEM) at a density of 8×10^3 cells per well, whereas Vero cells were maintained in RPMI 1640 Medium at a density of 1×10^4 cells per well in 96-well plates. The cell lines were subjected to extracts at concentrations of 5, 10, 20, 40, and 80 µg/mL, respectively. Doxorubicin was administered at concentrations of 0.625, 1.25, 2.5, 5, and 10 µM as positive controls over a period of 72 h. After that, the cells were washed with 1x PBS and incubated in 100 µL of 0.5 mg/mL MTT at 37 °C for 30 min. Then, 100 µL of DMSO was used to solubilize the formazan generated in the cells, and the mixture was incubated at 37 °C for an additional 30 min at ambient temperature. Absorbance was determined at 570 and 650 nm with a microplate reader. All tests were conducted in triplicate.

Clinical efficacy and safety of Thai herbal formulation for patients with borderline diabetes

Preparation of Thai herbal formulation capsules

The capsules comprising Thai herbal formulation powder and maize starch (Grade USP 42) as the placebo were manufactured in consistently regarding shape, size, and color by a Thai traditional medicine practitioner utilizing a semi-automatic capsule filling machine. Each capsule included 500 mg. The quality control of the capsules included appearance characteristics, weight variation, and disintegration time, in accordance with the techniques outlined in USP 41-NF 36 and THP 2018 [31,32]. Both the Thai herbal formulation capsule and the placebo were stored in sealed plastic containers. All containers were marked with labels (A or B) on the outside of the package to preserve participant blinding.

Study design and interventions

A randomized, double-blind, clinical trial was performed at 60th Anniversary Commemoration of Princess Mother Tambon Health Promotion Hospital, Ratsada, Trang, Thailand. Prior to participation, all participants provided written informed permission for the approved procedure by the Human Research Ethics Committee of Prince of Songkla University, Songkhla Province, Thailand (reference number: HSc-HREC-64-042-1-3). The protocol was also registered in Thai Clinical Trials Registry (registration ID: TCTR20241031003). The research procedures were executed in accordance with the stated requirements and ethical standards of the Helsinki Declaration.

The participants were randomly allocated to 2 groups: The intervention group (Thai herbal formulation group) and the control group. Participants in both the Thai herbal formulation group and the control group were instructed to take 3 capsules post-breakfast and 3

capsules post-dinner for a sustained period of 12 weeks. The daily intake of the Thai herbal mixture and placebo capsules was 3,000 mg, in accordance with traditional use.

All participants were given instructions following to their regular dietary and physical activity habits and to report any adverse effects during the intervention. The participant's adherence was assessed by the pill count method, and those utilizing over 80% of the therapies were included into the research.

Randomization and blinding

Eligible patients were randomly allocated to either the Thai herbal formulation group or the control group via a random number method (1:1 ratio using SAS 9.2 software with a block size of 4). All participants and researchers, including the data analyzers, were unaware of group allocation. The form, hue, and aroma of Thai herbal medicine were indistinguishable to maintain concealment, and the packaging stayed consistent. The random assignment was hidden behind sequentially numbered, opaque, sealed envelopes. The allocation group remained private until an investigator supplied data on a completely eligible patient, so maintaining the integrity of the randomization procedure. Both the patients and the investigators evaluating outcomes were unaware of the random assignment to the therapy group (double-blind). Blinding remained unaware of the created allocation codes throughout the trial. The blinding was to be maintained (unless in emergencies) until the data was gathered and validated for analysis.

Inclusion criteria and exclusion criteria

Forty participants of both genders aged 35 to 60 years had at least one of the following indices: Fasting plasma glucose varied between 100 and 125 mg/dL, whereas Hemoglobin A1c ranged from 5.7% to 6.4%. Participants utilizing pharmacological agents or supplements that influence glycemic levels, corticosteroids; participants with coronary vascular pathology; those with renal and hepatic impairments; hematological conditions; uncontrolled hypertension; pregnant or lactating women; and patients predisposed to potential adverse complications during the study, including cephalalgia, dizziness, nausea, irregular capsule administration, or allergic responses to the herb or any capsule constituent, were deemed ineligible according to the exclusion criteria.

Outcome measurements

Demographic characteristics related to age, weight, height, gender, medical and pharmacological history, familial history, and other factors were evaluated and documented via patient interviews. At baseline and at the end of the intervention, measurements were conducted for body weight (kg), body mass index (kg/m^2), fasting serum concentrations of blood glucose parameters (fasting plasma glucose, hemoglobin A1c), lipid parameters (total cholesterol, triglycerides, low-density lipoprotein, high-density lipoprotein), complete blood count, and toxicity tests (blood urea nitrogen, creatinine, alanine transaminase, and aspartate aminotransferase).

Safety assessment

The clinical trial's safety was assessed by monitoring adverse events during the study. This research characterized serious adverse effects as death, life-threatening conditions, hospitalization or extended hospitalization, and permanent or severe disability.

Statistical analysis

The descriptive statistics for categorical variables were presented as frequency and percentage, and continuous variables were indicated as mean and median/interquartile range. Inferential statistics were used to conduct a comparative study of the baseline data between the intervention and control groups. Categorical data were evaluated using the Chi-Square Test of Independence, and continuous variables were examined using Welch's t-test (depending on normal distribution) or the Wilcoxon rank sum test. A 2-way mixed analysis of variance (ANOVA) was used to examine the interaction effects between time (pre/post) and group (intervention/control), using repeated measures on the final factor. Bonferroni's pairwise comparisons were used for post hoc analysis. The influence of these features on dietary habits was assessed by a 3-way mixed ANOVA (dietary habits/group/time) and the Bonferroni comparison test. *p*-values less than 0.05 were deemed indicative of statistically significant findings. Multiple comparisons

were conducted with Tukey's post-hoc test, with significance determined at $p < 0.05$.

Results and discussion

Physicochemical characteristics of Thai herbal medicines

The physicochemical characteristics, such as foreign matter, total ash, acid-insoluble ash, moisture loss, ethanol-soluble extractives, and water-soluble extractives, are essential parameters in the assessment of the physicochemical compositions of Thai herbal medicines. The physicochemical properties of the herbal constituents are shown in **Table 2**. The major medicinal plants, including *P. indica*, *L. speciosa*, *P. sarmentosum*, *Z. mays*, and *C. mitis*, conformed to the standards established by the Thai and Myanmar herbal pharmacopoeias regarding foreign matter, total ash, acid-insoluble ash, moisture loss, ethanol-soluble extractives, and water-soluble extractives.

Table 2 Physicochemical specification of herbal components.

Parameters	Content (% by weight)				
	Caryota mitis	***Lagerstroemia speciosa*** [b]	***Piper sarmentosum*** [a]	***Pluchea indica*** [b]	***Zea mays*** [c]
Foreign matter	1.30 ± 0.06	1.78 ± 0.21	1.31 ± 0.09	1.25 ± 0.01	0.81 ± 0.10
	NA	(< 2)	ND	(< 2)	(< 2)
Total ash	4.69 ± 0.51	3.43 ± 0.16	11.23 ± 2.66	3.50 ± 0.15	3.70 ± 0.23
	NA	(< 7)	(< 20)	-	(< 7)
Acid-insoluble ash	0.07 ± 0 .01	3.09 ± 0.20	4.18 ± 0.09	0.03 ± 0.02	2.21 ± 0.16
	NA	(< 7)	(< 7)	(< 0.5)	(< 4)
Loss on drying	5.57 ± 0.08	4.80 ± 0.25	7.25 ± 0.07	2.78 ± 0.07	8.02 ± 0.01
	NA	(< 7)	(< 10)	(< 3)	(< 14)
Ethanol-soluble extractive	12.28 ± 0.10	15.54 ± 0.13	16.48 ± 0.29	18.34 ± 0.67	22.27 ± 1.52
	NA	(> 9)	(> 7)	(> 10)	(> 12)
Water-soluble extractive	10.98 ± 0.19	9.40 ± 0.29	25.27 ± 0.52	15.25 ± 0.92	15.30 ± 0.29
	NA	(>3)	(> 20)	(> 10)	(> 10)

NA, Not applicable; ND, Not determined. [a] The parameters were tested according to Thai herbal pharmacopeia; [b] The parameters were tested according to Ayurvedic herbal pharmacopoeia; [c] The parameters were tested according to Chinese Herbal Pharmacopeia.

Percentage yield of aqueous and ethanol crude extracts of herbal components and Thai herbal formulations

The 5 herbal medicines and formulations approximately 200 g, were extracted using a 95% ethanol solvent and distilled water. The results of the investigation revealed the proportion of coarse extract content (**Figure 1**). The yield of the ethanol crude extract from the Thai herbal formulation was 29.90%. During the ethanol extraction, *L. speciosa* extract demonstrated the highest percentage of coarse extract at 45.25%, followed by *C. mitis* extract at 30.38%, *P. indica* at 20.34%, *P. sarmentosum* at 19.50%, and *Z. mays* extract at 10.91% correspondingly. The aqueous extraction of the Thai herbal formulation yielded a coarse extract content of 27.84%. Similarly, the water extract of *L. speciosa* exhibited the highest coarse extract content at 39.89%, followed by *C. mitis* extract at 25.56%, *P. indica* at 18.12%, *P. sarmentosum* at 14.15%, and *Z. mays* extract at 8.12%, respectively.

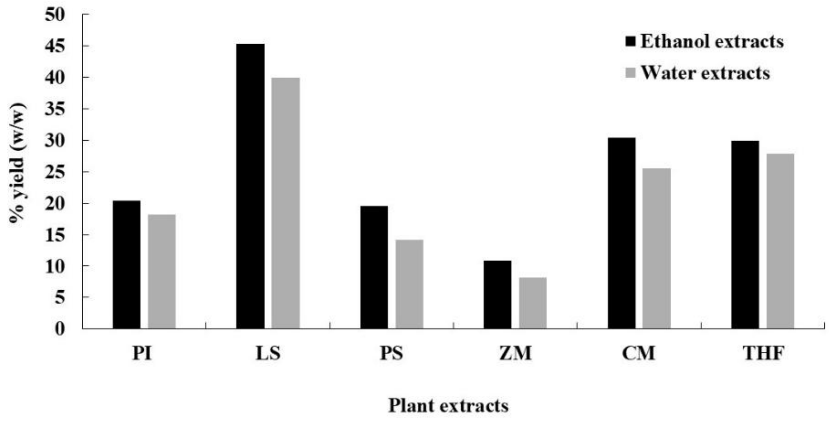


Figure 1 Extraction yields of Thai herbal formulation and its herbal components. CM, *Caryota mitis*; LS, *Lagerstroemia speciosa*; PS, *Piper sarmentosum*; PI, *Pluchea indica*; ZM, *Zea mays*; THF, Thai herbal formulation. The 5 herbal medicines and formulations approximately 200 grams, were extracted using a 95% ethanol solvent and distilled water. The results of the investigation revealed the proportion of coarse extract content.

Chemical composition of Thai herbal formulation using LC-QTOF MS

The evaluation of the chemical composition of ethanol and water extracts from a Thai herbal formulation employing LC-QTOF MS, specifically the 1290 Infinity II LC-6545 Quadrupole-TOF, utilizing an Electrospray Ionization (ESI) source in both negative and positive modes, related to chromatograms (**Figure 2**). The ethanol and water extracts of the Thai herbal formulation exhibited 40 identifiable components (**Table 3** and **Table 4**). The main chemical constituents of ethanol and water extracts from a Thai herbal formulation, demonstrating anti-oxidant properties and glucose-lowering effects, involved quinic acid, gallic acid, chlorogenic acid, 5z-caffeoylquinic acid, esculetin, caffeic acid, quercetin 3'-O-glucuronide, ellagic acid, maslinic acid, 1,3-dicaffeoylquinic acid, kaempferol, curcumin, and 3,4-dihydroxybenzoic acid (**Figure 3**).

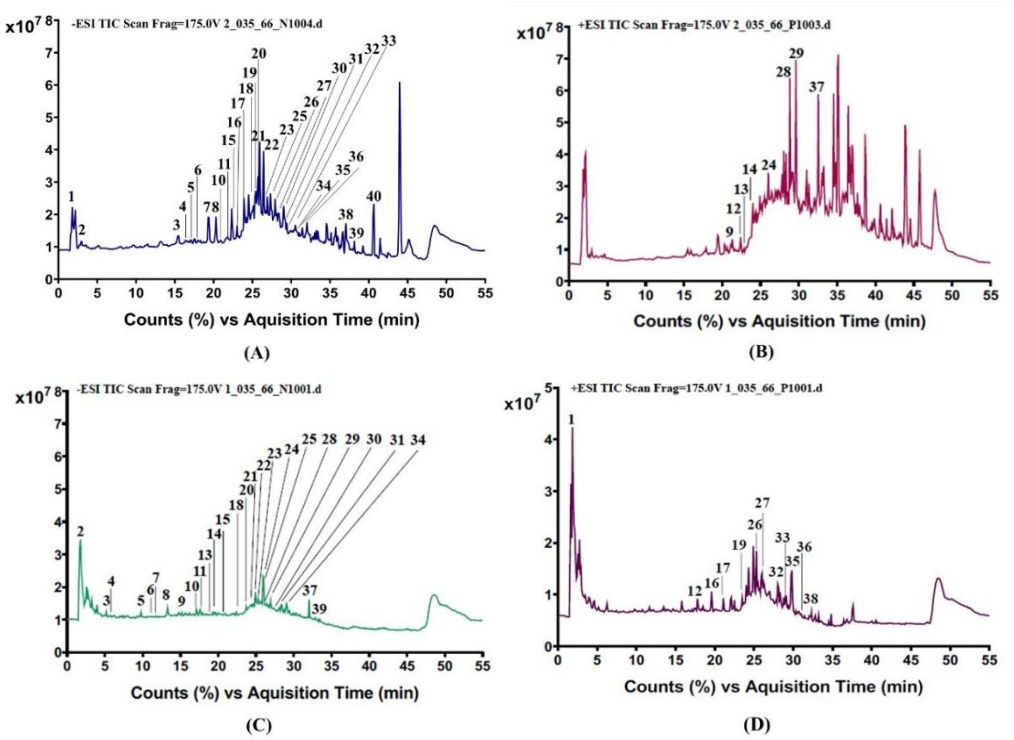


Figure 2 LC-QTOF-MS chromatogram of bioactive compounds from Thai herbal formulation; ethanol negative mode (A); ethanol positive mode (B); water negative mode (C); water positive mode (D). The evaluation of the chemical composition of ethanol and water extracts from a Thai herbal formulation employing LC-QTOF MS, specifically the 1290 Infinity II LC-6545 Quadrupole-TOF, utilizing an Electrospray Ionization (ESI) source in both negative and positive modes, related to chromatograms.

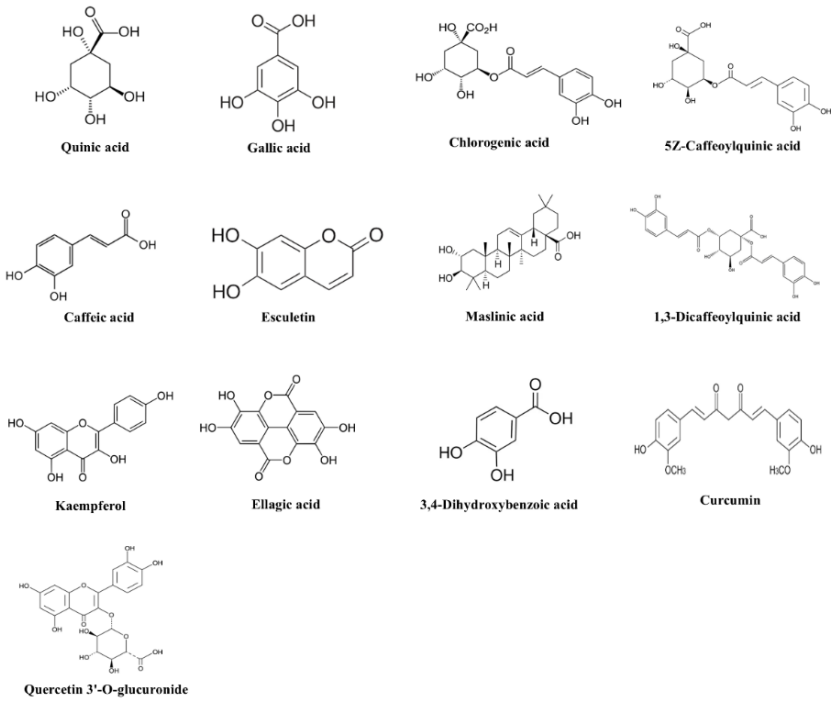


Figure 3 Chemical structure of bioactive compounds from Thai herbal formulation tentatively identified by LC-QTOF-MS. The main chemical constituents of ethanol and water extracts from a Thai herbal formulation, demonstrating anti-oxidant properties and glucose-lowering effects.

Table 3 Compounds identified in the ethanolic extract of Thai herbal formulation by LC-QTOF-MS.

No.	TR/min	Measured molecular w.		Molecular formula	Compound
		− ion (m/z)	+ ion (m/z)	Fragment ion	
1	2.018	191.0565		(−) 85, 191	$C_7H_{12}O_6$	Quinic acid
2	4.900	169.0142		(−) 81, 125	$C_7H_6O_5$	Gallic acid
3	15.46	353.0882		(−) 191, 353	$C_{16}H_{18}O_9$	Chlorogenic Acid
4	15.773	285.0619		(−) 153, 285	$C_{12}H_{14}O_8$	Uralenneoside
5	16.425	353.088		(−) 135, 173, 353	$C_{16}H_{18}O_9$	5Z-Caffeoylquinic acid
6	16.826	177.0196		(−) 133, 177	$C_9H_6O_4$	Esculetin
7	21.599	247.0251		(−) 112, 247	$C_{12}H_8O_6$	7-Deshydroxypyrogallin-4-Carboxylic Acid
8	21.988	197.0458		(−) 124, 197	$C_9H_{10}O_5$	3,4-O-Dimethylgallic acid
9	23.956		601.1522	(+) 371	$C_{27}H_{30}O_{14}$	Scutellarein 7,4’-dirhamnoside
10	23.641	593.1515		(−) 293, 413, 593	$C_{27}H_{30}O_{15}$	Saponarin
11	23.954	577.1568		(−) 293, 413, 577	$C_{27}H_{30}O_{14}$	Scutellarein 7,4’-dirhamnoside
12	24.232		433.1129	(+) 121, 283, 397	$C_{21}H_{20}O_{10}$	Vitexin
13	24.62		487.0841	(+) 127, 303	$C_{21}H_{20}O_{12}$	Quercetagetin 3-rhamnoside
14	24.808		197.1171	(+) 135	$C_{11}H_{16}O_3$	Isobutyl 2-furanpropionate
15	24.042	563.1414		(−) 293, 413, 563	$C_{26}H_{28}O_{14}$	Luteolin 3’-methyl ether 7,4’-dixyloside
16	24.130	477.0676		(−) 315, 477	$C_{21}H_{18}O_{13}$	Quercetin 3’-O-glucuronide
17	24.468	380.9923		(−) 151, 301, 380	$C_{15}H_{10}O_{10}S$	8-Hydroxyluteolin 8-sulfate
18	24.556	463.0889		(−) 300, 463	$C_{21}H_{20}O_{12}$	Quercetin 3-galactoside
19	24.568	300.9996		(−) 229, 300	$C_{14}H_6O_8$	Ellagic acid
20	24.844	461.0736		(−) 314, 461	$C_{21}H_{18}O_{12}$	3-Methylellagic acid 8-rhamnoside
21	25.495	515.1202		(−) 191, 353, 515	$C_{25}H_{24}O_{12}$	1,3-Dicaffeoylquinic acid
22	25.708	439.1076		(−) 96, 439	$C_{20}H_{24}O_9S$	Hallactone B
23	25.846	609.1259		(−) 327, 429, 609	$C_{30}H_{26}O_{14}$	Theasinensin C
24	26.988		303.0869	(+) 215, 303	$C_{16}H_{15}O_6$	5-Methyl-6-hydroxyluteolinidin
25	26.272	417.1195		(−) 99, 297, 417	$C_{21}H_{22}O_9$	Aloin A
26	26.397	431.0984		(−) 152, 431	$C_{21}H_{20}O_{10}$	Kaempferol 4’-rhamnoside
27	26.873	639.1356		(−) 89, 178, 300, 463	$C_{31}H_{28}O_{15}$	Patuletin 3-(6’’-p-coumaroylglucoside)
28	27.941		303.0501	(+) 165, 229	$C_{15}H_{10}O_7$	Herbacetin
29	27.512	375.1094		(−) 207, 375	$C_{19}H_{20}O_8$	5,2’-Dihydroxy-6,7,8,6’-tetramethoxyflavanone
30	27.901	301.0366		(−) 151, 301	$C_{15}H_{10}O_7$	Hieracin
31	28.452	329.0311		(−) 329	$C_{16}H_{10}O_8$	2,8-Di-O-methylellagic acid
32	28.978	425.0882		(−) 351, 425	$C_{22}H_{18}O_9$	Epigallocatechin 3-O-(4-hydroxybenzoate)
33	29.178	429.1196		(−) 325, 429	$C_{22}H_{22}O_9$	Ononin
34	29.341	285.0409		(−) 59, 239, 327	$C_{15}H_{10}O_6$	Kaempferol
35	32.711	367.1189		(−) 149, 217, 367	$C_{21}H_{20}O_6$	Curcumin

No.	TR/min	Measured molecular w.		Molecular formula		Compound
		− ion (m/z)	+ ion (m/z)	Fragment ion		
36	33.653		701.3721	(+) 347, 539	$C_{33}H_{58}O_{14}$	Gingerglycolipid B
37	36.132	471.3484		(−) 112, 417	$C_{30}H_{48}O_4$	Maslinic Acid
38	37.873	425.2702		(−) 95, 425	$C_{27}H_{38}O_4$	Azafrin
39	40.579	277.2177		(−) 277	$C_{18}H_{30}O_2$	Pinolenic Acid

Table 4 Compounds identified in the water extract of Thai herbal formulation by LC-QTOF-MS.

No.	TR/min	Measured molecular w.		Fragment ion	Molecular formula	Compound
		- ion (m/z)	+ ion (m/z)			
1	1.907		381.0796	(+) 57, 381	$C_{12}H_{22}O_{11}$	Kojibiose
2	2.747	191.0203		(−) 111, 191	$C_6H_8O_7$	Citric acid
3	5.265	169.0144		(−) 81, 125	$C_7H_6O_5$	Gallic acid
4	5.566	165.0194		(−) 165	$C_8H_6O_4$	3,4-Methylenedioxybenzoic acid
5	9.838	153.0196		(−) 109	$C_7H_6O_4$	3,4-Dihydroxybenzoic acid
6	13.333	167.0351		(−) 152	$C_8H_8O_4$	3-Methoxysalicylic acid
7	13.421	246.992		(−) 112, 167, 246	$C_8H_8O_7S$	Vanillic acid 4-sulfate
8	14.824	393.119		(−) 59, 161, 245	$C_{19}H_{22}O_9$	2’-Oxoaloesol 7-glucoside
9	15.851	285.0617		(−) 153, 205, 285	$C_{12}H_{14}O_8$	Uralenneoside
10	16.854	177.0194		(−) 133, 177	$C_9H_6O_4$	Esculetin
11	17.154	247.0248		(−) 112, 247	$C_{12}H_8O_6$	7-Deshydroxypyrogallin-4-Carboxylic Acid
12	17.417		251.125	(+) 251	$C_{12}H_{20}O_4$	5-Heptyltetrahydro-2-oxo-3-furancarboxylic acid
13	17.517	179.0351		(−) 135	C_9H_8O4	Caffeic Acid
14	18.056	197.0457		(−) 89, 153	$C_9H_{10}O_5$	3,4-O-Dimethylgallic acid
15	20.938	499.1279		(−) 96, 499	$C_{22}H_{28}O_{11}S$	Lyoniresinol 9’-sulfate
16	22.115		225.1119	(+) 77, 165, 193	$C_{12}H_{16}O_4$	3’,4’,5’-Trimethoxycinnamyl alcohol
17	22.328		605.2202	(+) 109, 186, 272, 397, 599	$C_{28}H_{38}O_{13}$	(+)-Lyoniresinol 9-glucoside
18	23.694	593.151		(−) 176, 293, 413, 530	$C_{27}H_{30}O_{15}$	Saponarin
19	24.032		234.1122	(+) 131	$C_{13}H_{12}O_3$	Ethyl 2-phenyl-3-furancarboxylate
20	24.095	563.1404		(−) 150, 293	$C_{26}H_{28}O_{14}$	Luteolin 3’-methyl ether 7,4’-dixyloside
21	24.546	300.999		(−) 145, 300	$C_{14}H_6O_8$	Ellagic acid
22	25.097	391.1403		(−) 122, 197, 391	$C_{20}H_{24}O_8$	Marmesin rhamnoside
23	25.435	445.1829		(−) 175	$C_{22}H_{30}O_8$	Valtratum

No.	TR/min	Measured molecular w.		Fragment ion	Molecular formula	Compound
		- ion (m/z)	+ ion (m/z)			
24	25.573	575.14		(−) 112, 196, 411, 575	$C_{27}H_{28}O_{14}$	Chamaemeloside
25	25.699	439.1073		(−) 96, 439	$C_{20}H_{24}O_9S$	Hallactone B
26	26.262		306.2634	(+) 81	$C_{16}H_{32}O_4$	9,10-dihydroxy-hexadecanoic acid
27	26.989		303.0863	(+) 131, 203, 273	$C_{16}H_{14}O_6$	5,2',3'-Trihydroxy-7-methoxyflavanone
28	26.350	589.1563		(−) 103, 327, 425, 589	$C_{28}H_{30}O_{14}$	Maysin 3'-methyl ether
29	27.014	257.0826		(−) 65, 163, 231	$C_{15}H_{14}O_4$	cis-4-Hydroxyequol
30	27.352	395.2076		(−) 331, 395	$C_{21}H_{32}O_7$	Isopetasoside
31	27.477	201.1135		(−) 57, 201	$C_{10}H_{18}O_4$	2,6-Dimethyl-1,8-octanedioic acid
32	28.166		290.2691	(+) 290	$C_{16}H_{32}O_3$	2-Hydroxyhexadecanoic acid
33	28.817		277.1773	(+) 77, 95	$C_{15}H_{26}O_3$	formyl 7-oxo-11E-tetradecenoate
34	28.756	215.1292		(−) 215	$C_{11}H_{20}O_4$	Butyl butyryllactate
35	29.444		433.2195	(+) 157, 239, 373	$C_{22}H_{34}O_7$	Colforsin
36	31.072		295.0937	(+) 79, 109, 182	$C_{16}H_{16}O_4$	Loureirin C
37	32.063	293.1765		(−) 71, 236	$C_{17}H_{26}O_4$	Myrsinone
38	33.177		209.1172	(+) 91, 121, 179	$C_{12}H_{16}O_3$	a-Asarone
39	33.466	293.1759		(−) 193, 293	$C_{17}H_{26}O_4$	Embelin

Phenolic and flavonoid contents and antioxidant capacity of Thai herbal formulation

Total phenolic and flavonoid contents of the individual herbs and Thai herbal formulation were demonstrated in **Table 5**. The total phenolic content varied among different extracts, ranging from 28.63 to 244.36 mg GAE/g of dry material. The total phenolic content of the ethanol extract from the Thai herbal formulation was 70.47 ± 0.58 mg GAE/g. The highest total phenolic levels among the herbal components were observed in *L. speciosa* (244.36 ± 9.90 mg GAE/g), followed by *C. mitis* (172.22 ± 5.84 mg GAE/g), *P. indica* (47.50 ± 1.89 mg GAE/g), *P. sarmentosum* (33.55 ± 1.56 mg GAE/g), and *Z. mays* (28.63 ± 0.43 mg GAE/g). In addition, the aqueous extract of Thai herbal formulation had 57.48 ± 0.56 mg GAE/g. *L. speciosa* also exhibited the greatest total phenolic content (199.40 ± 1.25 mg GAE/g), followed by *Z. mays* (36.44 ± 0.85 mg GAE/g), *C. mitis* (35.28 ± 0.18 mg GAE/g), *P. sarmentosum* (29.42 ± 0.37 mg GAE/g), and *P. indica* (29.31 ± 0.26 mg GAE/g).

The total flavonoid content of all extracts ranged from 19.51 to 80.98 mg QE/g of dry material. The ethanol extract of the Thai herbal formulation contained 70.98 ± 7.26 mg QE/g, whereas, *L. speciosa* exhibited the greatest total flavonoid content at 80.98 ± 0.01 mg QE/g, followed by *Z. mays* at 29.25 ± 0.53 mg QE/g, *C. mitis* at 26.16 ± 0.52 mg QE/g, *P. indica* at 23.48 ± 4.20 mg QE/g, and *P. sarmentosum* at 21.37 ± 0.66 mg QE/g, respectively. Moreover, the aqueous extracts revealed that the Thai herbal formulation extracts contained a total flavonoid content of 51.51 ± 1.43 mg QE/g. Similarly, *L. speciosa* exhibited the highest flavonoid content at 63.09 ± 2.52 mg QE/g, followed by *C. mitis* at 37.64 ± 0.24 mg QE/g, *P. sarmentosum* at 30.72 ± 1.18 mg QE/g, *Z. mays* at 23.70 ± 0.28 mg QE/g, and *P. indica* at 19.51 ± 0.71 mg QE/g.

Antioxidant properties including DPPH radical-scavenging assay, ABTS radical scavenging assay, and FRAP assay of the individual herbs and Thai herbal formulation were presented in **Table 5**. The DPPH radical scavenging activity of ethanol extracts from Thai herbal formulation exhibited an IC_{50} of 57.67 ± 3.47 µg/mL, which was lower than the IC_{50} value of 94.63 ± 1.68 µg/mL for aqueous extracts. For the ethanol and aqueous extracts of the medicinal plants, *L. speciosa* exhibited the most effective DPPH radical scavenging (15.25 ± 0.83 and 19.02 ± 0.94 µg/mL), in comparison to the standard ascorbic acid (6.61 ± 0.01 µg/mL). Furthermore, the ethanol extract of *C. mitis* exhibited superior efficacy compared to *P. indica*, followed by *P. sarmentosum* and *Z. mays*, with IC_{50} values of 22.11 ± 0.98, 55.98 ± 1.15, 116.26 ± 2.55, and 273.33 ± 1.43 µg/mL, respectively. In the aqueous extracts, *C. mitis* (142.13 ± 2.37 µg/mL) shown greater effectiveness than *P. indica* (155.46 ± 4.92 µg/mL), followed by *P. sarmentosum* (212.85 ± 2.27 µg/mL), and *Z. mays* (242.24 ± 1.37 µg/mL).

The ABTS radical scavenging activity of ethanol extracts from the Thai herbal formulation demonstrated an IC50 of 39.17 ± 0.01 µg/mL, which was lower than the IC_{50} value of 67.38 ± 0.03 µg/mL for aqueous extracts. For the medicinal plant extracts, the results indicated that both ethanol and aqueous extracts of *L. speciosa* (12.78 ± 0.01 and 10.84 ± 0.01 µg/mL) demonstrated higher antioxidant capacities relative to ascorbic acid (2.83 ± 0.02 µg/mL). Moreover, the ethanol extract of *C. mitis* presented more potency than *P. indica*, followed by *P. sarmentosum*, and *Z. mays*, with IC_{50} values of 18.33 ± 0.01, 36.47 ± 0.01, 111.79 ± 0.01, and 158.06 ± 0.01 µg/mL, respectively. In the aqueous extracts, *Z. mays* (64.55 ± 0.01 µg/mL) revealed stronger efficacy than *C. mitis* (77.81 ± 0.02 µg/mL), followed by *P. sarmentosum* (95.96 ± 0.01 µg/mL) and *P. indica* (99.07 ± 0.01 µg/mL).

The FRAP experiment demonstrated that the ethanol and aqueous extracts of Thai herbal formulation demonstrated ferric reduction effects comparable to the quercetin standard curve, measuring 57.14 ± 0.19 and 33.40 ± 0.30 mg/g dry extract, respectively. For the medicinal plant extracts, the results were presented that both the ethanol and aqueous extracts of *L. speciosa* exhibited the greatest FRAP values of 245.92 ± 1.37 and 210.91 ± 4.59 mg QE/g, respectively, followed by *C. mitis* with values of 131.74 ± 3.18 and 23.31 ± 0.60 mg QE/g, *P. indica* with values of 73.75 ± 0.85 and 15.92 ± 0.40 mg QE/g, *P. sarmentosum* with values of 32.32 ± 1.41 and 19.41 ± 1.19 mg QE/g, and *Z. mays* with values of 20.07 ± 0.98 and 16.82 ± 0.66 mg QE/g. Notably, the ethanol and aqueous extracts of all medicinal plants produced consistent the results in this investigation.

Table 5 Antioxidant properties of the individual herbs and Thai herbal formulation.

Agents	% yield (w/w)		Total phenolic (mg GAE/g)		Total flavonoid (mg QE/g)		DPPH (IC_{50}; µg/mL)		ABTS (IC_{50}; µg/mL)		FRAP (mg QE/g)	
	Ethanol	Water	Ethanol	Water	Ethanol	Water	Ethanol	Water	Ethanol	Water	Ethanol	Water
CM	30.38	25.56	172.22 ± 5.84^{b}	35.28 ± 0.18^{c}	26.16 ± 0.52^{c}	37.64 ± 0.24^{c}	22.11 ± 0.98^{b}	142.13 ± 2.37^{c}	18.33 ± 0.01^{a}	77.81 ± 0.02^{c}	131.74 ± 3.18^{b}	23.31 ± 0.60^{c}
LS	45.25	39.89	244.36 ± 9.90^{a}	199.40 ± 1.25^{a}	80.98 ± 0.01^{a}	63.09 ± 2.52^{a}	15.25 ± 0.83^{a}	19.02 ± 0.94^{a}	12.78 ± 0.01^{a}	10.84 ± 0.01^{a}	245.92 ± 1.37^{a}	210.91 ± 4.59^{a}
PS	19.50	14.15	33.55 ± 1.56^{e}	29.42 ± 0.37^{d}	21.37 ± 0.66^{c}	30.72 ± 1.18^{c}	116.26 ± 2.55^{d}	212.85 ± 2.27^{d}	111.79 ± 0.01^{c}	95.96 ± 0.01^{d}	32.32 ± 1.41^{e}	19.41 ± 1.19^{d}
PI	20.34	18.12	47.50 ± 1.89^{d}	29.31 ± 0.26^{d}	23.48 ± 4.20^{e}	19.51 ± 0.71^{e}	55.98 ± 1.15^{c}	155.46 ± 4.92^{c}	36.47 ± 0.01^{b}	99.07 ± 0.01^{d}	73.75 ± 0.85^{c}	15.92 ± 0.40^{e}
ZM	10.91	8.12	28.63 ± 0.43^{f}	36.44 ± 0.85^{c}	29.25 ± 0.53^{c}	23.70 ± 0.28^{d}	273.33 ± 1.43^{e}	242.24 ± 1.37^{e}	158.06 ± 0.01^{d}	64.55 ± 0.01^{b}	20.07 ± 0.98^{f}	16.82 ± 0.66^{e}
THF	29.90	27.84	70.47 ± 0.58^{c}	57.48 ± 0.56^{b}	70.98 ± 7.26^{b}	51.51 ± 1.43^{b}	57.67 ± 3.47^{c}	94.63 ± 1.68^{b}	39.17 ± 0.01^{b}	67.38 ± 0.03^{b}	57.14 ± 0.19^{d}	33.40 ± 0.30^{b}
AC	NA		NA		NA		6.61 ± 0.01		2.83 ± 0.02		NA	

DPPH, DPPH radical scavenging assay; ABTS, ABTS radical scavenging assay; FRAP, Ferric reducing antioxidant power; CM, *Caryota mitis*; LS, *Lagerstroemia speciosa*; PS, *Piper sarmentosum*; PI, *Pluchea indica*; ZM, *Zea mays*; THF, Thai herbal formulation; AC, Ascorbic acid; NA, Not applicable. Ascorbic acid was employed as a positive control for DPPH and ABTS radical scavenging activities. $^{a\text{-}e}$ Values in the same column with different superscripts are significantly different ($p < 0.05$; Tukey).

α-amylase and α-glucosidase inhibitory activities of Thai herbal formulation

In vitro α-amylase and α-glucosidase inhibitory activities of the herbal components and Thai herbal formulation extract were demonstrated in **Table 6** and **Figure 4**. The aqueous extracts of the Thai herbal formulation greatly inhibited both enzymes, exhibiting IC_{50} values of 9.06 ± 2.14 μg/mL for α-amylase and 16.85 ± 3.81 μg/mL for α-glucosidase. The ethanol extracts of the Thai herbal formulation demonstrated α-amylase inhibitory activity with an IC_{50} value of 14.97 ± 0.54 μg/mL and α-glucosidase inhibitory activity with an IC_{50} value of 24.34 ± 0.77 μg/mL. Acarbose was employed as a standard reference medicine, demonstrating α-amylase inhibitory action with an IC_{50} value of 6.71 ± 0.52 μg/mL and α-glucosidase inhibitory activity with an IC_{50} value of 3.71 ± 0.64 μg/mL. Among all, the aqueous and ethanol extracts of *L. speciosa* exhibited the highest α-amylase activity, with IC_{50} values of 5.64 ± 1.59 and 9.87 ± 1.54 μg/mL, respectively, while α-glucosidase inhibitory activities, presenting IC_{50} values of 10.29 ± 2.70 and 19.80 ± 0.86 μg/mL, respectively. The aqueous and ethanol extracts of *C. mitis* had moderate inhibitory effects on α-amylase, with IC_{50} values of 20.39 ± 1.67 μg/mL and 26.10 ± 2.28 μg/mL, respectively, and on α-glucosidase, with IC_{50} values of 32.03 ± 0.40 μg/mL and 29.70 ± 2.79 μg/mL, respectively. Additionally, the aqueous and ethanol extracts of *Z. mays* showed moderate inhibitory effects on α-amylase, with IC_{50} values of 64.54 ± 0.47 μg/mL and 25.40 ± 2.24 μg/mL, respectively, and revealed mild effects on α-glucosidase, with IC_{50} values of 123.89 ± 1.66 μg/mL and 106.26 ± 0.79 μg/mL, respectively. Conversely, other medicinal plants including *P. sarmentosum* and *P. indica* possessed little effects on both α-amylase and α-glucosidase enzymes.

Table 6 α-amylase and α-glucosidase inhibitory activities of Thai herbal formulation and herbal components.

Agents	α-amylase inhibitory activity ($IC_{50;}$ μg/mL)		α-glucosidase inhibitory activity ($IC_{50;}$ μg/mL)	
	Ethanol	Water	Ethanol	Water
CM	26.10 ± 2.28^{c}	20.39 ± 1.67^{b}	29.70 ± 2.79^{b}	32.03 ± 0.40^{b}
LS	9.87 ± 1.54^{a}	5.64 ± 1.59^{a}	19.80 ± 0.86^{a}	10.29 ± 2.70^{a}
PS	109.58 ± 0.69^{d}	114.77 ± 1.99^{d}	105.46 ± 2.70^{c}	96.07 ± 0.55^{c}
PI	246.35 ± 0.91^{e}	210.24 ± 0.19^{e}	116.07 ± 2.74^{d}	133.05 ± 1.48^{e}
ZM	25.40 ± 2.24^{c}	64.54 ± 0.47^{c}	106.26 ± 0.79^{c}	123.89 ± 1.66^{d}
THF	14.97 ± 0.54^{b}	9.06 ± 2.14^{a}	24.34 ± 0.77^{b}	16.85 ± 3.81^{a}
AB	6.71 ± 0.52		3.71 ± 0.64	

CM, *Caryota mitis*; LS, *Lagerstroemia speciosa*; PS, *Piper sarmentosum*; PI, *Pluchea indica*; ZM, *Zea mays*; THF, Thai herbal formulation; THF, Thai herbal formulation; AB, Acarbose. $^{a\text{-}e}$ Values in the same column with different superscripts are significantly different ($p < 0.05$; Tukey). Acarbose was employed as a positive control for α-glucosidase and α-amylase inhibitory activities.

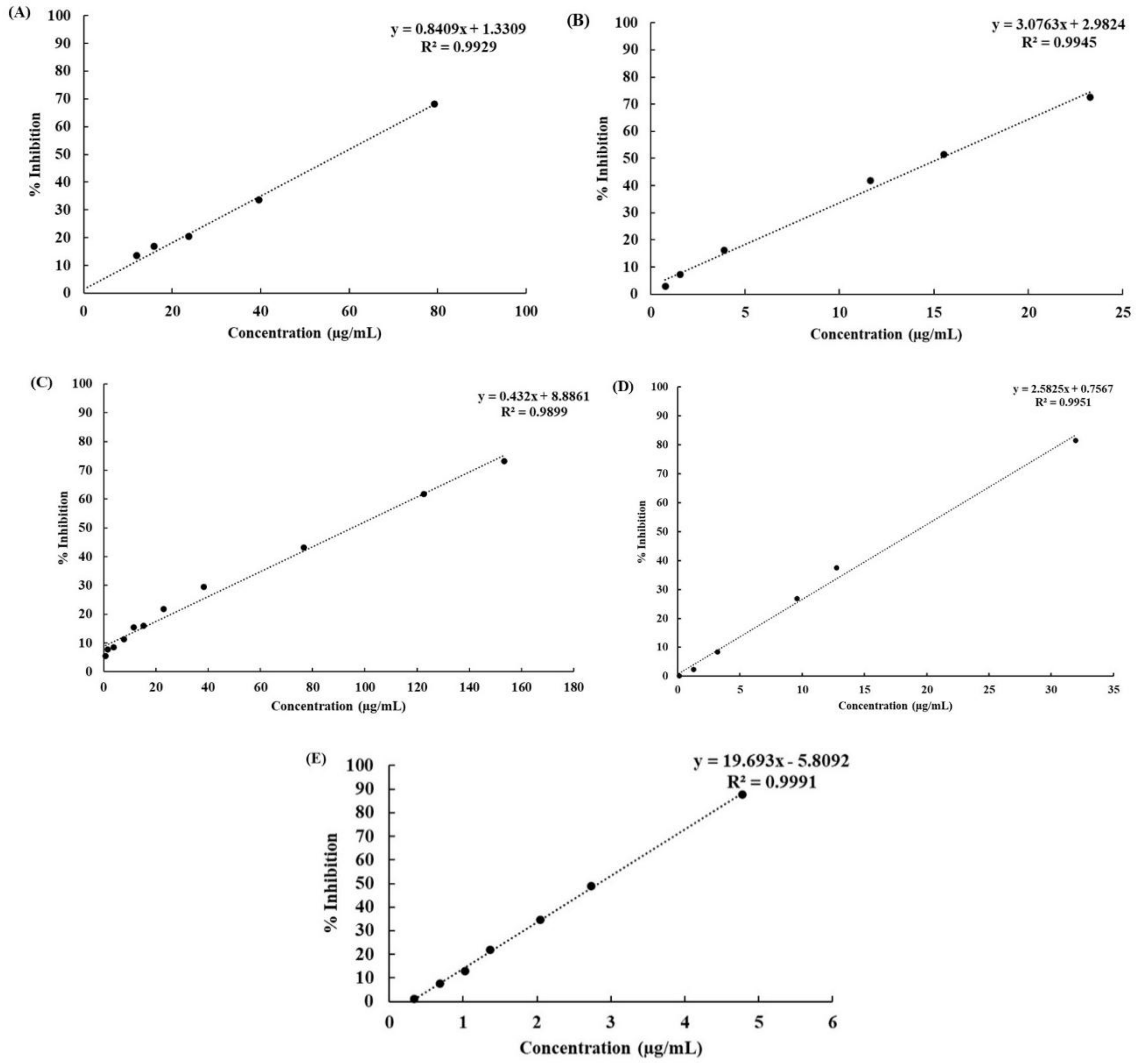


Figure 4 α-glucosidase and α-amylase inhibitory activity of Thai herbal formulation ethanol extract (A and B) and Thai herbal formulation aqueous extract (C and D) and Acarbose (E). *In vitro* α-amylase and α-glucosidase inhibitory activities of the herbal components and Thai herbal formulation extract were demonstrated. Acarbose was employed as a standard reference medicine.

Cytotoxic activity of Thai herbal formulation

The cytotoxic effect of herbal extracts was evaluated using the MTT assay using standard cell lines, namely L929 and Vero cells. The IC_{50} value is the concentration expected to inhibit 50% of cellular proliferation. The results demonstrated that the aqueous and ethanol extracts had no inhibitory effect on either cell line (IC_{50} values above 80 µg/mL). Doxorubicin was used as a positive control, exhibiting an IC_{50} of 4.16 µM in the Vero cell line and an IC_{50} of 1.17 µM in the L929 cell line.

Clinical efficacy and safety of Thai herbal formulation for patients with borderline diabetes

Quality evaluation of Thai herbal formulation

The evaluation of the exterior features showed no damage to the surface of the herbal capsules. The weight variation analysis of herbal capsules revealed an average weight of 505.25 mg (USP 41-NF 36), within an acceptable range of 456.71 to 556.21 mg, and an average weight of 507.82 mg (THP 2018), within an acceptable range of 461.32 to 559.52 mg. The assessed placebo capsules indicated an average weight of 501.46 mg (USP 41-NF 36), ranging within an acceptable range of 451.12 to 550.67 mg, and an average weight of 503.82

mg (THP 2018), within an acceptable range of 448.32 to 553.56 mg. The disintegration test demonstrated that the Thai herbal formulation capsule dissolved in 14.86 minutes, whereas the placebo capsule dissolved in 14.20 min. Consequently, none of the analyzed capsules exceeded the acceptable limits, and both the weight variation and disintegration time conformed to the standards established in USP 41-NF 36 and THP 2018.

Study participants and demographic characteristics

According to the framework of the study, 40 participants achieved the inclusion criteria during the screening evaluation. At the end of the study, all participants completed the inquiry (**Figure 5**). No significant adverse events were seen throughout this trial.

The demographic characteristics of participants were presented in **Table 7**. In the Thai herbal formulation and placebo groups, there were 40 patients, including 9 females and 11 men in each group. At baseline, there were no significant differences in demographic characteristics and glucose profiles between the 2 groups ($p < 0.05$).

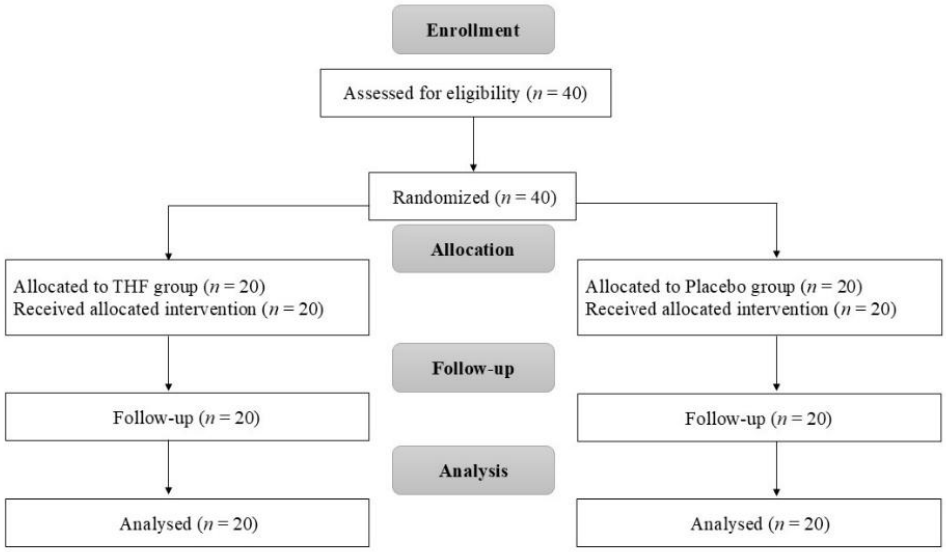


Figure 5 Flow chart of the study. According to the framework of the study, 40 participants achieved the inclusion criteria during the screening evaluation. At the end of the study, all participants completed the inquiry. No significant adverse events were seen throughout this trial.

Table 7 General characteristics and blood chemistry of participants in Thai herbal formulation and placebo groups.

Parameters	THF group (n = 20)	Placebo group (n = 20)	*p*-value
Gender[a]			
Male, n (%)	11 (55)	11 (55)	1
Female, n (%)	9 (45)	9 (45)	
Age (year)[b]	52.34 ± 5.24	52.79 ± 5.45	0.329
Family history of hyperlipidemia, n (%)[c]	4 (20)	3 (15)	1
Family history of hypertensive, n (%)[a]	8 (40)	7 (35)	1
Family history of Diabetes, n (%)[a]	14 (70)	15 (75)	1
Family history of CVD, n (%)[c]	3 (15)	4 (20)	1
Physical activity, n (%)[c]	5 (25)	4 (20)	1

Parameters	THF group (n = 20)	Placebo group (n = 20)	*p*-value
Consuming high-sugar foods, n (%)	-	-	-
Sugar (Dessert & Soft drink)[c]	15 (75)	16 (80)	1
Protein (Offal, Red meat and Seafood)[a]	11 (55)	10 (50)	1
Fat (Fried food & Grilled food)[a]	10 (50)	11 (55)	1
Carbohydrate (Snacks & Bread)[a]	11 (55)	12 (60)	1
Alcohol consumption every day, n (%)[c]	1 (5)	1 (5)	1
Smoking every day, n (%)	0 (0)	0 (0)	-
Weight (kg)[c]	59.86 ±0.76	60.18 ± 1.26	0.367
Height (cm)[d]	155.44 ± 8.53	158.90 ± 8.24	0.106
BMI (kg/m^2)[c]	24.29 ± 0.82	24.21 ± 0.73	0.590
Waist Circumference (cm)[c]	94.59 ± 12.89	93.34 ± 12.90	0.760
SBP (mmHg)[b]	122.55 ± 10.13	125.76 ± 6.86	0.594
DBP (mmHg)[b]	84.00 ± 5.02	84.50 ± 6.04	0.199
FBS (mg/dl)[c]	117.70 ± 4.79	119.40 ± 1.42	0.098
HbA1c (%)[c]	6.19 ± 0.10	6.29 ± 0.08	0.340
TC (mg/dl)[b]	207.10 ± 16.15	216.96 ± 16.07	0.902
TG (mg/dl)[b]	103.38 ± 7.73	109.00 ± 16.20	0.114
LDL-C (mg/dL)[c]	95.04 ± 6.15	101.60 ± 7.19	0.669
HDL-C (mg/dL)[c]	41.72 ± 3.94	46.18 ± 4.84	0.177
ALT (mg/dl)[b]	39.67 ± 1.05	39.77 ± 1.72	0.120
AST (mg/dl)[b]	25.63 ± 0.89	26.40 ± 1.50	0.112
BUN (mg/dl)[b]	11.99 ± 0.70	11.55 ± 0.83	0.408
CR (mg/dl)[b]	0.82 ± 0.07	0.65 ± 0.15	0.433
RBC[b]	4.73 ± 0.15	4.87 ± 0.33	0.262
WBC[b]	6.32 ± 0.43	6.64 ± 0.39	0.629
PLT[b]	253.37 ± 34.16	273.00 ± 13.40	0.747

Values are presented as Frequency (percentage) and mean ± SD. SD: standard deviation; IQR: interquartile range. [a]Chi-squared test of homogeneity, [b]Wilcoxon rank sum test, [c]Fisher's Exact Test, [d]Welch's t-test. Significant differences at $p < 0.05$. THF, Thai herbal formulation; SBP, Systolic blood pressure; DBP, Diastolic blood pressure; BMI, Body Mass Index; FBS, Fasting Blood Sugar; HbA1c, Hemoglobin A1c; TC, Total Cholesterol; TG, Triglyceride; LDL-C, low-density lipoprotein-cholesterol; HDL-C, high-density lipoprotein-cholesterol; ALT, Alanine transaminase; AST, Aspartate transaminase; BUN, Blood Urea Nitrogen; CR, Creatinine; RBC, Red blood cell; WBC, white blood cell; PLT, Platelet.

Efficacy and safety of Thai herbal formulation in borderline diabetes

The values of biochemical parameters in 2 groups at baseline and after 12 weeks of treatment were presented in **Table 8**. The results demonstrated that the Thai herbal formulation group had a significant decrease in fasting blood sugar (FBS) and hemoglobin A1C (HbA1c), together with a significant increase in high-density lipoprotein cholesterol (HDL) ($p < 0.05$), in comparison to the placebo group. In addition, neither group indicated any significant modifications in the blood levels of alanine transaminase, aspartate

aminotransferase, blood urea nitrogen, creatinine, and complete blood count throughout a 12-week period. The results indicated that the Thai herbal formulation was both effective and safe in lowering FBS and HbA1c **(Figure 6)**, resulting in exhibiting anti-diabetic characteristics in patients with borderline diabetes.

Table 8 Comparison of variables before and after the intervention in Thai herbal formulation and placebo groups.

Variables	THF group		Placebo group		*p*-value
	Before	After	Before	After	
SBP (mmHg)	122.55 ±10.13	121.62 ± 11.03	125.76 ± 6.86	127.26 ± 6.68	0.709
DBP (mmHg)	84.00 ± 5.02	83.00 ± 4.41	84.50 ± 6.04	80.45 ± 17.82	0.222
BMI	24.29 ± 0.82	23.98 ± 0.80	24.21 ± 0.73	24.13 ± 0.77	0.027
Weight (kg)	59.86 ± 0.76	59.41 ±1.68	60.18 ± 1.26	60.23 ± 0.28	0.208
FBS (mg/dL)	117.70 ± 4.79	92.95 ± 6.75	119.40 ± 1.42	120.27 ± 4.96	0.001*
HbA1c (%)	6.19 ± 0.10	5.11 ± 0.20	6.29 ± 0.08	6.33 ± 0.10	0.001*
TC (mg/dL)	207.10 ± 16.15	204.70 ± 19.95	216.96 ± 16.07	218.33 ± 12.43	0.600
TG (mg/dL)	103.38 ± 7.73	103.35 ± 6.47	109.00 ± 16.20	111.00 ± 15.70	0.080
HDL (mg/dL)	41.72 ± 3.94	50.72 ± 4.75	46.18 ± 4.84	46.21 ± 4.71	0.001*
LDL (mg/dL)	95.04 ± 6.15	94.60 ± 7.29	101.60 ± 7.19	101.34 ± 6.96	0.302
ALT (mg/dL)	39.67 ± 1.05	40.07 ± 1.35	39.77 ± 1.72	39.54 ± 2.05	0.625
AST (mg/dL)	25.63 ± 0.89	25.24 ± 1.24	26.40 ± 1.50	26.00 ± 1.44	0.604
BUN (mg/dL)	11.99 ± 0.70	12.18 ± 0.70	11.55 ± 0.83	11.56 ± 0.92	0.109
CR (mg/dL)	0.82 ± 0.07	0.77 ± 0.09	0.65 ± 0.16	0.64 ± 0.15	0.438
RBC	4.73 ± 0.15	4.64 ± 0.27	5.32 ± 0.57	5.18 ± 0.43	0.204
WBC	6.32 ± 0.43	6.23 ± 0.39	6.64 ± 0.39	6.52 ± 0.47	0.104
PLT	253.37 ± 34.16	248.64 ± 33.23	273.00 ± 13.40	272.00 ± 13.68	0.243

Values are mean ± SD. THF, Thai herbal formulation; SBP, systolic blood pressure; DBP, diastolic blood pressure; BMI, body Mass Index; FBS, fasting blood sugar; HbA1c, hemoglobin A1C; TC, total cholesterol; TG, triglyceride; LDL-C, Low-density lipoprotein cholesterol; HDL-C, High-density lipoprotein cholesterol; ALT, Alanine transaminase; AST, Aspartate transaminase; BUN, blood urea nitrogen; RBC, red blood cell; WBC, white blood cell; PLT, Platelet. * ignificant difference between the 2 groups by 2-way Mixed ANOVA by Bonferroni ($p < 0.05$).

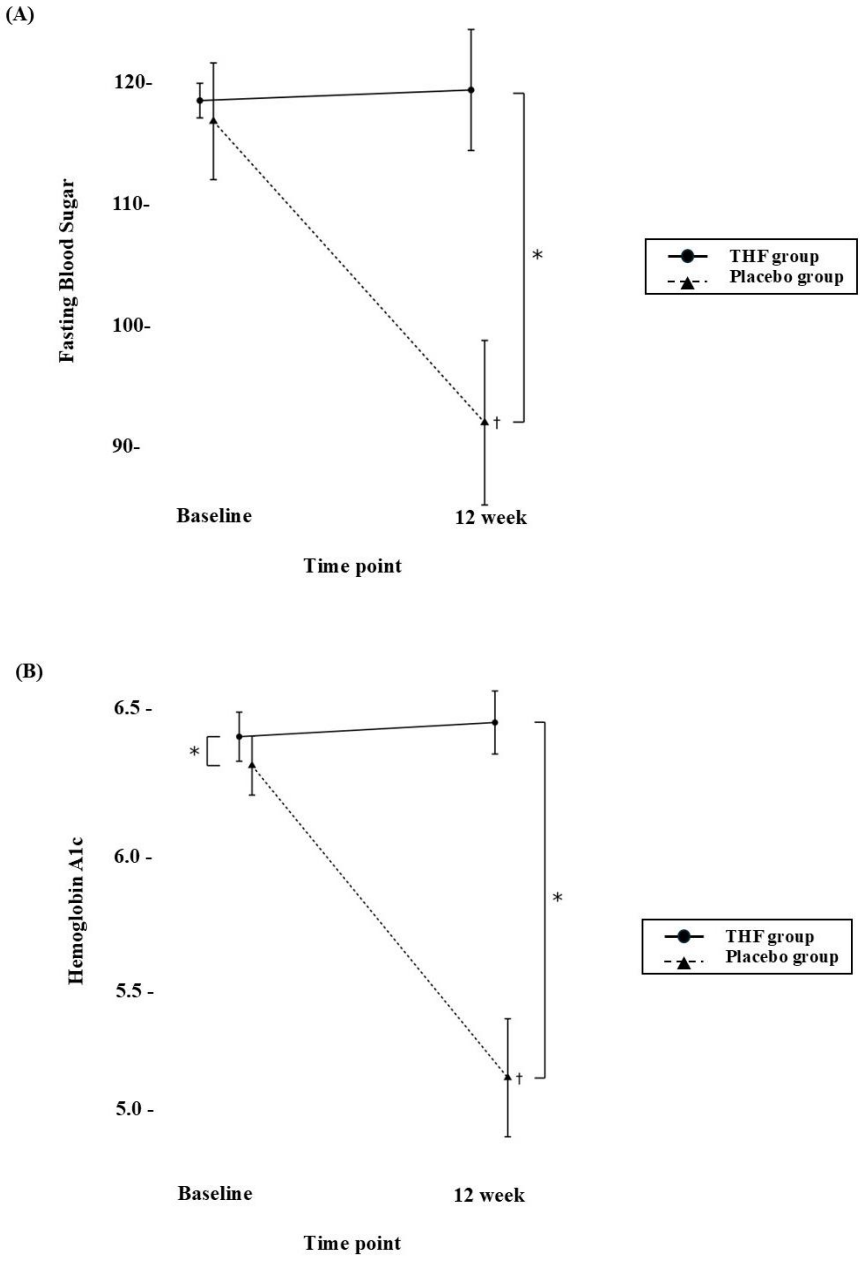


Figure 6 Trends in change in fasting blood sugar (A) and hemoglobin A1c (B) levels in participants before and after the intervention of the study. The results indicated that Thai herbal formulation was both effective and safe in lowering FBS and HbA1c, resulting in exhibiting anti-diabetic characteristics in patients with borderline diabetes.

Traditional Thai herbal therapy has been employed as an alternative and complementary method for diabetes management, simply because of its minimal adverse effects and therapeutic advantages [33,34]. Furthermore, multicomponent traditional Thai herbal medicine effectively addresses complicated diseases by targeting multiple pathways and biological systems due to its inherent variety and complexity [35]. A Thai herbal composition established in traditional Thai medicine, including *C. mitis*, *L. speciosa*, *P. sarmentosum*, *P. indica*, and *Z. mays*, has been recommended for patients with borderline diabetes (**Table 1**); nevertheless, there is a lack of scientific evidence to substantiate its therapeutic efficacy. Therefore, this study was to evaluate both the biological and clinical efficacy of a Thai herbal formulation in lowering blood glucose levels in patients with borderline diabetes.

Traditional Thai medicine employs a herbal formulation comprising *C. mitis*, *L. speciosa*, *P. sarmentosum*, *P. indica*, and *Z. mays*, which possesses properties that reduce blood sugar, nourish the body, avert hypoglycemia, stimulate pancreatic insulin secretion, restore liver function, and address urinary issues to enhance blood circulation and vitality, thereby regulating elemental balance. Furthermore, the chemical components included in 5 medicinal plants have been recognized for their beneficial health effects, namely as anti-diabetic, anti-hyperlipidemic, anti-oxidant, and anti-inflammatory compounds with potential therapeutic applications for diabetes (**Table 1**).

Prior investigations have shown that *L. speciosa* [12,13], *P. sarmentosum* [14,15], *P. indica* [16,17], and *Z. mays* [18,19] significantly reduced fasting blood glucose levels in *in vivo* research. Moreover, *L. speciosa* [12,13] and *P. indica* [16,17] also reduce fasting blood glucose and Hemoglobin A1c in clinical studies. Additionally, *L. speciosa* [36], *P. sarmentosum* [37], *P. indica* [38], and *Z. mays* [39] exhibited an elevation in HDL levels in previous studies. Consequently, the substantial elevation in HDL among the Thai herbal formulation group may result from the synergistic interactions among these plants. This research demonstrated that a Thai herbal formulation could regulate fasting blood glucose and Hemoglobin A1c levels with increasing HDL levels to improve diabetes management, mostly owing to the phenolic, flavonoid, tannin, alkaloid, and terpene components sourced from medicinal plants. As shown in **Tables 3** and **4** and **Figure 3**, the most important active constituents of the Thai herbal formulation for diabetes management included phenolic and flavonoid compounds, specifically quinic acid, gallic acid, chlorogenic acid, 5z-caffeoylquinic acid, esculetin, caffeic acid, ellagic acid, 1,3-dicaffeoylquinic acid, quercetin 3'-O-glucuronide, kaempferol, 3,4-dihydroxybenzoic acid, along with other bioactive components.

Plant-derived compounds have been investigated for the discovery and development of novel antidiabetic therapies [40-51]. In this study, antidiabetic action of phytochemicals compounds derived from a Thai herbal formulation, which encompasses several chemical classes, namely phenolics and flavonoids (quinic acid, gallic acid, chlorogenic acid, 5z-caffeoylquinic acid, esculetin, caffeic acid, ellagic acid, 1,3-dicaffeoylquinic acid, kaempferol, 3,4-dihydroxybenzoic acid, quercetin 3'-O-glucuronide), phenylpropanoids (curcumin) and others. These phytochemicals exhibited anti-diabetic properties that may facilitate hypoglycemic effects by inhibiting digestive enzymes such as alpha-amylase and alpha-glucosidase, emulating insulin action, reducing oxidative stress, displaying antihyperglycemic and hypolipidemic activities, inhibiting aldose reductase, and enhancing glucose uptake and insulin secretion [43,44].

Thai herbal formulation has been extensively studied as a treatment target for diabetes. The 6 mechanisms by which herbal medicines improve diabetic syndromes include enhanced insulin secretion and sensitivity, increased glucose uptake by muscle cells and adipose tissues, inhibition of glucose absorption from the intestine, reduction of glucose production by hepatocytes, and the demonstration of anti-inflammatory properties [45]. Thai herbal formulation may function as an anti-diabetic therapy, mostly because to its principal phenolics, flavonoids, and/or other components [46-51]. For example, gallic acid mitigated elevated glucose, insulin, inflammation, and oxidative stress in diabetes complications via stimulating radical generation, the GPX/GSH system, NO/iNOS production, AGE/ALE pathways, as well as JNK/ERK, GLUT4, and Nrf2 pathways [46]. Ellagic acid alleviated diabetes implications by decreasing inflammation, oxidative stress, hyperglycemia, apoptosis, insulin resistance, obesity, lipid profile, and histopathological alterations via the upregulation of Nrf2, HO-1, PPAR-γ, and protein kinase B, and downregulating NF-κB gene expression [47]. Maslinic acid decreased insulin resistance by inhibiting the peroxisome proliferator-activated receptor, α-amylase, α-glucosidase, glycogen phosphorylase, ghrelin, SGLT1, and GLUT2 genes, while also decreasing NF-κB, activating Nrf2, and regulating the AMPK/SIRT1 pathway [48]. Caffeic acid stimulated insulin secretion, inhibited α-amylase and β-glucosidase, obstructed sodium-dependent glucose transporter-1 from absorbing glucose in the intestine, and reduced hepatic glucose output [49]. Chlorogenic acid directly blocked G-6-Pase and reduced blood glucose via hepatic glycogenolysis and gluconeogenesis [50]. Curcumin enhanced insulin sensitivity, reduced glucose and insulin concentrations, increased adiponectin production, and decreased levels of leptin,

resistin, IL-6, IL-1β, and TNF-α in patients with Type 2 diabetes [51]. In conclusion, the oral administration of a Thai herbal formulation to patients with borderline diabetes significantly decreased fasting blood glucose and Hemoglobin A1c levels via potential anti-diabetic, anti-hyperlipidemic, anti-oxidative, and anti-inflammatory effects. Therefore, the Thai herbal formulation could have potential as an effective alternative therapy for diabetes management.

Conclusions

The findings demonstrated that the Thai herbal formulation including *C. mitis*, *L. speciosa*, *P. sarmentosum*, *P. indica*, and *Z. mays* had potential as a herbal combination therapy for diabetes management. The therapeutic advantages of the Thai herbal formulation may be attributed to phenolics and flavonoids as the major compounds in this formulation. However, further investigation is necessary to clarify the processes behind these findings.

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Ethics Approval and Consent to Participates

Ethical approval for a clinical study was obtained from the Human Research Ethics Committee of Prince of Songkla University, Songkhla Province, Thailand (reference number: HSc-HREC-64-042-1-3). The protocol was also registered in Thai Clinical Trials Registry (registration ID: TCTR20241031003).

Declaration of Generative AI in Scientific Writing

The authors acknowledge the use of QuillBot for language correction in the manuscript and confirm that the content was thoroughly reviewed and edited as needed. No generative AI or AI-assisted tools were employed for data analysis, interpretation, or generation of scientific content. The authors take full responsibility for the integrity and originality of the manuscript.

CRediT Author Statement

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