Pharmacogn Res. 2022; 14(1):89-99
A Multifaceted Journal in the field of Natural Products and Pharmacognosy
www.phcogres.com | www.phcog.net

Original Article

# Antidiabetic Activities of Medicinal Plants in Traditional Recipes and Candidate Antidiabetic Compounds from *Hydnophytum formicarum* Jack. Tubers

**Mingkwan Rachpirom[1,2], Louis R Barrows[3], Suriyan Thengyai[4], Chitchamai Ovatlarnporn[5], Chonlatid Sontimuang[6], Pitchanan Thiantongin[7], Panupong Puttarak[1,2,*]**

## ABSTRACT

**Introduction:** Wang-Nam-Yen hospital recipe used as a traditional antidiabetic for a long time, but its beneficial properties have not been described. **Materials and Methods:** Antidiabetic mechanisms including anti-α-glucosidase, anti-α-amylase, anti-dipeptidyl-peptidase-4, antioxidant, anti-inflammatory activities, and wound healing effects of 26 medicinal plants that make up the Wang-Nam-Yen preparation were investigated. **Results:** Interestingly, most plants in this study inhibited α-glucosidase and α-amylase at excellent levels with higher potency than standard acarbose (18.2% ± 0.5). *Hydnophytum formicarum*, *Urceola minutiflora*, and *Lagerstroemia speciosa*, inhibited DPP-4 with more than 70% inhibition at 50 µg/mL (82.8%± 0.8, 71.9% ± 0.3, and 71.1% ± 0.1, respectively compared with standard Diprotin A at 50 µg/mL, 90.1% ± 0.4). *Terminalia arjuna* showed the highest inhibition in all anti-oxidation assays. *Andrographis paniculata* inhibited NO production at 90.1% ± 2.4, which was more effective than indomethacin (34.3% ± 2.4). Most of the herbs contained high amounts of terpenoids and flavonoids, which might play an important role in antidiabetic activity. The results demonstrated that *H. formicarum* extract exhibited the highest anti-DPP-4 activity (IC50 = 33.87 ± 0.02 µg/mL). When *H. formicarum* ethanolic extract was isolated, Palmitic acid (1) exhibited DPP-4 inhibitory activity at IC50 value of 73.82 ± 2.64 µg/mL, and a mixture of stigmasterol (2) and ß-sitosterol (3) at 78.58 ± 0.92 µg/mL. **Conclusion:** Many herbs in the Wang-Nam-Yen preparation possessed properties predictive for antidiabetic treatment. The results also suggest the possibility of further use of *H. formicarum* and its isolated compounds as a standard diabetic drug in the future.

**Key words:** Anti-DPP-4 activity, Anti-α-glucosidase activity, Anti-α-amylase activity, antioxidant activity, Anti-inflammation activity, Antidiabetic recipe.

**Mingkwan Rachpirom[1,2], Louis R Barrows[3], Suriyan Thengyai[4], Chitchamai Ovatlarnporn[5], Chonlatid Sontimuang[6], Pitchanan Thiantongin[7], Panupong Puttarak[1,2,*]**

*[1]Department of Pharmacognosy and Pharmaceutical Botany, Faculty of Pharmaceutical Sciences, Prince of Songkla University, Hat-Yai, Songkhla, THAILAND.*
*[2]Phytomedicine and Pharmaceutical Biotechnology Excellence Center, Prince of Songkla University, Hat-Yai, Songkhla, THAILAND.*
*[3]Department of Pharmacology and Toxicology, College of Pharmacy, University of Utah, Salt Lake City, UT, USA.*
*[4]Drug and Cosmetics Excellence Center, Walailak University, Thasala, Nakhonsithammarat, THAILAND.*
*[5]Department of Pharmaceutical Chemistry, Faculty of Pharmaceutical Sciences, Prince of Songkla University, Hat-Yai, Songkhla, THAILAND.*
*[6]Department of Thai Massage and Midwifery, Faculty of Traditional Thai Medicine, Prince of Songkla University, Hat-Yai, Songkhla, THAILAND.*
*[7]Faculty of Thai Traditional and Alternative Medicine, Ubon-Ratchathani Rajabhat University, Meung, Ubon-Ratchathani, THAILAND.*

**Correspondence**

**Assoc. Prof. Dr. Panupong Puttarak**

Department of Pharmacognosy and Pharmaceutical Botany, Faculty of Pharmaceutical Sciences, Prince of Songkla University, Hat-Yai, Songkhla-90112, THAILAND.
Email id: panupong.p@psu.ac.th

**History**

- Submission Date: 31-10-2021;
- Review completed: 12-11-2021;
- Accepted Date: 21-12-2021

**DOI : 10.5530/pres.14.1.13**

**Article Available online**
http://www.phcogres.com



## INTRODUCTION

Diabetes mellitus (DM) is a public health syndrome in which up to 422 million adults worldwide have been diagnosed, and the number of DM patients is rising continuously. DM isa result of a disturbance in glucose metabolism and presents as an elevation of fasting blood sugar. The disease causes substantial morbidity, mortality, and long-term complications and leading to a significant risk factor for various chronic diseases such as cardiovascular disease.[1-4] In the early stages, DM does not distinctly affect the daily life of patients; however, long term elevations of blood glucose often leads to micro-and macro-vascular complications, including retinopathy, nephropathy, neuropathy, peripheral vascular disease, and cerebrovascular disease, all of which are associated with quality of life reduction.[1,5] Usually, the treatment of DM aims to maintain lower blood sugar levels and prevent those complications. Metformin is the first-line drug for mild to moderate type-2 DM (T2DM) as monotherapy or in combination with sulfonylurea and/or α-glucosidase inhibitor, and when unsatisfactory, a third agent, such as dipeptidyl-peptidase-4 (DPP-4), a thiazolidinedione, or glucagon-like peptide-1 (GLP-1) agonist is required.[2,6,7] α-glucosidase and α-amylase are digestive enzymes that play roles in breaking down the polysaccharide to monosaccharide. The inhibition of α-glucosidase and α-amylase has proven to be effective in lowering glucose uptake by reducing an absorbable monosaccharide.[2,8,9] Synthetic drugs or dietary supplements with their actions as α-glucosidase and α-amylase inhibitors have become alternatives in the treatment of DM.[6] Several α-glucosidase and α-amylase inhibitors are commercially available such as acarbose, voglibose, and miglitol.[9] Other than non-serious gastrointestinal side-effects,

**Cite this article:** Rachpirom M, Barrows LR, Thengyai S, Ovatlarnporn C, Sontimuang C, Thiantongin P, *et al.* Antidiabetic Activities of Medicinal Plants in Wang Nam Yen Hospital Recipes and Candidate Antidiabetic Compounds from *Hydnophytum formicarum* Jack. Tubers. Pharmacog Res. 2022;14(1):89-99.

these agents provide a helpful action in controlling DM. The incretin hormones, comprised of GLP-1 and glucose-dependent insulinotropic polypeptide (GIP, also known as a gastric inhibitory polypeptide), are secreted from L-cell and K-cell, respectively, in response to food ingestion. They regulate insulin release, improve insulin resistance, and lower gastric emptying time. Unfortunately, GLP-1 and GIP persist in the bloodstream for a very short time after secretion due to the degradation by DPP-4.[2,8,10] Therefore, inhibition of DPP-4 can prevent the hydrolysis of intact GLP-1 levels and has beenproven to be an effective mechanism in lowering blood sugar. Presently, DPP-4 is an attractive target for discovering additional oral antidiabetic drugs.

Moreover, it was reported that the antioxidant defense mechanisms in DM patients and in patients with DM-associated metabolic syndrome are attenuated and result in higher levels of oxidative stress in these individuals.[11,12] Increased oxidative stress is reported to promote the development and progression of DM and its complications.[11-13] The formation of advanced glycated end products from elevated glucose can promote free radicals and reactive oxygen species and worsen the disease.[11,12] The advanced glycated end products can activate the NF-kB transcription factor and increase nitric oxide level, contributing to the inflammatory condition.[14,15] Also, DM patients have been in trouble with wound complications from the impairment of microcirculation, immune function, collagen accumulation, the proliferation of keratinocytes, and fibroblast migration.[16]

Many Thai-traditional antidiabetic recipes are extensively accepted as alternatives for holistic care in DM patients to lower blood glucose levels, slowing the progress of DM complications, and even wound healing.[16-19] Although the acceptance by traditional doctors, patients, and routine usage over a decade reflects clinical effectiveness, scientific evidence that describes the biological activities associated with DM benefit of component plants is limited. This study aims to evaluate several antidiabetic activities of individual plant components of Wang Nam Yen recipes, including anti-α-glucosidase, anti-α-amylase, anti-DPP-4, antioxidant, anti- inflammatory, and skin proliferative activity. Twenty-six plants from the Thai-traditional recipe of Wang-Nam-Yen hospital, Thailand, were selected for the study. This preparation has been routinely used in the hospital. In addition, one plant that demonstrated exceptional anti- DPP-4 activity was extracted and fractionated, and pure compounds showing anti-DPP-4 activity were isolated using chromatographic methods. These bioactive compounds may be used as biomarkers for product quality assurance of Wang Nam Yen preparations in the future.

## MATERIALS AND METHODS

### Chemicals and reagents

DPP-4 enzyme from porcine kidney, trichloroacetic acid, Folin-Ciocalteu reagent, and 1,10- phenanthroline chloride monohydrate were obtained from Merck (Darmstadt, Germany). Indomethacin standard, gallic acid standard, quercetin standard, ascorbic acid standard, 2,2- diphenyl-1-picrylhydrazyl (DPPH), butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA), gly-pro-*p*-nitroanilide (GP-*p*NA) as *p*-toluene sulfonate salt, lipopolysaccharide (LPS) from *Escherichia coli* 0111:B4, RPMI-1640 medium, and 3-(4,5- dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) were from Sigma-Aldrich (MO, USA). Penicillin, streptomycin, fetal calf serum, trypsin–EDTA, and Dulbecco's Modified Eagle Medium (DMEM) from Thermo Fisher Scientific (CA, USA) were used for cell culture study. The solvents were purchased from RCI Labscan (Thailand). The mouse fibroblast L929 cells were obtained from the Chinese Academy of Preventive Medical Sciences, Beijing, China. Murine macrophage-like RAW264.7 cell-line (ATCC® TIB-71™) was kindly provided by the Medical Science Research and Innovation Institute, Prince of Songkla University.

### Plant materials and crude extracts preparation

The twenty-six medicinal plants used in this study are listed in Table 1. All plants were bought from local Thai-traditional drug stores in Songkhla, Thailand. Plant materials were authenticated by a botanist from the Department of Pharmacognosy and Pharmaceutical Botany, Faculty of Pharmaceutical Sciences, Prince of Songkla University. The plant specimens were also deposited, as shown in Supplement 1. After drying at 50 - 55°C, each dried plant was powdered with an electric grinder and stored the plant powder at -20°C until use. Each plant powder (30 g) was macerated in ethanol (~150 mL) at room temperature for two days. The macerated extract was dried under reduced pressure to yield the ethanolic crude extract. Each sample powder was refluxed in water over 90 min for aqueous extract preparation. After cooling, the extract was filtered through a filtering paper (Whatman® No. 1), and the filtrate was then freeze-dried to obtain the aqueous extract. All extracts were kept at - 20°C and protected from light until use.

### Phytochemicals analysis Determination of total phenolic content

The Folin-Ciocalteu method was used for determining total phenolic content.[20] A sample solution in MeOH (200 µL) and 0.25 N Folin-Ciocalteu reagent (1 mL) were mixed in a 2 mL Eppendorf tube using a vortex mixer. The mixture was allowed to react for 3 min and added 1 N $Na_2CO_3$ solution (0.8 mL). An aliquot of each mix (200 µL) was transferred into 96-well microplate and incubated at room temperature for 20 min. The absorbance of the incubated solution was determined at 765 nm using a microplate reader (Bio-Tec instruments, Inc., U.S.A). Total phenolic contents were reported as gallic acid equivalents (mg/g dry mass). Each sample was conducted in triplicate.

### Determination of total flavonoid content

The aluminum-chloride colorimetric method[21] was used to determine the total flavonoid content of all samples. Test sample solutions in MeOH (200 µL) were mixed with MeOH (600 µL), 10% w/v aluminum chloride (40 µL), 1 M potassium acetate (40 µL), and distilled water (1,120 µL). The mixture was left standing at room temperature for 30 min, and the absorbance measurement was then carried out at 415 nm using a microplate reader (Bio-Tec instruments, Inc., U.S.A). Total flavonoid contents were summarized as quercetin equivalents (mg/g dry mass). Each sample was conducted in triplicate.

### Determination of total anthocyanin content

The total anthocyanin content of all samples was analyzed by the pH differential method.[22] 500 µL of the test sample solution in MeOH was mixed with 3,500 µL of 25 mM KCl buffer solution pH 1. The mixture was left at room temperature for 15 min. Then the absorbance of the mixture was then measured at 510 and 700 nm. Following the same procedure, a test sample (500 µL) was added to 25 mM sodium acetate buffer solution pH 4.5 (3,500 µL), and the absorbance of the mixture was again measured at 510 and 700 nm after 15 min. Total anthocyanin values were calculated using the following equations (mg/L).

Total anthocyanin content (mg/L) = (A×MW×DF×1000) / (ε×C)Where A is absorbance of the sample calculated as:

A = (A515 ×A700) pH 1.0 - (A515 ×A700) pH 4.5,

MW= the molecular weight for cyanidin-3-glucoside = 449.2,DF = the dilution factor of the samples,

**Table 1: The list of 26 Thai medicinal plants used in this study.**

| Abbreviation | Scientific name | Part of uses |
|---|---|---|
| AHW | *Abutilon hirtum* (Lam.) | Whole plant |
| AEW | *Acanthus ebracteatus* Vahl. | Whole plant |
| AMH | *Albizia myriophylla* Benth. | Heartwood |
| APL | *Andrographis paniculata* (Burm. f.) Wall. Ex Nees. | Leaves |
| CMH | *Capparis micracantha* DC. | Heartwood |
| CMT | *Caryota mitis* Lour. | Tubers |
| CRR | *Cyperus rotundus* L. | Roots |
| HPR | *Harrisonia perforata* (Blanco) Merr. | Roots |
| HAS | *Homalomena aromatic* Schott. | Stem |
| HFT | *Hydnophytum formicarum* Jack. | Tubers |
| ICR | *Imperata cylindrica* (L.) P. Beauv. | Roots |
| LSL | *Lagerstroemia speciosa* (L.) Pers. | Leaves |
| OAW | *Orthosiphon aristatus* (Blume) Miq. | Whole plant |
| POS | *Pandanus odoratissimus* L. f. | Stem |
| RNW | *Rhinacanthus nasutus* (L) Kurz. | Whole plant |
| SCH | *Salacia chinensis* L. | Heartwood |
| SCR | *Smilax corbularia* Kunth | Rhizome |
| SGR | *Smilax glabra* Wall. Ex Roxb. | Rhizome |
| SIF | *Solanum indicum* L. | Fruits |
| TAF | *Terminalia arjuna* (Roxb.) Wight & Arn. | Fruits |
| TBF | *Terminalia bellirica* (Gaertn.) Roxb. | Fruits |
| TCF | *Terminalia chebula* Retz. var. chebula. | Fruits |
| TCV | *Tinospora cripa* (L.) Miers ex Hook.f & Thomson. | Vines |
| TTW | *Tribulus terrestris* L. | Whole plant |
| UMV | *Urceola minutiflora* (Pierre) D.J. Middleton. | Vines |
| URV | *Urceola rosea* (Hook. &Arn.) D.J. Middleton. | Vines |

ε = the molar absorptivity of cyanidin-3-glucoside = 26,900, andC = the concentration of sample buffer in mg/mL

The total anthocyanin content was reported as mg of cyanidin-3-glucoside equivalents (c-3- gE) for 100 g of sample. Each sample was conducted in triplicate.

## Determination of total alkaloid contents

Total alkaloid content was determined by using bromocresol green (BCG) reagent to form a yellow-color product.[23] The sample (1,000 µL, 2.5 mg/mL in 2 N HCl) was transferred to a 50 mL separatory funnel and washed with 10 mL of chloroform three times, and the pH was adjusted to neutral by 0.1 N NaOH. Then 5 mL of BCG solution (BCG was prepared by warming BCG (69.8 mg) in 3 mL of 2 N NaOH and diluted to 1,000 mL with distilled water) and 5 mL of phosphate buffer solution (pH 4.7) were mixed. Then the solution was extracted with 3 mL chloroform three times by vigorous shaking. Then, the volume was adjusted to 10 mL with chloroform by the volumetric flask. The absorbance of the solution was then measured at 470 nm with a microplate reader (Bio-Tec instruments, Inc., U.S.A). Total alkaloid values were reported as atropine equivalents (mg/g dry mass). Each sample was conducted intriplicate.

## Determination of total terpenoid content

Total terpenoid content was determined by using linalool as a positive control.[24] The sample or linalool solution (200 µL) and 1.5 mL of chloroform were combined in a 2 mL Eppendorf tube and mixed well using a vortex mixer. The mixture was allowed to stand for 3 min and 100 µL of conc. $H_2SO_4$ was then added to each tube. The mixture was placed in the dark at room temperature for 120 min (90 min for linalool). MeOH (1.5 mL) was then added and mixed using a vortex mixer. The absorbance of the final solution was measured at 538 nm (Bio- Tec instruments, Inc., USA). The total terpenoid content was reported as linalool equivalents (mg/g dry mass). Each sample was conducted in triplicate.

## Anti-α-amylase activity

The α-amylase inhibitory activity method was determined using the colorimetric method.[25,26] Briefly, 2 mg of starch azure was dissolved by boiling in 0.2 mL of 0.05 M Tris-HCl buffer (pH 6.9) containing 0.01 M $CaCl_2$ at 100°C for 10 min and then cooled down to 37°C. The sample (2 mg) was dissolved in a mixture of 1 mL of DMSO and 0.1 mL of porcine pancreas α-amylase (1.6 unit/mL) in 20 mM phosphate buffer (pH 6.9) containing 6.7 mM NaCl solution. The reaction was initiated by adding the sample solutions into a starch azure solution. After incubation at 37°C for 10 min, the chemical reaction was quenched by adding 0.5 mL of 50% acetic acid, and the mixture was centrifuged at 3000 rpm, 4°C for 5 min. The absorbance of the supernatant at wavelength 595 nm was monitored on the microplate reader (DTX 880, Multimode Detector, Beckman Coulter, Inc., Austria). Acarbose was used as a positive standard. Each sample and positive control at the same concentration (25 mg/mL) were screened to establish the % inhibition. All test samples were conducted in triplicate. The % inhibition was calculated according to the following equation;

$$\% \text{ Inhibition } = [(A_{control} - A_{sample}) / A_{control}] \times 100$$

Where $A_{control}$ = the absorbance of blank
$A_{sample}$ = the absorbance of the sample

## Anti α-glucosidase activity

The method of Kumar *et al*.[27] was used to evaluate α-glucosidase inhibitory activity. The sample solution in DMSO (50 µL) was mixed with 50 µL of α-glucosidase enzyme solution (0.57 unit/mL in 50 mM phosphate buffer, pH 6.9). After incubation at 37°C for 10 min, 50 µL of 5 mM *p*-nitrophenyl-α-*D*-glucopyranoside in phosphate buffer, pH 6.9, was added, and the incubation continued at 37°C for 20 min. The reaction was quenched by adding 50 µL of 1 M $Na_2CO_3$ solution. The absorbance of the final solution was measured at a wavelength of 405 nm (DTX 880, Multimode Detector, Beckman Coulter, Inc., Austria). Acarbose was used as a positive standard. Each sample and positive control at the same concentration (25 mg/mL) were screened to establish the % inhibition. All test samples were conducted in triplicate. The % inhibition was calculated according to the following equation;

$$\% \text{ Inhibition } = [(A_{control} - A_{sample}) / A_{control}] \times 100$$

Where $A_{control}$ = the absorbance of blank (without sample)
$A_{sample}$ = the absorbance of the sample

## Anti DPP-4 activity

In this study, the method of Rachipirom *et al*.[18] was used to determine anti-DPP-4 activity. Briefly, each sample was dissolved in 50 mM Tris-HCl buffer (pH 7.5) to a final concentration of 50 µg/mL. The sample

solution (40 µL) was mixed with 20 µL of DPP-4 enzyme (0.05 U/mL) in a 96-well plate. The 0.2 mM GP-pNA in Tris-HCl (100 µL) was added after incubation for 10 mins at 37°C. The mixture was placed at 37°C in the incubator for 30 min. Then, the reaction was terminated with 30 µL of 25% glacial acetic acid. The absorbance at wavelength 405 nm of the resulting yellow solution was measured on the microplate reader (DTX 880, Multimode Detector, Beckman Coulter, Inc., Austria). Diprotin A was used as a positive standard. The testing was conducted in triplicate for each sample. The % inhibition was calculated according to the following equation;

$$\% \text{ Inhibition} = [(A_{control} - A_{sample}) / A_{control}] \times 100$$

Where $A_{control}$ = the absorbance of blank
$A_{sample}$ = the absorbance of the sample

## Antioxidant activity

### DPPH radical scavenging assay

DPPH radical scavenging activity was determined by the method of Brand-Williams *et al*.[28] Briefly, DPPH solution at conc. 24% w/v (170 µL) was mixed with the test sample in MeOH (30 µL). The mixture was allowed to stand at room temperature for 30 min, avoiding light. The absorbance measurement at wavelength 515 nm was executed using MeOH as a blank and ascorbic acid as a positive control. Each sample was conducted in triplicate. The % inhibition was calculated according to the following equation;

$$\% \text{ Inhibition} = [(A_{control} - A_{sample}) / A_{control}] \times 100$$

Where $A_{control}$ = the absorbance of blank (without sample)
$A_{sample}$ = the absorbance of the sample

### Ferric reducing antioxidant power (FRAP)

The ferric reducing antioxidant power was determined the potassium ferricyanide method.[29]The 200 µL of 200 µg/mL extract was mixed with 500 µL phosphate buffer (0.2 M, pH 6.6) and 500 µL 1% w/v potassium ferricyanide. After incubation at 50°C for 30 min, 500 µL of 10% w/v trichloroacetic acid was added, and the mixture was then centrifuged at 3000 rpm for 30 min. The supernatant solution (600 µL) was mixed with 600 µL of distilled water and 120 µL 0.1% w/v ferric chloride. The absorbance of the final mixture was measured at a wavelength of 700 nm. The antioxidant activity was reported as quercetin equivalents (mg/g dry mass).

### Hydroxyl radical scavenging activity

The hydroxyl radical scavenging activity (HRSA) was modified from the method of Tian *et al*.[30] Briefly, the 300 µL of 1.87 mM 1,10-phenanthroline solution, 600 µL of 0.2 M phosphate buffer saline pH 7.4, and 300 µL of 30 µg/mL sample solution were mixed homogenously. The 300 µL of ferrous (II) sulfate solution (1.87mM) was then pipetted into the mixture. The reaction was initiated by adding 300 µL of 0.03% v/v $H_2O_2$. After incubation at 37°C for 60 min, the absorbance of the reaction mixture was measured at wavelength 536 nm. Each sample was conducted in triplicate. The percentage of HRSA was calculated by the following equation;

$$\% \text{ HRSA} = [(A_S - A_n) / (A_b - A_n)] \times 100$$

Where $A_b$ = the absorbances of blank,
$A_n$ = the absorbances of negative controls, and $A_s$ are the absorbances of the test sample

### Nitric oxide inhibitory activity

The inhibition of nitric oxide (NO) production in murine macrophage-like cell-line (RAW264.7) was evaluated by the method of Owolabi *et al*.[31,32] Cells were cultured in a culture flask at 37°C with a humidified atmosphere containing 5% $CO_2$ using RPMI medium as culture medium. The RPMI medium was supplemented with 0.1% $NaHCO_3$, 2 mM glutamine, penicillin G (100 units/mL), streptomycin (100 µg/mL), and 10% Fetal Bovine Serum (FBS). Cells ($1\times10^5$ cells/well) were seeded in 96-well plates and adhered to the bottom of the well in the incubator for 1 hr. Then, the medium was replaced with samples, and 25 µg/mL of lipopolysaccharide (LPS) and incubated for 24 hr. The supernatant (100 µL) was collected and reacted with Griess reagent (100 µL) to measure the nitrite accumulation in the supernatant. The reaction mixture was measured at wavelength 570 nm. Indomethacin was used as a standard control. Each sample was conducted in triplicate. The % inhibition was calculated based on the following equation.

$$\text{Inhibition (\%)} = [(A - B) / (A - C)] \times 100$$

Where $A$ = the absorbances of LPS solution,
$B$ = the absorbances of the sample with LPS, and
$C$ = the absorbances of the sample without LPS

### Cytotoxicity

Cytotoxicity was also determined using the MTT colorimetric method.[33] The cytotoxicity was used to determine the test samples in fibroblast (L929) proliferation activity. L929 cells ($5\times10^3$ cells/well) were seeded in 96-well plates and incubated for 24 hr. After that, the test samples were replaced in each well and incubated for 24 hr. Then the supernatant solution was removed and added 10 µL of MTT solution (5 mg/mL in PBS) then incubated for 2 hr. After that, the medium was removed and added DMSO to dissolve the formazan production in the cells. The formazan solution was measured with a microplate reader at 570 nm. The test samples were considered cytotoxic when the optical density of the sample-treated group was less than 80% of that in the control (vehicle-treated) group. While the percentage was more than 100% indicated that this sample could promote fibroblast proliferation.

## Evaluation of *Hydnophytum formicarum* Jack.

### Purification of DPP-4 inhibitory activities of HFT fractions

Dry powder of HFT was macerated with EtOH (950 g × 1.3 L) for 72 hr. The ethanolic extract was evaporated at 45°C by using a rotary vacuum evaporator. The crude ethanolic extract (25 g, % yield = 2.92%w/w) was dissolved in 10% methanol:$H_2O$ (3 L) to obtain a clear solution prior to fractionation by using a solvent partition method with hexane (2 L), ethyl acetate (EtOAc; 4 L), *n*-butanol (*n*-BuOH; 1.5 L), and water, respectively. Each partition fraction was evaporated by a rotary evaporator or freeze-drying. Finally, four fractions were obtained including HH (2.64 g, %yield = 9.52%w/w), HE (12.79 g, %yield = 46.13% w/w), HB (3.25 g, %yield = 11.75% w/w) and HW (7.27 g, %yield = 26.26% w/w), respectively.

Each fraction was analyzed for DPP-4 inhibitory activities, as the method described in section 2.6. The fraction that gave the highest DPP-4 inhibitory activity was further fractionated using bioassay-guided isolation of chromatographic peaks using silica gel, Sephadex LH-20, and Diaion HP-20 with various mobile phases such as hexane, EtOAc, MeOH, and $CHCl_3$. In the purification process using each chromatographic technique, fractions were collected. The purity of the fractions was determined by using silica gel TLC, detected by UV-lamp at 254 and 356 nm, and by spraying p-anisaldehyde- sulfuric acid solution.

The fractions that gave the same TLC patterns were combined, and the purity was checked again by TLC after combination.

### Structure elucidation

Spectroscopic methods including infrared (IR), Ultraviolet (UV), nuclear magnetic resonance (NMR), and mass spectra (MS) were used to characterize the chemical structures of the isolated compounds. Mass spectrometry and nuclear magnetic resonance spectroscopy ($^{1}H$ and $^{13}C$) were used to give structural information about the pure isolated compounds. The compounds isolated from *H. formicarum* tuber extracts were well-characterized, and their DPP-4 inhibitory activity was confirmed to find their $IC_{50}$ values.

### Statistical analysis

The $IC_{50}$ values were calculated from dose-response curves using Microsoft Excel. All results were shown as mean ± S.D. For statistical analysis, the data were statistically compared by one-way analysis of variance (ANOVA) at 95% significant level using SPSS (version 12) for windows. All bioassay experiments were performed in triplicate.

## RESULTS

### Phytochemical screening

Results on phytochemical screening of 26 selected plants on phenolic, flavonoid, terpenoid, anthocyanin, and alkaloid were indicated in Table 2. All plants contained detectable phenolic, flavonoid, and terpenoid components. *S. corbularia* (SCR), *T. arjuna* (TAF), and *H. formicarum* (HFT) showed the highest total phenolic content. *H. formicarum* (HFT) yielded the highest terpenoid content compared to the other plants.

### Anti-α-glucosidase activity

The inhibition of α-glucosidase activity of all extracts were illustrated in Figure 1. Most of all extracts exhibited very effective anti-α-glucosidase activity compared with the standard acarbose (18.59% ± 0.45 at 25 µg/mL). The range of anti-α-glucosidase activity in all tested samples was from 84.84% ± 0.56 to 4.46% ± 2.81 at a concentration of 25 µg/mL. The ethanolic extract showed inhibition (84.84% ± 0.56) that was 17 times higher than the aqueous extract of the recipe (4.46% ± 2.81). The most potent plants at inhibiting α-glucosidase (> 50%) were *S. chinensis* (SCH) (69.06% ± 0.78), *L. speciosa* (LSL) (67.05% ± 0.25), *U. minutiflora* (UMV) (63.53% ± 0.72), *I. cylindrica* (ICR) (61.74% ± 1.16), *H. formicarum* (HFT) (60.60% ± 1.49), and *S. glabra* (SGR) (59.91% ± 2.99).

### Anti-α-amylase activity

α-Amylase inhibitory activities of all extracts were shown in Figure 2. All tested samples at a concentration of 25 µg/mL inhibited α-amylase in different levels. The aqueous extract of the combined recipe exhibited slightly less inhibition (35.67% ± 1.89) than the ethanolic extract of the recipe (45.14% ± 0.45) and the same potency with acarbose (33.14% ± 0.79 at 25 µg/mL). More than 17 plants demonstrated effective inhibition (more than 50%). The plants that showed the highest inhibition were *O. aristatus* (OAW) (81.49% ± 12.80), *T. arjuna* (TAF) (79.61% ± 0.37), *C. mitis* (CMT) (78.10% ± 3.52), *C. rotundus* (CRR) (77.87% ± 4.71), and *T. terrestris* (TTW) (77.52% ± 1.57).

### Anti- DPP-4 activity

All samples showed DPP-4 inhibitory activity at a concentration of 50 µg/mL in the range of 9.81% - 82.75% (Figure 2). Diprotin A (as positive control) showed 90.07% inhibition at the same concentration. The aqueous extract of the recipe inhibited DPP-4 at moderate levels (41.43% ± 0.53), which was approximately two-fold higher than the ethanolic extract of the recipe (24.71% ± 0.50). For single plants,

**Table 2: Phytochemical screening of the 26 selected plants.**

| Plant | Phytochemical contents | | | | |
|---|---|---|---|---|---|
| | Phenolic[1] | Flavonoid[2] | Terpenoid[3] | Anthocyanin[4] | Alkaloid[5] |
| AHW | 22.54 ± 1.43 | 61.02 ± 0.27 | 1089.85 ± 1.59 | 9.83 ± 2.34 | ND |
| AEW | 54.25 ± 0.85 | 58.73 ± 0.61 | 901.94 ± 2.75 | 17.66 ± 4.03 | 8.31 ± 0.28 |
| AMH | 62.02 ± 1.72 | 70.99 ± 0.80 | 688.37 ± 1.59 | 5.31 ± 2.02 | ND |
| APL | 35.81 ± 1.56 | 82.95 ± 0.38 | 2346.51 ± 1.59 | 12.95 ± 2.66 | 1.59 ± 0.07 |
| CMH | 166.69 ± 2.18 | 31.59 ± 0.51 | 864.36 ± 4.20 | 10.22 ± 1.88 | 0.41 ± 0.05 |
| CMT | 34.20 ± 0.66 | 28.71 ± 0.70 | 967.94 ± 2.75 | 5.12 ± 2.87 | ND |
| CRR | 101.92 ± 1.82 | 67.97 ± 0.83 | 1235.59 ± 4.20 | 13.56 ± 1.14 | ND |
| HPR | 132.95 ± 1.03 | 66.87 ± 1.42 | 1613.23± 1.59 | 5.75 ± 3.96 | ND |
| HAS | 21.79 ± 1.19 | 58.49 ± 0.93 | 578.38 ± 1.59 | 19.69 ± 6.10 | ND |
| HFT | 246.56 ± 1.24 | 15.21 ± 0.22 | 9413.56 ± 1.59 | 10.83 ± 5.10 | ND |
| ICR | 14.25 ± 1.03 | 61.56 ± 0.22 | 1381.33 ± 1.59 | 4.09 ± 0.35 | ND |
| LSL | 211.83 ± 2.60 | 65.39 ± 0.41 | 2157.69 ± 1.59 | 15.64 ± 8.55 | 6.00 ± 0.22 |
| OAW | 93.96 ± 1.61 | 52.33 ± 0.81 | 1384.99 ± 1.59 | 23.55 ± 5.83 | ND |
| POS | 22.34 ± 0.38 | 11.94 ± 0.67 | 398.72 ± 2.75 | 13.27 ± 4.15 | 0.04 ± 0.05 |
| RNW | 34.17 ± 0.54 | 50.99 ± 0.44 | 1835.96 ± 1.59 | 23.69 ± 5.84 | 0.49 ± 0.06 |
| SCH | 143.10 ± 2.53 | 7.17 ± 0.48 | 2411.59 ± 2.75 | 6.86 ± 3.24 | ND |
| SCR | 388.22 ± 1.92 | 33.72 ± 1.18 | 1176.01 ± 1.59 | 3.04 ± 0.75 | ND |
| SGR | 30.83 ± 1.18 | 21.12 ± 0.54 | 1690.22 ± 4.20 | 17.04 ± 5.39 | ND |
| SIF | 6.26 ± 0.42 | 75.91 ± 1.61 | 945.94 ± 2.75 | 29.46 ± 7.68 | ND |
| TAF | 383.37 ± 4.45 | 76.85 ± 1.71 | 833.20 ± 2.75 | 1.94 ± 0.54 | 0.17 ± 0.41 |
| TBF | 43.05 ± 2.36 | 27.87 ± 0.62 | 1187.92 ± 2.75 | 21.21 ± 3.56 | ND |
| TCF | 152.44 ± 3.52 | 14.37 ± 0.11 | 832.28 ± 1.59 | 12.09 ± 4.62 | ND |
| TCV | 51.08 ± 0.94 | 38.98 ± 0.80 | 925.77 ± 1.59 | 22.14 ± 3.21 | 0.14 ± 0.04 |
| TTW | 29.71 ± 1.85 | 32.43 ± 0.86 | 1493.15± 2.75 | ND | ND |
| UMV | 340.65 ± 6.74 | 30.99 ± 0.69 | 2173.27 ± 4.20 | 10.85 ± 0.64 | ND |
| URV | 95.93 ± 2.81 | 6.58 ± 0.27 | 1386.83 ± 4.20 | 10.04 ± 2.64 | ND |

The values represent as mean ± S.D. (n = 4). ND = not detect
[1] Gallic acid equivalents (µg/g dry mass).
[2] Quercetin equivalents (mg/g dry mass).
[3] Linalool equivalents (g/g dry mass).
[4] Cyanidin-3-glucoside equivalents (mg) for 100 g of sample.
[5] Atropine equivalents (mg/g dry mass).

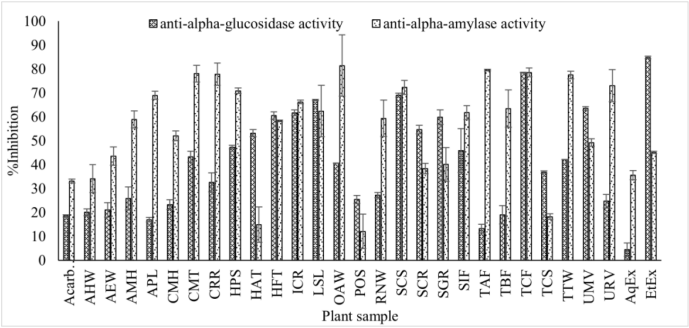



**Figure 1:** Anti-α-glucosidase, and anti-α-amylase activities of the extracts from 26 selected plants in this study. The % inhibition was observed at the concentration 25 µg/mL for anti-α-glucosidase and anti-α-amylase activities are shown. Sample codes are referred to Table 1. AqEx and EtEx are the aqueous and ethanolic extracts of the recipe. The acarbose and sample were chosen the concentration at 25 µg/mL for compare the activity in the same concentration.

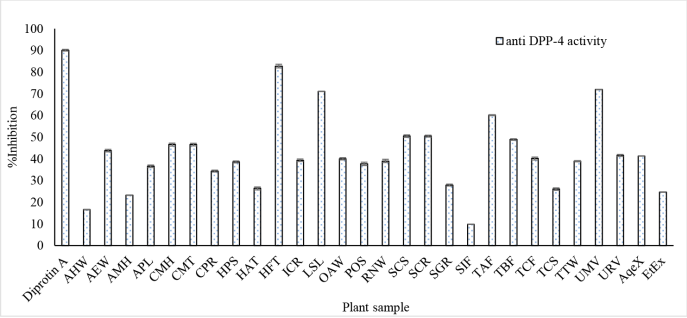



**Figure 2:** Anti-DPP-4 activities of the extracts from 26 selected plants in this study. The % inhibition was determined at the concentration of 50 µg/mL. Positive control diprotiin A was tested at the concentration of 50 µg/mL.[18] Sample codes are referred to Table 1. AqEx and EtEx are the aqueous and ethanolic extracts of the recipe.

*H. formicarum* (HFT) exhibited the most DPP-4 inhibitory activity with the inhibition 82.75% ± 0.83 was displayed by other plants that exhibited DPP-4 inhibition at a high level (> 50%) included *U. minutiflora* (UMV) (71.89% ± 0.34), *L. speciose* (LSL) (71.07% ± 0.07), and *T. arjuna* (TAF) (60.11% ± 0.22).

## Antioxidant activity

DPPH radical scavenging assay, ferric reducing anti-oxidation power assay, and hydroxyl radical scavenging assays were performed to investigate the inhibition of different kinds of reactive radical species. The antioxidant activity results were shown in Table 3. *T. arjuna* (TAF), *U. minutiflora* (UMV), T. *bellirica* (TBF), *C. micracantha* (CMH), and *S. corbularia* (SCR) showed good scavenging DPPH radicals with $IC_{50}$ 3.77, 6.42, 7.02, 9.17 and 9.24 µg/mL, respectively, compared with the standards ascorbic acid and BHT (4.28 and 4.82 µg/mL, respectively). Only TAF could neutralize DPPH radical equipotent to ascorbic acid and BHT. In FRAP assay, *T. arjuna*, *L. speciosa, T. bellirica, C. micracantha,* and *U. minutiflora* exhibited ferric-reducing effects equivalent to quercetin standard curve, 136, 629, 594, 448, and 444 mg/g dry mass, respectively. Meanwhile, *T. arjuna, S. chinensis, A. ebracteatus, C. micracantha, and A. hirtum* indicated a hydroxyl radical formation quite a bit better than the standard quercetin with percent inhibitions of 50.86, 34.94, 32.27, 27.36, and 25.28, respectively. Overall, *T. arjuna* very effectively inhibited all reactive species assessed in this study.

## Anti-inflammatory activity

The ability to reduce nitric oxide production from RAW264.7 cells was used to assess inhibition of the inflammation process (Table 4). Due to their toxicity to RAW264.7 (data of % viability of RAW264.7 was showed in Table 4), the nitric oxide inhibition of *A. myriophylla* and *T. terrestris* could not be compared with others. *A. paniculata*, *A. hirtum*, *R. nasutus*, *A. ebracteatus*, and *H. perforata* effectively inhibited NO production with percent inhibition of 90.1% ± 2.4, 73.5% ± 2.8, 61.9% ± 1.9, 60.8% ± 3.6, and 54.0% ± 1.1, respectively. All of which were higher than the standard indomethacin (34.3% ± 2.4) at the concentration of 50 µg/mL. The ethanolic extract of the recipe also showed satisfactory activity (44.1% ± 2.3), slightly more potent than indomethacin.

## Cytotoxicity

The cell proliferation and viability of L929 fibroblast cells were performed with all plants using the concentration at 0, 3, 10, 30, 100 µg/mL as shown in Table 5.[33] *U. minutiflora, T. terrestris*, *A. hirtum*, *A. myriophylla*, *S. chinensis*, and *T. arjuna* can promote the L292 cell proliferation at all concentrations (more than 100%), whereas *A. ebracteatus* can promote cell proliferation only at low concentrations (3 µg/mL). Most of the samples showed lower % cell proliferation when treated with a high concentration of the sample (100 µg/mL). All extracts were not toxic to the cells and classified as safe because the cell viability remained higher than 80% of the control after being tested with the sample.[34] The promotion of fibroblast proliferation of the sample could promote wound healing in the proliferation phase.[33]

**Table 3: The antioxidant activities of the twenty-six selected plants in this study. For DPPH assay the plant extracts and ascorbic acid tested at concentration of 0.15-75 µg/mL (Brand-Williams et al., 1995). For FRAP assay the plant extracts tested at 200 µg/mL compared with the standard curve of quercetin (Yıldırım *et al*., 2001). For HRSA assay the plant extracts and positive control tested at concentration of 30 µg/mL (Tian *et al*., 2009).**

| Plants | DPPH[1] | FRAP[2] | HRSA[3] |
|---|---|---|---|
| Ascorbic acid | 4.28 ± 0.02 | NT | NT |
| BHT | 4.82 ± 0.02 | NT | NT |
| Quercetin | NT | NT | 5.67 ± 6.05 |
| AHW | NA | 0.02 ± 0.02 | 25.28 ± 1.98 |
| AEW | 59.45 ± 1.79 | 0.03 ± 0.01 | 32.27 ± 3.22 |
| AMH | NA | 0.07 ± 0.01 | 20.59 ± 10.21 |
| APL | NA | NA | 3.27 ± 6.18 |
| CMH | 9.17 ± 0.68 | 0.45 ± 0.01 | 27.36 ± 7.79 |
| CMT | 109.21 ± 6.16 | 0.08 ± 0.01 | 7.14 ± 13.61 |
| CRR | 39.96 ± 0.59 | 0.13 ± 0.01 | 11.30 ± 2.52 |
| HPR | 20.33 ± 0.28 | 0.23 ± 0.07 | NA |
| HAS | NA | 0.09 ± 0.01 | 11.38 ± 2.51 |
| HFT | 10.06 ± 1.04 | 0.39 ± 0.01 | 14.05 ± 5.22 |
| ICR | NA | 0.01 ± 0.01 | NA |
| LSL | 10.25 ± 0.23 | 0.63 ± 0.01 | 17.47 ± 1.98 |
| OAW | 39.26 ± 1.21 | 0.21 ± 0.01 | NA |
| POS | NA | NA | NA |
| RNW | NA | 0.11 ± 0.01 | NA |
| SCH | 21.83 ± 0.40 | 0.13 ± 0.01 | 34.94 ± 12.48 |
| SCR | 9.24 ± 1.71 | 0.26 ± 0.01 | 4.61 ± 5.39 |
| SGR | 132.54 ± 3.43 | 0.02 ± 0.01 | NA |
| SIF | NA | 0.04 ± 0.01 | 10.48 ± 2.06 |
| TAF | 3.77 ± 0.17 | 1.14 ± 0.06 | 50.86 ± 8.21 |
| TBF | 7.02 ± 0.08 | 0.59 ± 0.04 | 25.80 ± 3.59 |
| TCF | 9.59 ± 0.40 | 0.33 ± 0.04 | 4.16 ± 3.34 |
| TCV | 106.46 ± 9.44 | NA | 0.45 ± 1.88 |
| TTW | NA | NA | NA |
| UMV | 6.42 ± 0.24 | 0.44 ± 0.02 | NA |
| URV | 28.17 ± 4.82 | 0.07 ± 0.01 | 8.18 ± 3.87 |

* NA = Not active

[1] The half-maximal inhibitory concentration ($IC_{50}$) (µg/mL).

[2] Quercetin equivalents (mg/g dry mass).

[3] % hydroxyl radical scavenging activity (%HRSA).

**Table 4: The percent inhibition of NO production and percent cell viability using RAW264.7 cells when treated with sample or positive control at a concentration of 50 µg/mL.**

| Plants | % Inhibition of NO | % Viability |
|---|---|---|
| AHW | 73.5±2.8* | 95.0±1.1 |
| AEW | 60.8±3.6 | 101.5±3.8 |
| AMH | 100.9±0.7* | 20.4±1.5[a] |
| APL | 90.1±2.4* | 143.2±8.7 |
| CMH | 33.9±0.6 | 134.5±3.1 |
| CMT | 15.8±1.2 | 130.7±2.7 |
| CRR | 30.5±1.2 | 173.7±3.4 |
| HPR | 54.0±1.1 | 134.0±1.2 |
| HAS | 32.3±1.4 | 114.3±2.6 |
| HFT | 20.8±1.0 | 148.1±4.3 |
| ICR | 27.9±2.2 | 112.8±2.0 |
| LSL | 7.5±1.0 | 104.4±1.4 |
| OAW | 42.5±1.5 | 129.1±3.7 |
| POS | 26.5±0.5 | 123.8±1.5 |
| RNW | 61.9±1.9 | 95.6±2.6 |
| SCH. | 26.2±2.3 | 121.1±2.6 |
| SCR. | 6.2±1.8 | 140.7±4.4 |
| SGR | 16.2±0.8 | 145.5±3.3 |
| SIF | 17.3±0.8 | 133.6±3.3 |
| TAF | 21.2±1.7 | 128.5±4.0 |
| TBF | NA | 107.1±5.4 |
| TCF | 3.9±1.1 | 135.0±1.3 |
| TCV | 20.0±2.6 | 126.0±3.2 |
| TTW | 40.8±2.7 | 71.8±1.1[a] |
| U.M.V. | NA | 125.5±3.6 |
| URV | 12.6±1.9 | 120.6±5.8 |
| Aqueous extract | 28.3±1.9 | 106.6±3.3 |
| Ethanolic extract | 44.1±2.3 | 152.4±7.8 |
| Indomethacin | 34.3±2.4 | 117.0±2.2 |

Value represents mean ± SEM (n = 4); NA = no activity; a cytotoxic effect was observed (% cell viability less than 80%)

[a] Cell viability less than 80%

*Significantly different higher activity than obtained with indomethacin, 50 µg/mL (The concentration of indomethacin and samples was used at 50 µg/mL for screen to establish the % inhibition).[32]

**Table 5: The percent cell proliferation of L929 cells treated with DM plant extracts at a concentration of 3, 10, 30, and 100 µg/mL.**

| Plants | % cell proliferation | | | |
|---|---|---|---|---|
| | 3 µg/mL | 10 µg/mL | 30 µg/mL | 100 µg/mL |
| AHW | 120.58±6.4 | 114.09±11.6 | 107.38±6.3 | 105.12±7.9 |
| AEW | 122.23±12.4 | 107.58±1.7 | 105.88±3.8 | 84.26±3.2 |
| AMH | 121.68±14.8 | 115.67±7.5 | 115.67±7.5 | 115.60±0.9 |
| APL | 112.12±17.8 | 104.95±14.62 | 107.41±21.0 | 79.34±13.0 |
| CMH | 97.95±11.0 | 100.95±13.8 | 93.97±12.0 | 92.62±12.8 |
| CMT | 100.9±6.0 | 95.85±10.5 | 96.22±10.2 | 114.02±3.2 |
| CRR | 110.7±10.7 | 101.81±13.0 | 112.76±19.9 | 95.93±29.9 |
| HPR | 115.18±14.5 | 106.56±18.5 | 93.04±10.5 | 97.95±3.9 |
| HAS | 98.71±18.1 | 103.06±12.2 | 101.37±8.0 | 114.53±2.1 |
| HFT | 132.66±35.4 | 99.45±15.1 | 102.14±3.5 | 105.89±4.1 |
| ICR | 121.22±14.6 | 106.88±14.5 | 98.39±5.8 | 83.25±7.2 |
| LSL | 96.18±2.4 | 107.59±14.3 | 91.93±3.7 | 85.78±4.6 |
| OAW | 123.14±2.3 | 112.53±2.9 | 106.46±4.6 | 90.50±8.6 |
| POS | 110.49±9.8 | 99.42±11.31 | 90.48±3.3 | 80.42±5.1 |
| RNW | 116.09±9.8 | 108.86±11.8 | 96.93±13.2 | 93.92±10.74 |
| SCH | 140.27±6.6 | 128.95±5.2 | 123.10±8.3 | 113.71±4.1 |
| SCR | 122.07±2.3 | 124.45±6.5 | 118.04±7.2 | 96.40±6.8 |
| SGR | 116.73±15.2 | 106.20±12.3 | 116.95±7.5 | 90.49±10.4 |
| SIF | 105.19±8.8 | 102.72±13.7 | 92.96±9.7 | 97.02±8.8 |
| TAF | 139.42±11.3 | 134.93±11.78 | 120.19±6.9 | 111.16±3.48 |
| TBF | 120.71±15.3 | 103.23±15.3 | 101.80±13.9 | 96.77±6.9 |
| TCF | 104.02±14.1 | 92.44±16.3 | 88.27±8.9 | 79.85±18.0 |
| TCV | 116.23±6.4 | 109.39±8.3 | 98.58±5.7 | 91.18±7.1 |
| TTW | 128.83±12.6 | 122.8±7.6 | 135.06±18.4 | 132.289±11.1 |
| UMV | 143.43±20.9 | 146.75±18.0 | 152.58±12.9 | 136.91±13.3 |
| URV | 99.89±9.3 | 88.62±9.3 | 96.87±10.05 | 72.16±10.4 |

## Evaluation of the most potential antidiabetic plants (*Hydnophytum formicarum* Jack.)

After screening the antidiabetic plants, it was found that *H. formicarum* tuber showed the highest DPP-4 inhibitory activity (82.75±0.83 % inhibition at the concentration of 50 µg/mL) as well as moderately high in anti-α-glucosidase and antioxidant activities. At the same time, it showed no toxicity to L929 cells. Thus, *H. formicarum* tuber was studied further to identify possible chemical components that contribute to its biological activities.

## Purification and assessment of anti-DPP-4 activities of HFT fractions

The ethanol crude extract of *H. formicarum* (HFT extract) was subjected to further partition using different solvents resulting in four fractions, including HH, HE, HB, and HW. The physical appearances and % yield of all extracts are summarized in Table 6. All fractions were obtained as a dark brown-off red viscous liquid. The isolated yields and percentage yields of HH, HE, HB, and HW extract were 2.64 g (9.52% w/w), 12.79 g (46.13% w/w), 3.25 g (11.75% w/w), and 27.01 g (21.43% w/w), respectively, when compared to the crude ethanolic extract. The anti-DPP-4 activity was used for bioassay-guided isolation of purified active compounds. The four fractions were analyzed for anti-DPP-4 activity and compared with diprotin A, the positive standard. 50 µg/mL of diprotin A showed significant inhibitory activity of 90.07 ± 0.39%. The most inhibitory HFT fractions were the HB fraction (78.99 ± 0.40 %), the HW fraction (71.46 ± 0.61 %) and the HE fraction (67.59 ± 0.48 %). However, the inhibitory activity of these fractions was less than the crude extract (80.75 ± 0.83 %). The HH fraction did not give good DPP-4 inhibitory activity (1.78 ± 0.20 %). Thus, the extracts that yielded efficient inhibitory activity were from high polar solvents. (Table 7).

## Structure elucidation of pure compounds

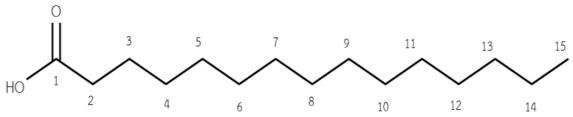
Bioassay-guided isolation was used to obtain pure compounds. When the HH fraction (1,000 mg) was subjected to silica gel column chromatography using a sequential of hexane, EtOAc, and MeOH, it yielded 10 fractions: HH 1 (461.40 mg), HH 2 (20.90 mg), HH 3 (10.00 mg),

**Table 6: The percentage yields and physical appearance of the extracts obtained from partition of *H. formicarium*.**

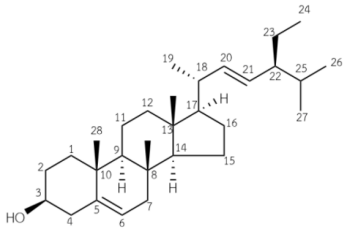
| Extract | Physical appearance | % Yield (%w/w) |
|---|---|---|
| **Crude extract (HFT)** | **Dark brown off red viscous liquid** | **-** |
| Hexane extract (HH) | Dark brown off red viscous liquid | 9.52 |
| EtOAc extract (HE) | Dark brown off red viscous liquid | 46.13 |
| n-BuOH extract (HB) | Dark brown off red viscous liquid | 11.75 |
| $H_2O$ extract (HW) | Dark brown off red viscous liquid | 26.26 |

**Table 7: The DPP-4 inhibitory activity (% inhibition) of 50 $\mu$g/mL of the extracts.**

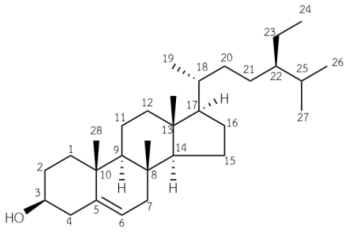
| Samples | % Inhibition of DPP-4 (at conc. 50 µg/mL) |
|---|---|
| **Diprotin A** | 90.07 ± 0.39 |
| **Crude extract (HFT)** | 80.75 ± 0.83 |
| **Hexane extract (HH)** | 1.78 ± 0.20 |
| **EtOAc extract (HE)** | 67.59 ± 0.48 |
| ***n*-BuOH extract (HB)** | 78.99 ± 0.40 |
| **H2O extract (HW)** | 71.46 ± 0.61 |



Palmitic acid (1)



Stigmasterol (2)



ß-sitosterol (3)

**Figure 3:** Structures of isolated compounds of *Hydnophytum formicarum* Jack.

HH 4 (46.00 mg), HH 5 (22.40 mg), HH 6 (7.70 mg), HH 7 (40.30 mg), HH 8 (53.90 mg), HH 9 (187.00 mg), HH 10 (515.10 mg). HH 1 (461.40 mg) fraction was selected to be further purified by a silica gel column chromatography using hexane and EtOAc in a gradient mode to give 6 sub-fractions: HH 1.1 (203.70 mg), HH 1.2 (301.40 mg), HH 1.3 (12.40 mg), HH 1.4 (46.50 mg), HH 1.5 (58.70 mg) and HH 1.6 (88.10 mg). Of these, two fractions, HH 1.2 and HH 1.4 were further purified by silica gel column chromatography with gradient mode using sequential hexane and EtOAc. HH 1.2 was isolated to give 8 fractions: HH 1.2.1 (15.30 mg), HH 1.2.2 (26.20 mg), HH 1.2.3 (71.10 mg), HH 1.2.4 (7.40 mg), HH 1.2.5 (47.60 mg), HH 1.2.6 (8.50 mg), HH 1.2.7 (1.20 mg) and HH 1.2.8 (19.10). HH 1.2.3 (71.10 mg) was further purified by Sephadex LH-20 using chloroform to give 6 fractions: HH 1.2.3.1 (480 mg), HH 1.2.3.2 (1.30mg), HH 1.2.3.3 (54 mg), HH 1.2.3.4 (5.30 mg), HH 1.2.3.5 (2.20 mg) and HH 1.2.3.6 (2.50 mg). Among these obtained fractions, fraction HH 1.2.3.4 provided white solid residue after drying by the evaporation process as shown the structure in Figure 3(1).

HH 1.4 was also isolated to give 9 fractions: HH 1.4.1 (3.40 mg), HH 1.4.2 (3.60 mg), HH 1.4.3 (9.80 mg), HH 1.4.4 (3.70 mg), HH 1.4.5 (1.80 mg), HH 1.4.6 (0.90 mg), HH 1.4.7 (0.90 mg), HH 1.4.8 (4.70 mg) and HH 1.4.9 (10.30 mg). HH 1.4.3 was also further purified by Sephadex LH-20 column using the mixture of MeOH in EtOAc (1:1) to give 4 fractions: HH 1.4.3.1 (4.40 mg), HH 1.4.3.2 (5.90 mg), HH 1.4.3.3 (10 mg) and HH 1.4.3.4 (1.30 mg). HH 1.4.3.2 (5.90 mg) showed a clear pattern with an off-white solid after drying from this separation fraction as shown the structure in Figure 3(2) and (3).

The purified residues were further characterized by H1-NMR, FT-IR, UV, and mass spectroscopy. The spectral data were utilized for comparison with known compounds. HH 1.2.3.4 Yielded spectral and mass data that matched palmitic acid, as previously reported,[35] (Supplement 2). The HH 1.4.3 solid was also characterized by H1-NMR, FT-IR, and EIMS techniques and matched a report by Chaturvedula and Prakash,[36] which is a mixture of stigmasterol and ß-sitosterol (Supplement 3).

**Table 8: $IC_{50}$ of pure compounds for DPP-4 inhibitory activities.**

| Compounds | $IC_{50}$ ± SD (µg/mL) |
|---|---|
| Palmitic acid (1) | 73.82 ± 2.64 |
| Stigmasterol (2) and ß-sitosterol (3) | 78.58 ± 0.92 |
| Sugar fraction | 624.98 ± 11.99 |
| Diprotin A | 2.07 ± 0.01 |

## Assessment of anti-DPP-4 activities of isolated compounds

HH 1.2.3.4 and HH 1.4.3 were tested to quantify their inhibition activities against DPP-4 activity using a similar procedure to that used with the extracts. Diprotin A was used as a positive control in each experiment. The results demonstrated that palmitic acid (1) and the mixture of stigmasterol (2) and ß-sitosterol (3) gave more inhibition than the sugar from fraction HW, but they showed much less activity than a positive standard, diprotin A. We, therefore, suggest that these components of HFT could be useful as marker compounds for HFT, rather than as DPP-4 inhibitors in their own right. Their $IC_{50}$ values are summarized in Table 8. The compounds with high anti-DPP-4 activity may be considered biomarkers for quality control of the HFT standardized extract preparation for antidiabetic activity.

# DISCUSSION

Herbal antidiabetic medicines are extensively accepted as alternatives for DM patients in lowering blood sugar. In this study, a traditional antidiabetic recipe, which is being commonly prescribed at the Thai-traditional medicine department in Wang-Nam-Yen hospital, Thailand, was investigated for potentially beneficial DM-related activities. These included anti-DPP-4, anti-α-glucosidase, and anti-α-amylase, anti-inflammation, antioxidant, and fibroblast proliferation activity. Our previous report showed that some plants in this recipe, such as *S. chinensis*, *L. speciosa*, *U minutiflora*, *I. cylindrica*, and *H. formicarum* showed promising anti- α-glucosidase, and anti-α-amylase activities.[19] In this study, additional potential antidiabetic mechanisms were evaluated to support the broader clinical utilization of this remedy. Wang- Nam-Yen hospital formulation is administered

via the oral route. Firstly, the aqueous extract of the recipe was tested for the inhibitory activities against DPP-4, α-glucosidase, and α- amylase enzymes. The results showed that the aqueous extract inhibited α-amylase and DPP-4 enzymes at a moderate level but only slightly inhibited α-glucosidase. This suggests that anti- α-glucosidase activities may not be a major mechanism of this extract. The ethanolic extract, compared with the aqueous extract, provided better inhibition of α-glucosidase and α-amylase enzymes, suggesting that ethanol extraction might improve the remedy's targeting of these enzymes.

A number of medicinal plants with DPP-4 inhibitory activity have been previously reported, such as *Castanospermum austral*,[37,38] *Mangiferia indica*,[39] *Withania somnifera*,[40] *Trigonella foenum-graecum*,[40] *Urena lobate*,[41] and *Berberis aristata*.[42] *H. formicarum* is also used as the constituent in Thai traditional medicine, and some of its biological activities have been described previously.[43-45] Sinapinic acid has been reported to be an active compound isolated from the rhizome of *H. formicarum,* which can inhibit the growth of HeLa and HT29 cells via histone deacetylase inhibition.[46] In this study, *H. formicarum* showed the most potent DPP-4 inhibitory activity among all screened plants with 82.72% inhibition, nearly an equally potent to diprotin A. Jeli and Makiyah,[47] reported *H. formicarum* was able to increase the size of Langerhans islet, and the number of β-cells; they concluded that *H. formicarum* could minimize the damage of pancreases of alloxan-induced diabetic rats. Our results could support the use of *H. formicarum* as herbal medicine for antidiabetic.

Triterpenoids have been reported as anti-diabetes activities by several targets, such as α- glucosidase, α-amylase, aldose reductase, protein tyrosine phosphatase 1B, glycogen phosphorylase.[48] Due to the high contents of terpenoids, both α-glucosidase and α-amylase were inhibited in most of the plants tested. In addition, another study suggested flavonoids, particularly luteolin, apigenin, and resveratrol, could act as natural DPP-4 inhibitors.[49] This is consistent with our results because most of the plants we studied contained high levels of terpenoids and flavonoids, and most of them displayed an inhibition of all tested enzymes at a significant level. This work shows that *H. formicarum*, *L. speciose*, *T. arjuna*, and *U.minutiflora* provided superior inhibition profiles against DPP-4, α-glucosidase, and α-amylaseand are good candidates for further development in antidiabetic formulations.

As noted above, increased oxidative stress and inflammation was associated with the development and progression of diabetes and its complications. Both aqueous and ethanolic extracts of Wang-Nam-Yen and its component plants gave significant inhibition of DPPH radical and NO production. Our results agreed with other studies that propose that various phenolics and flavonoids could attenuate the oxidative radicals and NO.[20,31,32] *T. arjura* was the most active of the plants tested in this regard and gave the superior inhibition in all anti- oxidation assays, while *A. hirtum*, *A. myriophylla,* and *A. paniculata* exhibited considerable significant reductions in NO production.

*H. formicarum* was selected for further study of chemical components because it exhibited the best anti-DPP-4 activity. Successes in isolation of purified chemical constituents were obtained from hexane partitioned fractions. The two compounds identified in the hexane fraction were palmitic acid and a mixture of stigmasterol and ß-sitosterol. These constituents have been reported in previous studies of other plants.[45,50,51]

Unfortunately, neither palmitic acid nor the mixture of stigmasterol and ß-sitosterol showed low activity against DPP-4 compared to diprotin A. However, previous studies have shown sterols like stigmasterol and β-sitosterol to reduce blood glucose level, cholesterol absorption, and body weight in STZ induced diabetic mice.[52] Moreover, β-sitosterol was shown to normalize the altered levels of blood glucose in the diabetic rat by induced β-cells regeneration, glucose uptake, reduced toxicity to the β-cells by inhibition of ROS, reduction of β -cells apoptosis, activation of insulin receptor (IR), and glucose transporter 4 (GLUT4) proteins in the adipose tissue, and to increased insulin release.[53-56] Furthermore, β-sitosterol reduced blood glucose through various signaling pathways, including the reduction of PTP1B (Protein tyrosine phosphate 1B), which increases leptin and insulin signaling, reduction of glucose production by inhibition of phosphoenolpyruvate carboxykinase (PEPCK), inhibition of alpha-glucosidase and amylase enzyme activities, activation of AMPK pathway through AMPK, GSK-3, and ACC phosphorylation, and increasing of glycogen synthesis.[54,55]

Our results do not confirm the inhibition of DPP-4 enzyme by β-sitosterol and stigmasterol suggested in the report of Purnomo *et al*.[41] using *in silico* techniques.

Palmitic acid also exhibited antidiabetic activity by acute stimulation of glucose uptake via activation of Akt and ERK1/2 in skeletal muscle cells.[57] We suggested that palmitic acid, stigmasterol, and ß-sitosterol may also serve as useful biomarkers of *H. formicarum,* for quality control purposes.

## CONCLUSION

Many of the plants in the Wang-Nam-Yen traditional anti-diabetes recipe displayed potent inhibition not only against α-glucosidase and α-amylase. Some also exhibited potent activity against DPP-4, anti-oxidation and anti-inflammation assays. The terpenoid and flavonoid contents of the constituent plants were quantified and are possibly related to the reported beneficial DM-related activities. *H. formicarum* was chosen for chemical evaluation, and the purified compounds. Palmitic acid, stigmasterol, and β-sitosterol were extracted from the hexane fraction. These chemicals have reported anti-DM activity but showed only weak activity in the specific DPP-4 inhibition assay. The potentially beneficial anti-DM assay results obtained from the constituent plants of Wang-Nam-Yen are consistent with its traditional use and argue for wider dissemination of its use in the clinical setting.

## ACKNOWLEDGEMENT

The authors acknowledge the cooperation from all staff in the Department of Pharmacognosy and Pharmaceutical Botany, and Department of Pharmaceutical Chemistry, Faculty of Pharmaceutical Sciences, Prince of Songkla University. The authors would like to thank Mr. Patarachai Pornpanyanukul from Pharmacy Division, Wangnamyen hospital, Sakeao Province, Thailand, for the material and data support. Thank you to the Prince of Songkla University research grant (Grant Number PHA6000985) for financial support. Moreover, the research was supported from Prince of Songkla University and Ministry of Higher Education, Science, Research, and Innovation under the Reinventing University Project (Grant Number REV64035).

## CONFLICT OF INTEREST

The authors declare no conflict of interest.

## ABBREVIATIONS

**BCG:** bromocresol green; **BHA:** butylated hydroxyanisole; **BHT:** butylated hydroxytoluene; **n-BuOH:** n-butanol; **DMEM:** Dulbecco's Modified Eagle Medium; **DPPH:** 2,2-diphenyl-1-picrylhydrazyl; **EtOAC:** ethyl acetate; **EtOH:** Ethanol; **FRAP:** Ferric reducing antioxidant power; **GP-pNA:** gly-pro-p-nitroanilide; **HB:** n-butanol extract; **HE:** ethyl acetate extract; **HH:** Hexane extract; **HRSA:** hydroxyl radical scavenging activity; **HW:** aqueous extract; **IR:** infrared; **LPS:** lipopolysaccharide from *Escherichia coli*; **MeOH:** methanol; **MS:** mass spectra; **MTT:** 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-

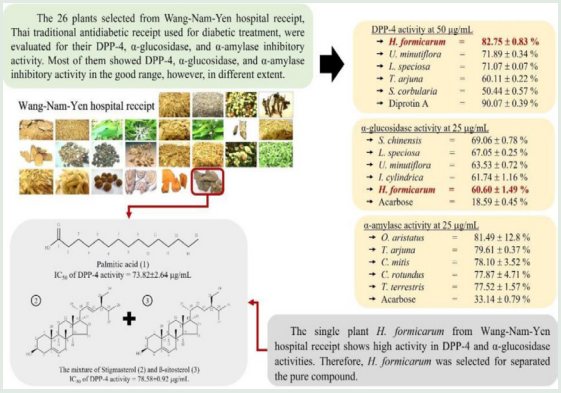
tetrazolium bromide; **NMR:** nuclear magnetic resonance; **NO:** nitric oxide; **RAW264.7:** murine macrophage-like cell-line; **UV:** Ultraviolet.

## REFERENCES


1. American Diabetes Association. 4. Comprehensive medical evaluation and assessment of comorbidities: Standards of medical care in Diabetes-2020. Diabetes Care. 2020a;43(Suppl 1);Suppl 1 [Suppl 1:S37-47]:S37-47. doi: 10.2337/dc20-S004, PMID 31862747.
2. Artasensi A, Pedretti A, Vistoli G, Fumagalli L. Type 2 diabetes mellitus: A review of multi-target drugs. Molecules. 2020;25(8). doi: 10.3390/molecules25081987, PMID 32340373.
3. Wang J, Huang M, Yang J, Ma X, Zheng S, Deng S, *et al*. Antidiabetic activity of stigmasterol from soybean oil by targeting the GLUT4 glucose transporter. Food Nutr Res. 2017;61(1). PMID 1364117.
4. World Health Organization. Diabetes. 2020.
5. Glasheen WP, Renda A, Dong Y. Diabetes Complications Severity Index (DCSI)-Update and ICD-10 translation. J Diabetes Complications. 2017;31(6):1007-13. doi: 10.1016/j.jdiacomp.2017.02.018, PMID 28416120.
6. International Diabetes Federation Guideline Development Group. Global guideline for type 2 diabetes. Diabetes Res Clin Pract. 2014;104(1):1-52. doi: 10.1016/j.diabres.2012.10.001, PMID 24508150.
7. Skliros NP, Vlachopoulos C, Tousoulis D. Treatment of diabetes: Crossing to the other side. Hellenic J Cardiol. 2016;57(5):304-10. doi: 10.1016/j.hjc.2016.07.002, PMID 27687958.
8. American Diabetes Association. 9. Pharmacologic approaches to glycemic treatment: standards of medical care in Diabetes-2020. Diabetes Care. 2020b;43(Suppl 1);Suppl 1 [Suppl 1:S98-S110]:S98-S110. doi: 10.2337/dc20-S009, PMID 31862752.
9. Hedrington MS, Davis SN. Considerations when using alpha-glucosidase inhibitors in the treatment of type 2 diabetes. Expert Opin Pharmacother. 2019;20(18):2229-35. doi: 10.1080/14656566.2019.1672660, PMID 31593486.
10. Marathe CS, Rayner CK, Jones KL, Horowitz M. Relationships between gastric emptying, postprandial glycemia, and incretin hormones. Diabetes Care. 2013;36(5):1396-405. doi: 10.2337/dc12-1609, PMID 23613599.
11. Bajaj S, Khan A. Antioxidants and diabetes. Indian J Endocrinol Metab. 2012;16(Suppl 2);Suppl 2 [Suppl 2:S267-71]:S267-71. doi: 10.4103/2230-8210.104057, PMID 23565396.
12. Karunakaran U, Park KG. A systematic review of oxidative stress and safety of antioxidants in diabetes: Focus on islets and their defense. Diabetes Metab J. 2013;37(2):106-12. doi: 10.4093/dmj.2013.37.2.106, PMID 23641350.
13. Rodríguez ML, Pérez S, Mena-Mollá S, Desco MC, Ortega ÁL. Oxidative stress and microvascular alterations in diabetic retinopathy: Future Therapies. Oxid Med Cell Longev. 2019;2019:4940825. doi: 10.1155/2019/4940825.
14. Bhatt D, Ghosh S. Regulation of the NF-κB-mediated transcription of inflammatory genes. Front Immunol. 2014;5:71. doi: 10.3389/fimmu.2014.00071, PMID 24611065.
15. Jurjus A, Eid A, Al Kattar S, Zeenny MN, Gerges-Geagea A, Haydar H, *et al*. Inflammatory bowel disease, colorectal cancer and type 2 diabetes mellitus: The links. BBA Clin. 2016;5:16-24. doi: 10.1016/j.bbacli.2015.11.002, PMID 27051585.
16. Soliman AM, Teoh SL, Ghafar NA, Das S. Molecular concept of diabetic wound healing: Effective role of herbal remedies. Mini Rev Med Chem. 2019;19(5):381-94. doi: 10.2174/1389557518666181025155204, PMID 30360709.
17. Chayarop K, Peungvicha P, Temsiririrkkul R, Wongkrajang Y, Chuakul W, Rojsanga P. Hypoglycaemic activity of Mathurameha, a Thai traditional herbal formula aqueous extract, and its effect on biochemical profiles of streptozotocin-nicotinamide-induced diabetic rats. BMC Complement Altern Med. 2017;17(1):343. doi: 10.1186/s12906-017-1851-8, PMID 28662699.
18. Rachpirom M, Ovatlarnporn C, Thengyai S, Sontimuang C, Puttarak P. Dipeptidyl peptidase-IV (DPP-IV) inhibitory activity, antioxidant property and phytochemical composition studies of herbal constituents of Thai folk anti-diabetes remedy. Walailak J Sci Technol. 2016;13(10):803-14.
19. Thengyai S, Thiantongin P, Sontimuang C, Ovatlarnporn C, Puttarak P. α-glucosidase and α-amylase inhibitory activities of medicinal plants in Thai antidiabetic recipes and bioactive compounds from Vitex glabrata R.Br. Stem bark. J Herb Med. 2020;19. doi: 10.1016/j.hermed.2019.100302, PMID 100302.
20. McDonald S, Prenzler PD, Antolovich M, Robards K. Phenolic content and antioxidant activity of olive extracts. Food Chem. 2001;73(1):73-84. doi: 10.1016/S0308-8146(00)00288-0.
21. Jiao H, Wang SY. Correlation of antioxidant capacities to oxygen radical scavenging enzyme activities in blackberry. J Agric Food Chem. 2000;48(11):5672-6. doi: 10.1021/jf000765q, PMID 11087537.
22. Prior RL, Cao G, Martin A, Sofic E, McEwen J, O'Brien C, *et al*. Antioxidant capacity as influenced by total phenolic and anthocyanin content, maturity, and variety of Vaccinium species. J Agric Food Chem. 1998;46(7):2686-93. doi: 10.1021/jf980145d.
23. Tambe VD. B, R. Estimation of total phenol, tannin, alkaloid, and flavonoid in Hibiscus tiliaceus Linn. Wood extracts. Res Rev J Pharmacogn Phytochem. 2014;2(4):41-7.
24. Ghorai N, Chakraborty S, Gucchait S, Saha SK, Biswas S, 2012. Estimation of total Terpenoids concentration in plant tissues using a monoterpene, linalool as standard reagent. Protoc. Exch. n. Pag.
25. Gao H, Kawabata J. α-glucosidase inhibition of 6-hydroxyflavones. Part 3: Synthesis and evaluation of 2, 3, 4-trihydroxybenzoyl-containing flavonoid analogs and 6- aminoflavones as α-glucosidase inhibitors. Bioorg Med Chem. 2005;13(5):1661-71. doi: 10.1016/j.bmc.2004.12.010, PMID 15698784.
26. Hansawasdi C, Kawabata J, Kasai T. Alpha-amylase inhibitors from roselle (*Hibiscus sabdariffa* Linn.) tea [*Hibiscus sabdariffa* Linn]. Biosci Biotechnol Biochem. 2000;64(5):1041-3. doi: 10.1271/bbb.64.1041, PMID 10879476.
27. Kumar D, Ghosh R, Pal BC. α-glucosidase inhibitory terpenoids from *Potentilla fulgens* and their quantitative estimation by validated HPLC method. J Funct Foods. 2013;5(3):1135-41. doi: 10.1016/j.jff.2013.03.010.
28. Brand-Williams W, Cuvelier ME, Berset C. Use of a free radical method to evaluate antioxidant activity. LWT Food Sci Technol. 1995;28(1):25-30. doi: 10.1016/S0023-6438(95)80008-5.
29. Yıldırım A, Mavi A, Kara AA. Determination of antioxidant and antimicrobial activities of *Rumex crispus* L. extracts. J Agric Food Chem. 2001;49(8):4083-9. doi: 10.1021/jf0103572, PMID 11513714.
30. Tian F, Li B, Ji B, Yang J, Zhang G, Chen Y, *et al*. Antioxidant and antimicrobial activities of consecutive extracts from *Galla chinensis*: The polarity affects the bioactivities. Food Chem. 2009;113(1):173-9. doi: 10.1016/j.foodchem.2008.07.062.
31. Owolabi IO, Chakree K, Takahashi Yupanqui C. Bioactive components, antioxidative and anti-inflammatory properties (on RAW 264.7 macrophage cells) of soaked and germinated purple rice extracts. Int J Food Sci Technol. 2019;54(7):2374-86. doi: 10.1111/ijfs.14148.
32. Sudsai T, Prabpai S, Kongsaeree P, Wattanapiromsakul C, Tewtrakul S. Anti-inflammatory activity of compounds from *Boesenbergia longiflora* rhizomes. J Ethnopharmacol. 2014;154(2):453-61. doi: 10.1016/j.jep.2014.04.034, PMID 24786574.
33. Sudsai T, Wattanapiromsakul C, Nakpheng T, Tewtrakul S. Evaluation of the wound healing property of *Boesenbergia longiflora* rhizomes. J Ethnopharmacol. 2013;150(1):223-31. doi: 10.1016/j.jep.2013.08.038, PMID 23994340.
34. Langdon SR, Mulgrew J, Paolini GV, Van Hoorn WP. Predicting cytotoxicity from heterogeneous data sources with Bayesian learning. J Cheminform. 2010;2(1):11. doi: 10.1186/1758-2946-2-11, PMID 21143909.
35. Keat NB, Umar RU, Lajis NH, Chen TY, Li TY, Rahmani M, *et al*. Chemical constituents from two weed species of Spermacoce (Rubiaceae). Malays J Anal Sci. 2010;14:6-11.
36. Chaturvedula VSP, Prakash I. Isolation of Stigmasterol and?-Sitosterol from the dichloromethane extract of Rubus suavissimus. Int Curr Pharm J. 2012;1(9):239-42. doi: 10.3329/icpj.v1i9.11613.
37. Bharti SK, Krishnan S, Kumar A, Rajak KK, Murari K, Bharti BK, *et al*. Antihyperglycemic activity with DPP-IV inhibition of alkaloids from seed extract of *Castanospermum australe*: Investigation by experimental validation and molecular docking. Phytomedicine. 2012a;20(1):24-31. doi: 10.1016/j.phymed.2012.09.009, PMID 23063145.
38. Bharti SK, Sharma NK, Kumar A, Jaiswal SK, Krishnan S, Gupta AK, *et al*. Dipeptidyl peptidase IV inhibitory activity of seed extract of *Castanospermum australe* and molecular docking of their alkaloids. Topclass. J Herb Med. 2012b;1(1):1-7.
39. Yogisha S, Raveesha KA. Dipeptidyl peptidase IV inhibitory activity of Mangifera indica. J Nat Prod. 2010;3:76-9.
40. Singh AK, Joshi J, Jatwa R. Dipeptidyl peptidase IV (DPP-IV/CD26) inhibitory and free radical scavenging potential of *W. somnifera* and *T. foenum-Graecum* extract. Int J Phytomed. 2014;5(4):503-9.
41. Purnomo Y, Soeatmadji DW, Sumitro SB, Widodo MA. Anti-diabetic potential of *Urena lobata* leaf extract through inhibition of dipeptidyl peptidase IV activity. Asian Pac J Trop Biomed. 2015;5(8):645-9. doi: 10.1016/j.apjtb.2015.05.014.
42. Al-Masri IM, Mohammad MK, Tahaa MO. Inhibition of dipeptidyl peptidase IV (DPP IV) is one of the mechanisms explaining the hypoglycemic effect of berberine. J Enzyme Inhib Med Chem. 2009;24(5):1061-6. doi: 10.1080/14756360802610761, PMID 19640223.
43. Itharat A, Houghton PJ, Eno-Amooquaye E, Burke PJ, Sampson JH, Raman A. *In vitro* cytotoxic activity of Thai medicinal plants used traditionally to treat cancer. J Ethnopharmacol. 2004;90(1):33-8. doi: 10.1016/j.jep.2003.09.014, PMID 14698505.
44. Manosroi J, Boonpisuttinant K, Manosroi W, Manosroi A. Anti-proliferative activities on HeLa cancer cell line of Thai medicinal plant recipes selected from Manosroi II database. J Ethnopharmacol. 2012;142(2):422-31. doi: 10.1016/j.jep.2012.05.012, PMID 22626926.
45. Prachayasittikul S, Buraparuangsang P, Worachartcheewan A, Isarankura-Na-Ayudhya C, Ruchirawat S, Prachayasittikul V. Antimicrobial and antioxidative activities of bioactive constituents from *Hydnophytum formicarum* Jack. Molecules. 2008;13(4):904-21. doi: 10.3390/molecules13040904, PMID 18463592.

46. Senawong T, Misuna S, Khaopha S, Nuchadomrong S, Sawatsitang P, Phaosiri C, *et al*. Histone deacetylase (HDAC) inhibitory and antiproliferative activities of phenolic-rich extracts derived from the rhizome of *Hydnophytum formicarum* Jack.: Sinapinic acid acts as HDAC inhibitor. BMC Complement Altern Med. 2013;13(1):232. doi: 10.1186/1472-6882-13-232, PMID 24053181.
47. Jeli MM, Makiyah SN. Pengaruh pemberian infusa tumbuhan sarang semut (*Hydnophytum formicarum*) terhadap gambaran histologi Pankreas pada tikus (*Rattus norvegicus*) diabetes terinduksi aloksan. Majalah Kesehatan Pharmmedika. 2011;3(1):200-4.
48. Nazaruk J, Borzym-Kluczyk M. The role of triterpenes in the management of diabetes mellitus and its complications. Phytochem Rev. 2015;14(4):675-90. doi: 10.1007/s11101-014-9369-x. PMID 26213526.
49. Fan J, Johnson MH, Lila MA, Yousef G, De Mejia EG. Berry and citrus phenolic compounds inhibit dipeptidyl peptidase IV: implications in diabetes management. Evid Based Complement Alternat Med. 2013;2013:479505. doi: 10.1155/2013/479505.
50. Abdullah NS, Ahmad WYW, Sabri NA. The chemical constituents from young tubers of *Hydnophytum formicarum*. Malays J Anal Sci. 2017;21(2):291-7. doi: 10.17576/mjas-2017-2102-03.
51. Abdullah NS, Ahmad WYW, Sabri NA. New compounds from *Hydnophytum formicarum* young tubers. Malays J Anal Sci. 2017b;21(4):778-83.
52. Luo C, Zhang W, Sheng C, Zheng C, Yao J, Miao Z. Chemical composition and antidiabetic activity of *Opuntia milpa* Alta extracts. Chem Biodivers. 2010;7(12):2869-79. doi: 10.1002/cbdv.201000077, PMID 21161999.
53. Gupta R, Sharma AK, Dobhal MP, Sharma MC, Gupta RS. Antidiabetic and antioxidant potential of β-sitosterol in streptozotocin-induced experimental hyperglycemia. J Diabetes. 2011;3(1):29-37. doi: 10.1111/j.1753-0407.2010.00107.x, PMID 21143769.
54. Munhoz ACM, Frode TS. Isolated compounds from natural products with potential antidiabetic activity - A systematic review. Curr Diabetes Rev. 2018;14(1):36-106. doi: 10.2174/1573399813666170505120621, PMID 28474555.
55. Ponnulakshmi R, Shyamaladevi B, Vijayalakshmi P, Selvaraj J. *In silico* and *in vivo* analysis to identify the antidiabetic activity of beta sitosterol in adipose tissue of high fat diet and sucrose induced type-2 diabetic experimental rats. Toxicol Mech Methods. 2019;29(4):276-90. doi: 10.1080/15376516.2018.1545815, PMID 30461321.
56. Babu S, Krishnan M, Rajagopal P, Periyasamy V, Veeraraghavan V, Govindan R, *et al*. Beta-sitosterol attenuates insulin resistance in adipose tissue via IRS-1/Akt mediated insulin signaling in high fat diet and sucrose induced type-2 diabetic rats. Eur J Pharmacol. 2020;873:173004. doi: 10.1016/j.ejphar.2020.173004.
57. Pu J, Peng G, Li L, Na H, Liu Y, Liu P. Palmitic acid acutely stimulates glucose uptake via activation of Akt and ERK1/2 in skeletal muscle cells. J Lipid Res. 2011;52(7):1319-27. doi: 10.1194/jlr.M011254, PMID 21518696.


## GRAPHICAL ABSTRACT




## SUMMARY


- The 26 plants selected from Wang-Nam-Yen hospital receipt, Thai traditional antidiabetic receipt used for diabetic treatment, were evaluated for their DPP-4, α-glucosidase, and α- amylase inhibitory activity. Most of them showed DPP-4, α-glucosidase, and α-amylase inhibitory activity in the good range, however, in different extent.
- The ethanolic extract of *H. formicarum* tubers was identified as the highest potential antidiabetic effect by inhibition of DPP-4 enzymes and showed high potential for inhibit enzyme α-glucosidase.
- Palmitic acid and the mixture of stigmasterol and β-sitosterol were extracted from the hexane fraction. These chemicals have reported antidiabitic activity in the specific DPP-4 inhibition assay.


**Cite this article:** Rachpirom M, Barrows LR, Thengyai S, Ovatlarnporn C, Sontimuang C, Thiantongin P, *et al*. Antidiabetic Activities of Medicinal Plants in Wang Nam Yen Hospital Recipes and Candidate Antidiabetic Compounds from *Hydnophytum formicarum* Jack. Tubers. Pharmacog Res. 2022;14(1):89-99.