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Research article

Anti-diabetic compounds from *Uvaria dulcis* Dunal

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ABSTRACT

Medicinal plants have long been a source of lead compounds for drug discovery. Among these, the Annonaceae family has gained recognition for its potential to yield novel compounds, particularly those that can be used in the development of drugs targeting chronic diseases like diabetes mellitus (DM). We employed various chromatographic methods to isolate bioactive compounds from the roots, leaves, and twigs of *Uvaria dulcis* Dunal. We used spectroscopic methods to determine the chemical structures of these compounds. We successfully identified twelve known compounds from various parts of *U. dulcis*: patchoulenon, polygochalcone, 2′3′-dihydroxy-4′,6′-dimethoxydihydrochalcone, 2′,3′-dihydroxy-4′,6′-dimethoxychalcone, chrysin, techochrysin, 8-hydroxy-5,7-dimethoxyflavanone, pinocembrin, 3-farnesylindole, onysilin, cinchonain la, and cinchonain lb. Interestingly, cinchonain la and cinchonain lb exhibited more potent anti-α-glucosidase activity than acarbose (standard drug), with IC_{50} values of 11.88 ± 1.41 μg/mL and 15.18 ± 1.19 μg/mL, respectively. Cinchonain la inhibited the DPP-IV enzyme, with IC_{50} value lower than the standard compound (diprotin A) at 81.78 ± 1.42 μg/mL. While 2′,3′-dihydroxy-4′,6′-dimethoxychalcone show more potent inhibitory effect than standard drug with IC_{50} value of 8.62 ± 1.19 μg/mL. Additionally, at a concentration of 10 μg/mL, cinchonain lb and 2′,3′-dihydroxy-4′,6′-dimethoxychalcone promoted glucose uptake in L6 myotubes cells to the same extent as 100 nM insulin. These findings suggest that cinchonain la, cinchonain lb, and 2′,3′-dihydroxy-4′,6′-dimethoxychalcone are the *U. dulcis*-derived bioactive compounds that hold promise as potential structures to use in the development of anti-diabetic drugs.

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1. Introduction

Diabetes mellitus (DM) is one of noncommunicable diseases (NCDs) and a major cause of deaths worldwide. DM is the condition of glucose metabolic disorder resulting in abnormal high blood glucose levels [1]. The number of DM patients worldwide has been projected to increase up to 380 million of type 2 diabetes mellitus (T2DM) patients in 2025 [2,3]. T2DM is the most common type of DM cause by impaired insulin secretion and action contributing to hyperglycemia [2,4]. Long term DM leads to increasing mortality and reducing life quality and healthcare costs [5]. Thus, the effective glycemic control represents a main strategy of managing DM and play an importance role for preventing the complications including, cardiovascular disease, nephropathy, neuropathy, retinopathy, and DM foot [6,7]. Nowadays, numerous scientists pay their efforts towards anti-DM drug development due to the lack of a single successful drug for achieving the goal of blood glucose control. The recommendation for diabetes treatment is to combine drugs with different modes of action for controlling blood glucose effectively [8,9]. Hence, the preference is shifting towards exploring new lead compounds from herbal medicine, which may offer various modes of action and effective results with less side effects [10,11].

Uvaria is a genus within the Annonaceae family, known for its potential as a source of novel compounds that are promising for development as potential anti-DM drug candidates [12]. *Uvaria chamae* is one of the genera *Uvaria* used as herbal medicine in Africa for DM. The ethanolic extract of *U. chamae* root reduced blood glucose in the alloxan-induced DM rat model. The fraction of *U. chamae* root extract also exhibited α-amylase, beta cell regeneration and α-glucosidase inhibition which may be modulated by its bioactive compounds such as alkaloids, flavonoids, phenols, terpenoids and tannins [13]. *Uvaria grandiflora,* a Philippine medicinal plant, was reported for its anti-DM activities via *in vitro* and *in silico* studies. *U. grandiflora* bioactive compounds including uvagrandol and (−)-zeylenone inhibited the activities of α-glucosidase, lipase, and dipeptidyl peptidase-IV (DPP-IV) [14]. Moreover, *Uvaria narum* leaf extract revealed the anti-DM potential by 3T3L1 cell model through glucose uptake and glucose transport [15].

Uvaria dulcis Dunal. is a tropical scandent plant distributed in Southeast Asia and Thailand. Previous reports on the chemical component of *U. dulcis* consisted of alkaloids, acetogenins, phenyl propanoid and flavonoids some of which showed cytotoxic, antimicrobial and anticancer activities [16]. However, the anti-DM potential of *U. dulcis* has not been reported. Thus, in this study, the anti-DM effects of the extract and pure isolated compounds from different parts of *U. dulcis* were evaluated. The results of this study could support the use of *U. dulcis* and its bioactive compounds as herbal medicine or lead compounds for DM drug development in the future.

2. Materials and methods

2.1. Chemicals and reagents

Analytical grade solvents, acetone, dichloromethane, ethanol, ethyl acetate, methanol, and hexane were purchased from Labscan Asia Co., Thailand. Silica gel 60 (5–40 μm) and silica gel 100 (63–200 μm) were used for quick column chromatography (QCC) and column chromatography (CC), respectively. Sephadex LH-20, when indicated, was also used for CC. Precoated thin-layer chromatography (TLC) plates of silica gel 60 F_{254} were used for analytical purposes. Those materials were purchased from SiliCycle Inc., Thailand. Dulbecco's Modified Eagle Medium: high glucose (DMEM: high glucose), fetal bovine serum (FBS), trypsin-EDTA, penicillin-streptomycin, trypan blue dyes, 3-(4, 5-dimethylthiazol-2-yl)-2, 5 diphenyltetrazolium bromide (MTT) were obtained from Thermo Fisher Scientific (CA, USA). *Para*-nitrophenyl-α-D-glucopyranoside (pNPG), α-glucosidase from *Saccharomyces cerevisiae*, dipeptidyl peptidase-IV (DPP-IV) from porcine kidney, acarbose, Gly-Pro-*p*-nitroanilide (GP-p-NA), diprotin A, dimethyl sulfoxide (DMSO), dexamethasone (DEX), human insulin, and glucose (GO) assay kit were purchased from Sigma Aldrich (MO, USA).

2.2. Plant materials

Roots, twigs, and leaves of *U. dulcis* were collected in May 2020 from Mae Fah Luang University Health Park, Chiang Rai Province, Thailand (N: 20.055125°, E: 99.893839°). The plant was identified by Prof. Dr. Surat Laphookhieo, and a voucher specimen (No. MFU-NPR0211) was deposited at the Natural Products Research Laboratory, School of Science, Mae Fah Luang University. Each plant part was cleaned, cut into small pieces, dried, and ground into powder. The plant material was kept at room temperature until used.

2.3. U. dulcis extraction and isolation

The powder of air-dried roots (1.1 kg), air-dried twigs (1.1 kg), and air-dried leaves (1.5 kg) of *U. dulcis* were individually extracted with EtOAc (3 × 5 L) at room temperature for three days. Each extracted solution was concentrated under reduced pressure to yielded 75.0, 40.2, and 167.2 g of root, twig, and leaf extracts, respectively.

The root extract (75.0 g) was subjected to quick column chromatography (QCC) (100% hexanes to 100% acetone) to give four fractions (RA−RD). Fraction RB (2.2 g) was further separated by silica gel column chromatography (CC) (1:1 v/v, CH_2Cl_2−hexanes), yielding compound **1** (288.3 mg), while fraction RC (2.0 g) was further purified by CC over silica gel (100% CH_2Cl_2), to give compound **2** (8.3 mg) (Fig. S1).

The leaf extract (167.2 g) was subjected to QCC over silica gel (100% hexanes to 100% acetone) yielding six fractions (LA−LF). Fraction LC (7.8 g) was subjected to CC using reversed-phase silica gel (4:1 v/v, MeOH–H_2O), to obtain fraction LCR (1.5 g), which was further separated by CC over silica gel (1:4 v/v, EtOAc−hexanes), to give three fractions (LCRA−LCRC). Fraction LCRC (130.1 mg) was further purified by CC over silica gel (1:4 v/v, EtOAc−hexanes) to obtain compound **6** (5.8 mg). Fraction LD (6.6 g) was separated by

CC using reversed-phase silica gel (4:1 v/v, MeOH–H_2O), yielding fraction LDR (1.2 g). Fraction LDR upon standing at room temperature, solid were formed and washed with acetone to yield compound **3** (32.7 mg). The remaining solid from fraction LDR was further washed with MeOH to obtain compound **5** (7.5 mg). Fraction LE (7.3 g) was subjected to CC using reversed-phase silica gel (4:1 v/v, MeOH–H_2O) to yield fraction LER (1.4 g). Compounds **4** (24.3 mg) and **7** (18.3 mg) were obtained from fraction LER by washing it with acetone and MeOH, respectively. Compound **8** (10.2 mg) was obtained from the remaining part of fraction LER via repeated silica gel CC (2:3 v/v, EtOAc−hexanes) (Fig. S2).

The twig extract (40.2 g) was subjected to QCC over silica gel (100% hexanes to 100% acetone) to yield five fractions (TA−TE). Fraction TB (3.1 g) was separated by CC over silica gel (1:4 v/v, EtOAc−hexanes) to obtain three fractions (TBA−TBC). Fraction TBA (130.1 mg) was purified using silica gel CC (1:4 v/v, EtOAc−hexanes), yielding compound **9** (10.1 mg). Fraction TBC (195.3 mg) was purified by CC over silica gel (1:4 v/v, EtOAc−hexanes), to obtain compound **10** (9.2 mg). Fraction TD (4.2 g) was purified by CC over Sephadex LH-20 (100% MeOH), yielding three fractions (TDA−TDC). Fraction TDC (95.1 mg) was further separated by semi-preparative RP-HPLC (2:3 v/v, MeCN–H_2O, 2 mL/min) to yield compounds **11** (2.8 g, t_R 30.2 min) and **12** (2.1 g, t_R 42.1 min) (Fig. S3). All isolated compounds from roots, twigs, and leaves of *U. dulcis* were presented in Fig. 1.

2.4. α-Glucosidase inhibitory assay

The anti-diabetic activity potential of the crude extract and all isolated pure compounds, through the inhibition of α-glucosidase enzyme, was determined following the previous assay [17]. Briefly, each sample at various concentrations in 50 μL of 0.57 U/mL α-glucosidase enzyme was equally pre-mixed with 50 mM phosphate buffer, pH 6.9 for 10 min at 37 °C. Subsequently, 50 μL of a 5 mM pNPG substrate was added to the reaction mixture and incubated for 30 min. The reaction was stopped by 50 μL of a 1 M sodium carbonate solution (Na_2CO_3), and the absorbance at 405 nm was immediately measured using a microplate reader (DTX880

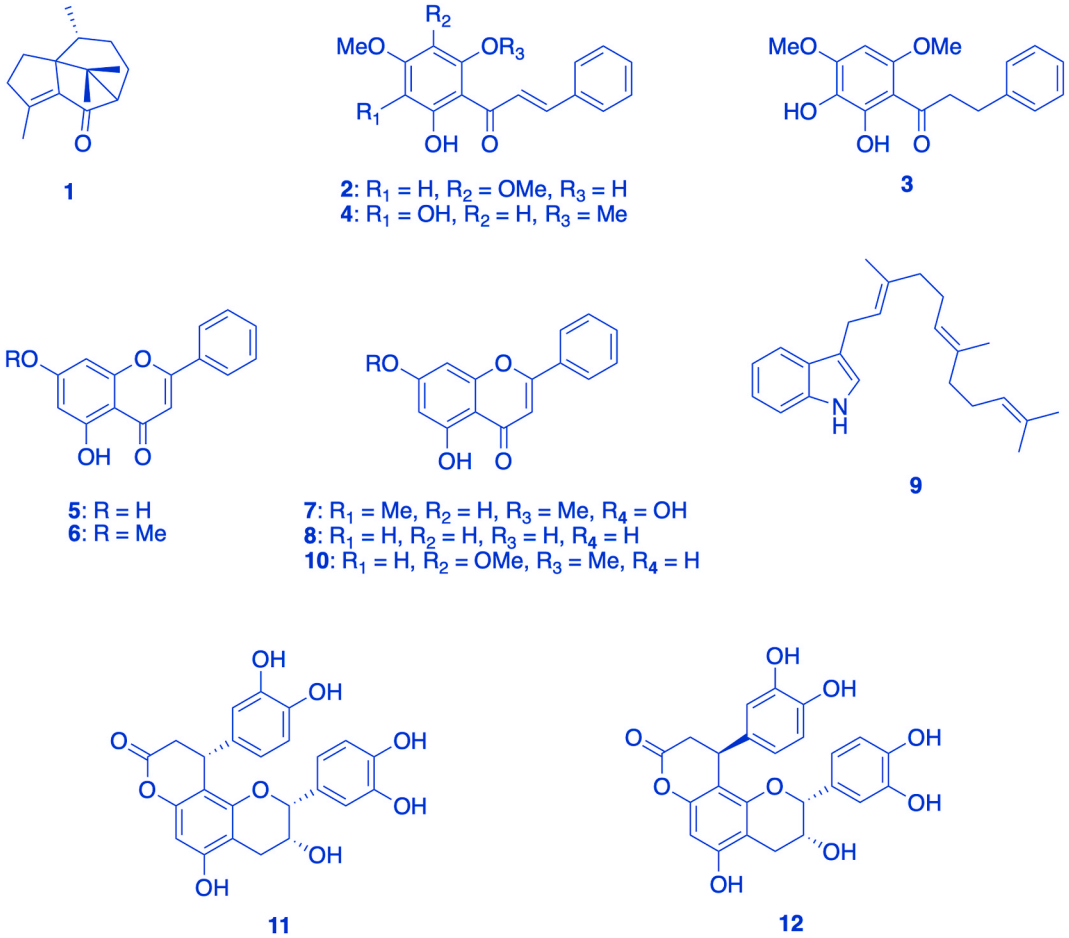

Fig. 1. Isolated compounds from *U. dulcis*. Patchoulenon (1) and polygochalcone (2) were isolated from the root. 2′3′-Dihydroxy-4′,6′-dimethoxydihydrochalcone (3), 2′,3′-dihydroxy-4′,6′-dimethoxychalcone (4), chrysin (5), techochrysin (6), 8-hydroxy-5,7-dimethoxyflavanone (7), and pinocembrin (8) were found within the leaf crude extract. 3-Farnesyl indole (9), onysilin (10), cinchonain Ia (11), and cinchonain Ib (12) were identified from the twig of *U. dulcis*.

Multimode Detector, Beckman Coulter®, Austria). The percentage of inhibition was calculated using the following equation: [($A_{control}$ − A_{sample})/$A_{control}$] *100 where $A_{control}$ and A_{sample} represent the absorbance of the reaction without and with the sample, respectively. The results were expressed as the concentration of the sample that could inhibit enzyme activity by 50% (IC_{50}). Acarbose served as a positive control, and each sample was analyzed in triplicate.

2.5. Dipeptidyl peptidase-IV (DPP-IV) inhibitory assay

The inhibition of the DPP-IV enzyme, leading to an increase in incretin hormones and subsequently elevating insulin secretion, is one of the mechanisms of action of antidiabetic drugs. In this study, the anti-diabetic potential of crude extracts and isolated pure compounds was examined through the inhibition of the DPP-IV enzyme, following the previous procedure [17]. Each sample was diluted with 50 mM Tris-HCl buffer, pH 8.0 to obtain the final concentration of 50 μg/mL and then pre-incubated with 0.05 U/mL of DPP-IV enzyme for 10 min at 37 °C. Subsequently, 0.2 mM GP-p-NA substrate was added to the reaction mixture and then incubated for 30 min. The reaction was stopped by 30 μL of 25% acetic acid, and the absorbance at 405 nm was immediately measured using a microplate reader. The percentage of inhibition was calculated using the following equation: [($A_{control}$ − A_{sample})/$A_{control}$] *100 where $A_{control}$ and A_{sample} represent the absorbance of the reaction without and with the sample, respectively. Each sample was conducted in triplicate and the results were expressed as the concentration of the sample that could inhibit enzyme activity by 50% (IC_{50}). Diprotin A was employed as a positive control.

2.6. Cell culture

Rat skeletal muscle cells (L6 myoblasts) were provided from the Medical Science Research and Innovation Institute, Prince of Songkla University and cultured in DMEM. The medium low glucose was supplemented with 10% FBS and 1% penicillin-streptomycin at 37 °C under a humidified atmosphere containing 5% CO_2. The cells subcultured once the cells reached 70% confluence and the medium was changed every 2 days.

2.7. Cell differentiation

L6 myoblast cells were cultured in 96 wells plates at density of 8000 cells/well and incubated at 37 °C under a humidified atmosphere containing 5% CO_2. After reaching 70 cell confluence, the old media were changed into 2% FBS in DMEM every 48 h, for 8–10 days to fully differentiate L6 myoblasts into multinucleated myotubes.

2.8. Cell viability assay

The cytotoxicity of crude extract and isolated compounds was assessed in L6 myotubes cells according to previous methods [17]. L6 myoblast cells were seed at a density of 8×10^3 cells/well and differentiated following the method in Section 2.7. Afterwards, the completed L6 myotubes were incubated overnight with each sample at various concentrations. The treated medium was transferred and further incubated with MTT solution at the final concentration of 0.5 mg/mL for 3 h. Finally, the MTT-containing medium was removed and the formazan crystals were dissolved with 100 μL of DMSO. Cell viability presented as the percentage of control was observed by measuring the absorbance at 570 nm. Each sample was conducted in triplicate.

2.9. Glucose uptake assay

One of the most important mechanisms of action of anti-diabetic drugs is the promotion of glucose uptake into the cell to reduce blood glucose levels. The glucose uptake stimulatory activity of both crude extracts and isolated compounds was evaluated according to the method described previously [18]. Briefly, differentiated rat skeleton muscles following the method in Section 2.7 were cultured in 96 wells plate with DMEM media containing 2% FBS. The cells were then washed and incubated with various concentrations of each sample in a complete DMEM medium. The treated medium was individually transferred into 96 wells plate at 24 h after incubation and mixed with the reagent of GO kit. The reaction was performed at 37 °C for 30 min and then stopped by 6 M H_2SO_4. The ability to enhance glucose uptake refers to the remaining glucose in the media which was observed by measuring the absorbance at 540 nm. The percentage of glucose uptake stimulation was calculated using the following equation: [($A_{control}$ − A_{sample})/$A_{control}$] *100 where $A_{control}$ and A_{sample} represent the absorbance of the reaction without and with the sample, respectively. Insulin was used as positive control. Each sample was conducted in triplicate.

2.10. Statistics

All data were performed in triplicate and reported as mean ± standard deviation. Any significant difference was indicated by one-way analysis of variance (one-way ANOVA) at $p < 0.05$ unless stated differently using the GraphPad Prism 9 statistical package (GraphPad Software Inc., La Jolla, CA, USA).

3. Results

3.1. U. dulcis extraction and isolation

The EtOAc extract of the root, leaf, and twig extract of *U. dulcis* were separated by several chromatographic techniques resulting in the isolation of 12 compounds (Fig. 1), including one terpenoid (**1**), three chalcones (**2**–**4**), five flavonoids (**5**–**8** and **10**), one alkaloid (**9**), and two flavan-3-ols (**11** and **12**). The known compounds were identified as patchoulenon (**1**) [19], polygochalcone (**2**) [20], 2′3′-dihydroxy-4′,6′-dimethoxydihydrochalcone (**3**) [12], 2′,3′-dihydroxy-4′,6′-dimethoxychalcone (**4**) [16], chrysin (**5**) [21], techochrysin (**6**) [22], 8-hydroxy-5,7-dimethoxyflavanone (**7**) [23], pinocembrin (**8**) [24], 3-farnesyl indole (**9**) [25], onysilin (**10**) [26], cinchonain la (**11**), and cinchonain lb (**12**) [27] by comparisons made with the literature reported spectroscopic data.

3.2. Anti-DM activities determination of crude U. dulcis and its isolated compounds

3.2.1. α-Glucosidase inhibitory assay

As shown in Table 1, the leaf crude extract demonstrated greater α-glucosidase inhibition than the twig crude extract. Interestingly, both crude extracts exhibited IC_{50} values approximately 10–20 times lower than the standard drug (Acarbose), which had an IC_{50} of 361.35 ± 1.75 μg/mL. These results highlight the potential of crude *U. dulcis* extracts as anti-α-glucosidase activity agents.

Each of the isolated compounds was tested at a concentration of 10 μg/mL and displayed potent anti-α-glucosidase activity within the inhibition range of 18.40–45.16%. Cinchonain la exhibited the highest α-glucosidase inhibition at 45.16 ± 1.87%, followed by cinchonain lb, 2′,3′-dihydroxy-4′,6′-dimethoxychalcone, pinocembrin, and polygochalcone. Cinchonain la and cinchonain lb demonstrated greater anti-α-glucosidase activity than the standard drug (Acarbose), with IC_{50} values of 11.88 ± 1.41 μg/mL and 15.18 ± 1.19 μg/mL, respectively. Cinchonain is a flavonolignan and appears to be a potent bioactive compound in *U. dulcis* in terms of its α-glucosidase inhibitory activity, suggesting its potential for reducing glucose absorption and postprandial hyperglycemia.

3.2.2. DPP-IV inhibitory assay

The potential anti-DPP-IV activity of the crude extracts and isolated compounds of *U. dulcis* was evaluated. As illustrated in Table 2, all test samples at a concentration of 50 μg/mL exhibited varying anti-DPP-IV enzyme activity, ranging from 23.66 to 97.37%. Of these, 2′,3′-dihydroxy-4′,6′-dimethoxychalcone demonstrated the greatest inhibition at 97.37 ± 1.01%, which was superior to that of the positive control of diprotin A (77.60 ± 1.18%). Although 2′,3′-dihydroxy-4′,6′-dimethoxychalcone demonstrated the highest inhibition, polygochalcone and 2′,3′-dihydroxy-4′,6′-dimethoxydihydrochalcone exhibited the lowest. The different chemical substitutions of these three chalcone derivatives appear to influence their anti-DDP-IV activities. Regarding the IC_{50} values, cinchonain la shown lower potent anti-DPP-IV activity than diprotin A used as a positive control (IC_{50} value of 11.36 ± 0.62 μg/mL). It demonstrated moderate anti-DPP-IV activity, with values of 81.78 ± 1.42 μg/mL. Whereas 2′,3′-dihydroxy-4′,6′-dimethoxychalcone with IC_{50} value of 8.62 ± 1.19 μg/mL demonstrated potent DPP-IV activity higher than diprotin A.

3.3. Cell viability

As presented in Fig. 2, the cell viability of the L6 myotubes was over 80% when the concentration of all samples did not exceed 2.5 μg/mL. A cytotoxic effect was observed when the concentration of the crude extract was 5 μg/mL and cinchonain la was 10 μg/mL. Cinchonain lb and 2′,3′-dihydroxy-4′,6′-dimethoxychalcone did not demonstrate any cytotoxic effects at any tested concentration. These results highlight the safety levels of the samples that could be used for future glucose uptake experiments.

Table 1
α-Glucosidase inhibitory activity of isolated compounds from *U. dulcis*.

No.	Code	Name	% Inhibition at 10 μg/mL	IC_{50} (μg/mL)
1	UDR-C1	Patchoulenon	34.46 ± 1.30	>10
2	UDR-C2	Polygochalcone	37.24 ± 2.80	>10
3	UDL-C1	2′3′-Dihydroxy-4′,6′-dimethoxydihydrochalcone	32.90 ± 1.77	>10
4	UDL-C4	2′,3′-Dihydroxy-4′,6′-dimethoxychalcone	38.04 ± 1.10	>10
5	UDL-C2	Chrysin	27.31 ± 1.04	>10
6	UDL-C8	Techochrysin	31.98 ± 1.07	>10
7	UDL-C3	8-Hydroxy-5,7-dimethoxyflavanone	34.57 ± 1.43	>10
8	UDL-C5	Pinocembrin	37.30 ± 1.03	>10
9	UDT-C1	3-Farnesyl indole	18.40 ± 1.39	>10
10	UDT-C2	Onysilin	28.13 ± 1.46	>10
11	UDT-C5	Cinchonain la	45.16 ± 1.87	11.88 ± 1.41[a]
12	UDT-C9	Cinchonain lb	40.07 ± 0.27	15.18 ± 1.19[a]
13	The twig crude extract	Crude from twig	–	34.47 ± 1.06[a]
14	The leaf crude extract	Crude from leaf	–	16.15 ± 1.20[a]
15	Positive control	Acarbose	–	361.35 ± 1.75

Note: The results were represented as the mean ± SD of three independent determination (N = 3).
[a] Indicates statistical difference, when compared with other samples.

Table 2
DPP-IV inhibitory activity of isolated compounds from *U. dulcis*.

No.	Code	Name	% Inhibition at 50 μg/mL	IC_{50} (μg/mL)
1	UDR-C1	Patchoulenon	23.95 ± 0.17	>50
2	UDR-C2	Polygochalcone	28.92 ± 0.94	>50
3	UDL-C1	2′3′-Dihydroxy-4′,6′-dimethoxydihydrochalcone	23.66 ± 3.48	>50
4	UDL-C4	2′,3′-Dihydroxy-4′,6′-dimethoxychalcone	97.37 ± 1.01[a]	8.62 ± 1.19[a]
5	UDL-C2	Chrysin	41.19 ± 1.03	>50
6	UDL-C8	Techochrysin	26.10 ± 3.05	>50
7	UDL-C3	8-Hydroxy-5,7-dimethoxyflavanone	27.75 ± 2.96	>50
8	UDL-C5	Pinocembrin	30.38 ± 1.90	>50
9	UDT-C1	3-Farnesyl indole	27.07 ± 0.73	>50
10	UDT-C2	Onysilin	24.54 ± 2.38	>50
11	UDT-C5	Cinchonain la	41.29 ± 2.53	81.78 ± 1.42
12	UDT-C9	Cinchonain lb	37.78 ± 1.01	>50
13	The twig crude extract	Crude from twig	43.43 ± 1.75	>50
14	The leaf crude extract	Crude from leaf	41.28 ± 1.07	>50
15	Positive control	Diprotin A	77.60 ± 1.18[a]	11.36 ± 0.62[a]

Note: The results were represented as the mean ± SD of three independent determination (N = 3).
[a] Indicates statistical difference, when compared with other samples.

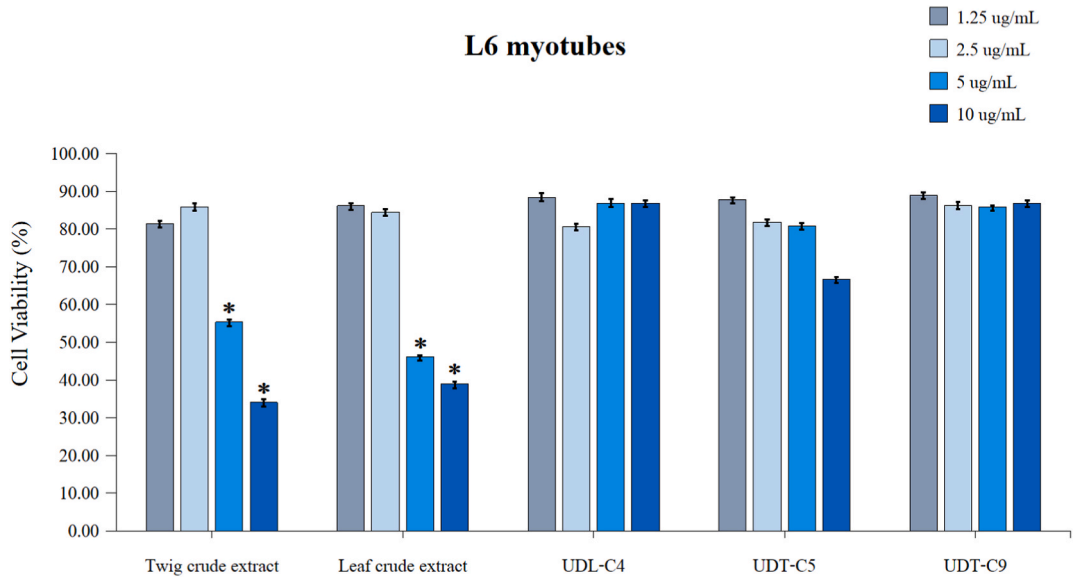

Fig. 2. Cell viability of L6 myotubes by MTT assay. Cell viability (%) after treating L6-myotubes with the twig and leaf extracts of *U. dulcis* and its bioactive compounds UDL-4 (2′,3′-dihydroxy-4′,6′-dimethoxychalcone), UDT-C5 (cinchonain la), and UDT-C9 (cinchonain lb) at various concentration (1.25, 2.5, 5, 10 μg/mL). *$p < 0.05$ when compared to the other concentrations of the same compound.

3.4. Glucose uptake in L6 myotube cells

The crude extracts and their three potent compounds that were evaluated in the current study are presented in Fig. 3. All test compounds enhanced glucose uptake in L6 myotube cells in a dose-dependent manner. However, at high concentrations of both the crude extracts (5 and 10 μg/mL) and cinchonain la (10 μg/mL), glucose uptake was not activated due to cytotoxicity. Cinchonain lb displayed the highest glucose uptake stimulation (80.93 ± 3.26%) at its highest concentration (10 μg/mL); however, this was not statistically different from the positive controls of 100 nM insulin (88.44 ± 1.04%), and 2′,3′-dihydroxy-4′,6′-dimethoxychalcone (74.77 ± 1.19%). At a concentration of 5 μg/mL, cinchonain lb also demonstrated potent glucose uptake stimulation compared with the other compounds at any concentration and the positive control.

4. Discussions

This study explored the anti-DM potential of both the crude extracts of *U. dulcis* and its 12 pure isolated compounds through various mechanisms to advance healthcare product development. We investigated the anti-DM potential of the crude extracts and isolated compounds of *U. dulcis* based on their anti-α-glucosidase activity. Glucosidase inhibitors, including acarbose, voglibose, and miglitol,

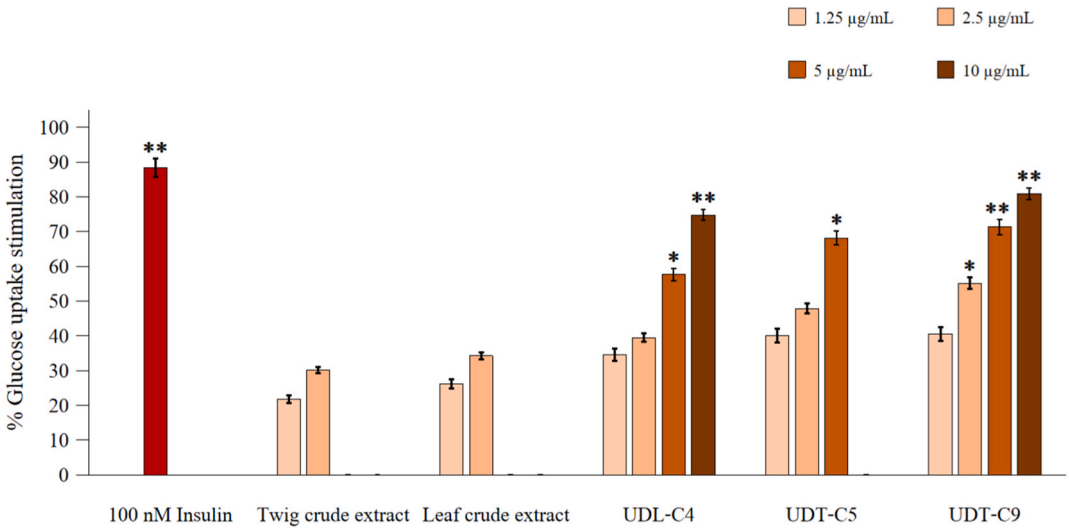

Fig. 3. Glucose uptake stimulation by glucose oxidase assay. Percentage of glucose uptake stimulation after treating L6-myotubes with insulin (100 nM), the twig and leaf extracts of *U. dulcis* and its bioactive compounds UDL-4 (2′,3′-dihydroxy-4′,6′-dimethoxychalcone), UDT-C5 (cinchonain la), and UDT-C9 (cinchonain lb) at various concentration (1.25, 2.5, 5, 10 µg/mL). Values with a different symbol are significantly different ($p < 0.05$) and values having the same symbol are not statistically significant.

are a class of anti-DM drugs frequently recommended in combination therapies for blood glucose level management. They function by competitively inhibiting the α-glucosidase enzyme at the brush border of the small intestine, delaying complex carbohydrate digestion. This reduces the absorption of glucose, resulting in decreased post-meal hyperglycemia. Moreover, we demonstrated anti-DPP-IV activity of the crude extracts and its isolated compounds. DPP-IV is an enzyme of the serine protease class that is naturally produced in the intestine. It is associated with incretin hormone degradation, which is a gut hormone that helps regulate glucose homeostasis after oral intake. DPP-IV inhibition increased active incretin hormone levels, leading to elevated insulin secretion [28–30]. Sitagliptin, linagliptin, saxagliptin, and alogliptin are examples of synthetic drugs belonging to the class of DPP-IV inhibitors. They play a crucial role in regulating blood glucose levels among diabetic patients when used in combination with other anti-DM medications. To confirm the anti-DM potential of *U. dulcis*, the glucose uptake of L6 myotubes cells was used as a model of insulin function. Metformin is the first line drug for patients with type 2 diabetes [31]. Its primary mechanism of action involves stimulating the activation of AMP-activated protein kinase (AMPK), which, in turn, promotes increased cellular glucose uptake in the muscles, liver, and adipose tissues.

Based on our results, cinchonain la, cinchonain lb, and 2′,3′-dihydroxy-4′,6′-dimethoxychalcone were selected for cell viability and glucose uptake assessment due to their potent inhibition of either α-glucosidase or DPP-IV enzyme compared to the other pure compounds. Therefore, these compounds could be recommended as potent lead compounds for the further development of anti-DPP-IV agents or the novel anti-DM drugs that target glucose uptake stimulation. This is the first preliminary report on the impact of *U. dulcis* extracts and their bioactive compounds on cellular glucose uptake. Our findings are consistent with those of a previous study that reported that cinchonain lb exerts anti-DM activity by enhancing insulin secretion in a rat insulinoma cell (INS-1) model [32]. Cinchonain la, cinchonain lb, and 2′,3′-dihydroxy-4′,6′-dimethoxychalcone have also exhibited antioxidant and anti-inflammation activities through various mechanisms, which could alleviate the complications associated with long-term DM [33–35]. The isolated compounds, in particular, could serve as chemical structure candidates for new drug development. However, using the crude extracts from twigs and leaves may be superior to using the isolated compounds alone due to the synergistic interactions between the various compounds present in the extracts [36].

5. Conclusions

This study was the first investigation into the potential anti-DM properties of *U. dulcis* and its isolated compounds, which were explored through various mechanisms. We extracted and isolated compounds from the roots, twigs, and leaves of *U. dulcis* using a variety of chromatographic methods, yielding 12 pure isolated compounds. The anti-DM potential of these compounds was assessed and compared against crude *U. dulcis* extracts and standard references. Cinchonain la, cinchonain lb, and 2′,3′-dihydroxy-4′,6′-dimethoxychalcone exhibited promising results across various anti-DM activities, including anti-α-glucosidase activity, anti-DPP-IV activity, and glucose uptake stimulation in muscle cells. Our findings suggest that these are potent bioactive compounds derived from *U. dulcis* and could contribute to blood glucose management through diverse pathways. Further experiments are warranted to assess the safety and efficacy of these compounds and their derivatives in the development of anti-DM drugs.

Data availability statement

The original data in this article will be made available on request or directed to the corresponding author.

CRediT authorship contribution statement

Passakorn Teerapongpisan: Writing – original draft, Visualization, Validation, Investigation, Formal analysis, Data curation. **Rachanida Praparatana:** Validation, Investigation, Formal analysis, Data curation. **Benjaporn Noppradit:** Writing – review & editing, Visualization, Investigation. **Surat Laphookhieo:** Writing – review & editing, Writing – original draft, Validation, Supervision, Software, Resources, Project administration, Methodology, Data curation, Conceptualization. **Panupong Puttarak:** Writing – review & editing, Writing – original draft, Validation, Supervision, Software, Resources, Project administration, Methodology, Data curation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Abbreviations

A	absorbance
ANOVA	analysis of variance
°C	degree Celsius
DMEM	Dulbecco's modified Eagle's medium
DMSO	dimethyl sulfoxide
GP-p-NA	Gly-Pro-*p*-nitroanilide
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetra-zolium bromide
nm	nanometer
NMR	nuclear magnetic resonance
NO	nitric oxide
pNPG	*para*-nirophenyl-α-D-glucopyranoside
RPMI	Roswell Park Memorial Institute
UV	ultraviolet

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e26962.

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