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In vitro antidiabetic and advanced glycation end products inhibitory activity of methanol extracts of various *Dendrobium* species

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ABSTRACT

Dendrobium orchids are one of the most varied species. In traditional Chinese medicine, *Dendrobium* has been reported to have an antidiabetic effect and has been used in the preparation of herbal medicines for a long time. This study aimed to investigate the *in vitro* antidiabetic (α-amylase and α-glucosidase assay) activities and advanced glycation end products (AGEs) inhibitory activity of the methanol extracts of 20 *Dendrobium* species. Phytochemical constituents and total phenolic and total flavonoid contents of the extracts were evaluated, as well. The total phenolic content was exhibited to be in the range of 31.73–132.70 mg GAE/g, and total flavonoid content was found in the range of 58.80–237.57 mg QE/g. The highest inhibitory activity of α-amylase was reported in *D. parishii* Rchb.f. (IC_{50} 46.57 µg/ml. The most effective inhibitory activity of α-glucosidase was found in *D. ellipsophyllum* Tang & Wang. (IC_{50} 128.69 µg/ml) and *D. brymerianum* Rchb.f. (IC_{50} 138.27 µg/ml). *Dendrobium brymerianum* Rchb.f. and *D. scabrilingue* Lindl. were shown to have the most potent inhibited AGEs formation with IC_{50} values of 34.44 and 46.95 µg/ml, respectively, compared to aminoguanidine (IC_{50} 49.81 µg/ml). As a result, several *Dendrobium* species displayed strong antidiabetic and AGEs inhibitory activity, and they have the potential for further development.

INTRODUCTION

Diabetes and its complications had affected approximately 415 million people in 2015, and the number of diabetic patients has been projected to grow to 642 million by 2040 (Ogurtsova *et al.*, 2017). An individual with diabetes mellitus has an increase in blood sugar due to faulty insulin from the pancreas or a malfunction in the cells' response to the insulin (Devaki *et al.*, 2016). As a result of long-term hyperglycemia in diabetes mellitus, advanced glycation end products (AGEs) are formed, which can lead to diabetic complications (Singh *et al.*, 2014). To reduce postprandial hyperglycemia, enzyme inhibitors that act on carbohydrate metabolism, such as α-amylase and α-glucosidase, are effective (Wang *et al.*, 2013; You *et al.*, 2012). Recently, there has been much interest in using antidiabetic and antiglycation products for relieving diabetes and its complications. Synthetic drugs have been reported to cause several side effects. Therefore, many studies have been focused on finding alternative therapies, especially plant-derived natural products that are used in managing diabetes and its complications due to low toxicity and low side effects.

Natural phytochemicals found in plants have a multitude of health-promoting properties, including antidiabetic, antioxidant, anti-inflammatory, antibacterial, antihypertensive, and anti-inflammatory activities. A large genus in Orchidaceae, *Dendrobium*, has about 1,400 species distributed throughout the Himalayas, Asia, Australia, Tasmania, and the Pacific Islands (Xu *et al.*, 2006). More than 150 species of this plant genus in Thailand have been identified (Seidenfaden, 1985). Recent pharmacological investigations have shown that the plants of the genus *Dendrobium* produce structurally different components resulting in a wide range of biological properties, including anticancer, antioxidant, antimalarial, and antidiabetic activities (Ng *et al.*, 2012). Inhibitory potential was shown for the whole plant extracts of *D. formosum* Roxb. ex Lindl. against

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α-glucosidase and pancreatic lipase enzymes (Inthongkaew *et al.*, 2017). Two new dihydrophenanthrenes, dendroinfundin A and dendroinfundin B, were isolated from the whole plant of *D. infundibulum* Lindl. and showed strong α-glucosidase inhibitory activity (Na Ranong et al., 2019). In addition, a methanol extract from the root of *D. christyanum* Rchb.f. exhibited α-glucosidase inhibitory activity and glucose uptake stimulatory effect (San *et al.*, 2020); also, the whole plant of *D. scabrilingue* Lindl. displayed the most potent α-glucosidase inhibitory activity (Sarakulwattana *et al.*, 2018). A *Dendrobium* species is considered an important phytochemical used in antidiabetic products; several species have not yet been reported in antidiabetic and antiglycation products. The main aim of this study was focused on investigating the *in vitro* antidiabetic and AGEs inhibitory activity of the methanol extracts of 20 *Dendrobium* species.

MATERIALS AND METHODS

Plant materials and plant extracts

The whole plant of *Dendrobium* orchids was purchased from Chatuchak Market, Bangkok, Thailand, and identified by one of the authors (B. Sritularak). The dried and powdered whole plant of samples was extracted with methanol (MeOH commercial grade) at room temperature by the maceration method. The sample was extracted three times in MeOH using a ratio of 1 kg/10 l. The methanol extracts of 20 species of *Dendrobium* orchids were obtained after removal of the solvent by using an evaporator and desiccator.

Phytochemical screening

Phytochemical analysis

The methanolic extracts of 20 species of *Dendrobium* orchids were subjected to qualitative chemical tests to detect the presence of various classes of phytoconstituents, including alkaloids, flavonoids, anthraquinones, coumarins, saponins, tannins, terpenoids, steroids, and glycosides. The qualitative analysis of secondary metabolites was carried out by following the methods of Yadav and Agarwala (2011) and Singh *et al*. (2015).

Quantification of bioactive compounds

The bioactive compounds phenols and flavonoids were quantified according to the standard procedure in a 96-well plate with a microplate reader (BioTek, USA Model Epoch 2 Gen5). A total of 20 species of *Dendrobium* orchids extracts were subjected to quantify the total phenolic and total flavonoid contents. The total phenolic contents in the *Dendrobium* orchids extracts were measured using the Folin-Ciocalteu method explained by a modified Miliauskas *et al*. (2004) method and were reported as mg gallic acid equivalents (GAE) per gram of dry extract. The total flavonoid content of *Dendrobium* orchids extracts was considered by the aluminum chloride colorimetric assay by a modified Chatatikun and Chiabchalard (2013) method and was reported as mg quercetin equivalents (QE) per gram of dry extract.

In vitro antidiabetic assays

α-amylase inhibitory assay by CNPG3

The *in vitro* α-amylase inhibition activity of *Dendrobium* orchids extracts from 20 species was determined based on the 2-chloro-4-nitrophenol (CNP) colorimetric assay using acarbose as the positive control by a modified Kumar *et al*. (2011) method. The plant extracts were dissolved in a sodium phosphate buffer (pH 6.9) to give concentrations in the range of 6.25–100 µg/ml. The enzyme α-amylase solution was prepared by dissolving of 0.5 U/ml of α-amylase in 40 mmol/l phosphate buffer, pH 6.9. The assays were conducted by mixing 80 µl of *Dendrobium* orchids extracts, 20 µl of the α-amylase solution, and 50 µl of CNPG3. The mixture was incubated at 37°C for 10 minutes. Finally, 50 µl of a 1 mM Na_2CO_3 solution was added to terminate the reaction. The absorbance was measured at 405 nm with a microplate reader (BioTek, USA Model Epoch 2 Gen5). A control reaction without the plant extract/acarbose was also conducted. From the graph, the IC_{50} values for α-amylase inhibition were determined based on the percent inhibition plotted against extract concentration.

α-glucosidase inhibition assay

The activity of α-glucosidase was measured as described previously, with slight modifications (Inthongkaew *et al.*, 2017). By hydrolyzing, specifically by α-glucosidase, p-nitrophenyl-α-D-glucopyranoside (pNPG) can be converted into p-nitrophenol (a yellow-colored product). In brief, 50 µl of 20 species of *Dendrobium* orchids extracts (25–250 µg/ml) was mixed with 80 µl of a 0.1 M phosphate buffer (pH 6.8), and 20 µl of 0.2 U/ml α-glucosidase in a 0.1 M phosphate buffer (pH 6.8) was added to a 96-well plate, and the mixture was preincubated at 37°C for 10 minutes. Then, 50 µl of 2 mM pNPG was added, and the reaction mixture was further incubated for 20 minutes. Finally, 50 µl of a 0.1 M Na_2CO_3 solution was added to terminate the reaction. The absorbance was measured at 405 nm with a microplate reader (BioTek, USA Model Epoch 2 Gen5). Acarbose was used as the positive control. IC_{50} values were calculated by plotting percent inhibition of α-glucosidase against the extract.

Inhibition of AGEs formation

The inhibitory effects of the extracts of 20 *Dendrobium* orchids species on AGEs formation were analyzed by a modified Jung *et al*. (2015) method. The AGEs reaction solution that consisted of bovine serum albumin (BSA) (10 mg/ml), D-glucose (0.2 M), D-fructose (0.2 M), and 0.02% *w/v* of sodium azide in a phosphate buffer (0.05 M, pH 7.4) was prepared. The reaction mixture (900 µl) of the AGEs reaction solution was incubated at 37°C for 7 days (in the dark) with or without 100 µl of *Dendrobium* orchids extracts (31.25–250 µg/ml) dissolved in the phosphate buffer. Aminoguanidine was used as the positive control (15.625–250 µg/ml). The fluorescence intensity of the reaction products was evaluated using a fluorescence spectrometer (PerkinElmer model LS-55) with respective excitation and emission wavelength at 355 and 450 nm. Based on the graph, the IC_{50} of the extracts were measured based on the percent inhibition of AGEs formation.

Statistical analysis

At least three experiments were performed, and the results were expressed as mean ± SD. Statistically significant differences were determined by Student's *t*-test, and *p*-values < 0.05 were considered significantly different.

RESULTS

Phytochemical constituents

Various species of *Dendrobium* orchids in this research are shown in Figure 1. The dried and powdered whole plant of

20 species of *Dendrobium* orchids was macerated with MeOH to obtain the MeOH extracts. The preliminary phytochemicals were identified in the MeOH extracts using various chemical tests. The presence of phytochemicals is shown in Table 1. The methanolic extract of 20 species of *Dendrobium* orchids shows the presence of major phytoconstituents like alkaloids, flavonoids, coumarins, tannins, terpenoids, and glycosides.

Total phenolic and flavonoid contents

The total phenolic and total flavonoid contents in the whole plant of 20 *Dendrobium* orchids extracts are presented in Table 2; it could be noticed that *D. formosum* Roxb. ex Lindl. showed the highest phenolic compounds at a value of 132.70 ± 3.41 mg GAE/g. The total phenolic contents of *D. tortile* Lindl., *D. williamsonii* Day & Rchb. f., *D. scabrilingue* Lindl., and *D. bellatulum* Rolfe. were evaluated in the range of 111.48–119.92 mg GAE/g.

The highest total flavonoid content was recorded in the methanol extract of *D. brymerianum* Rchb.f. at a value of 237.57 ± 1.19 mg QE/g, followed by *D. tortile* Lindl. and *D. scabrilingue* Lindl. at the values of 228.82 ± 7.64 mg QE/g and 214.93 ± 1.43 mg QE/g, respectively. Moreover, the total flavonoid content in the high value was found in *D. formosum* Roxb. ex Lindl., *D. williamsonii* Day & Rchb. f., and *D. ochreatum* Lindl. in the range from 179.51 to 197.73 mg QE/g.

***In vitro* α-amylase inhibitory activity**

In the preliminary evaluation, the α-amylase inhibitory activity of *Dendrobium* orchids extracts at the concentration of 100 µg/ml exhibited higher than 70% inhibition (Fig. 2). The IC_{50} values were calculated (Table 3). The MeOH extract of *D. parishii* Rchb.f. exhibited the lowest IC_{50} of 46.57 ± 0.33 µg/ml, and the IC_{50} values of the *D. albosanguineum* Lindl., *D. ochreatum* Lindl., and *D. brymerianum* Rchb.f. extracts were 51.87 ± 0.61, 54.45 ± 0.47, and 67.12 ± 0.41 µg/ml, respectively. The standard positive control, acarbose, showed an IC_{50} of 17.43 ± 0.67 µg/ml.

***In vitro* α-glucosidase inhibitory activity**

Six methanolic extracts of *Dendrobium* orchids exhibited higher than 50% of α-glucosidase inhibitory activities at the concentration of 250 µg/ml (Fig. 3). The IC_{50} values were calculated (Table 3). Surprisingly, the α-glucosidase inhibitory potential of *D. ellipsophyllum* Tang & Wang. (IC_{50} 128.69 ± 1.16 µg/ml) and *D. brymerianum* Rchb.f. (IC_{50} 138.27 ± 1.60 µg/ml) was significantly higher than acarbose (IC_{50} 166.83 ± 1.96 µg/ml) of the analyzed standard positive control. In addition, the MeOH extract of *D. signatum* Rchb. f. and *D. scabrilingue* Lindl. was characterized by the high inhibitory potential with IC_{50} of 166.86 ± 1.26 and 169.96 ± 0.83 µg/ml, respectively.

The formation of AGEs inhibition

The methanol extracts of all *Dendrobium* orchids, excluding the five crude extracts of *D. tortile* Lindl., *D. cretaceum* Lindl., *D. palpebrae* Lindl., *D. formosum* Roxb. ex Lindl., and *D. williamsonii* Day & Rchb. f., inhibited the formation of AGEs in a dose-dependent manner (data not shown). The IC_{50}

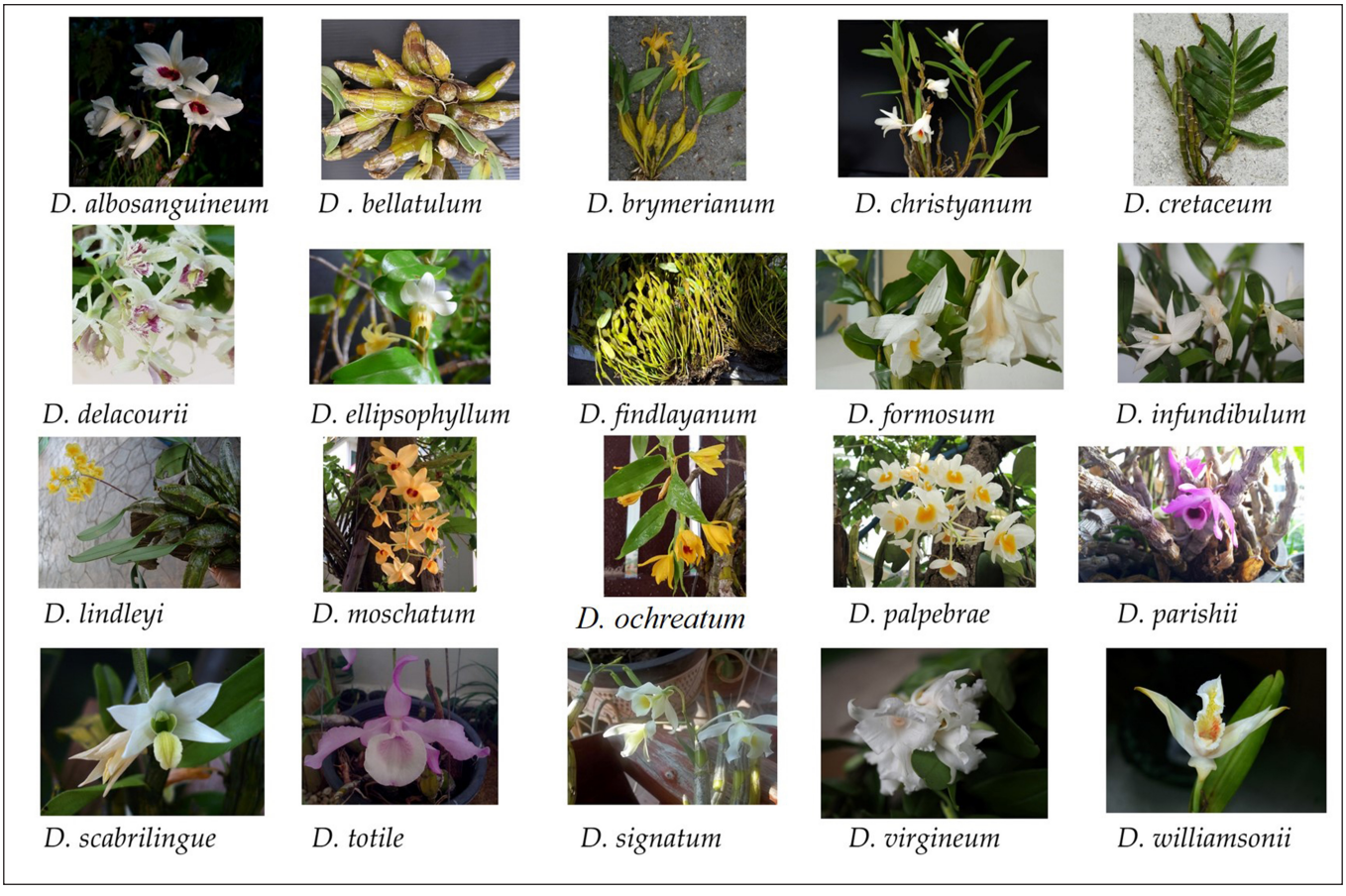

Figure 1. *Dendrobium* orchid species used in this study.

values of AGEs formation inhibitory activities are presented in Table 4 and Figure 4. The inhibition for the formation of AGEs of *D. brymerianum* Rchb.f. (IC_{50} 34.44 ± 1.29 µg/ml) and *D. scabrilingue* Lindl. (IC_{50} 46.95 ± 0.91 µg/ml), involved in the inhibition of the sugar-adding reaction of BSA molecules or anti-AGEs, is more effective than aminoguanidine (IC_{50} 49.81 ± 4.50 µg/ml). Moreover, the MeOH extracts of *D. infundibulum* Lindl., *D. albosanguineum* Lindl., and *D. ellipsophyllum* Tang & Wang. were considered by the high AGEs inhibitory potential with IC_{50} of 51.50 ± 2.25, 55.00 ± 2.06, and 59.05 ± 1.62 µg/ml, respectively.

Table 1. Phytochemical constituents of the methanolic extract of 20 species of *Dendrobium* orchids.

Phytochemical constituents	Methanolic extract of 20 species of *Dendrobium* orchids
Alkaloids	+
Flavonoids	+
Anthraquinones	−
Coumarins	+
Saponins	−
Tannins	+
Terpenoids	+
Steroids	−
Glycosides	+

(+): detected, (−): not detected.

In this report, a strong AGEs inhibitory effect of the nine methanol extracts of *Dendrobium* orchids was indicated, as well (IC_{50} lower than 100 µg/ml) (Table 4, Fig. 4).

DISCUSSION

In traditional Chinese medicine, the stem of several species of *Dendrobium* known as "Shi-Hu" has been used as a tonic and antipyretic to promote body fluid production and reduce fever (Lam *et al.*, 2015; Xu *et al.*, 2013). Previous studies have shown that *Dendrobium* plants produce many classes of metabolites, including polysaccharides, alkaloids, coumarins, bibenzyls, phenols, phenanthrenes, and terpenoids constituents (Cakova *et al.*, 2017; Xu *et al.*, 2013). Several chemical structures of these compounds showed pharmacological activities, including antioxidant, anticancer, antimicrobial, antifungal, antiherpetic, antidiabetic, anti-inflammatory, and antimalarial activities (Hei *et al.*, 2021). Recently, much research has reported *Dendrobium* possess outstanding antidiabetic activity, for example, *D. nobile* Lindl., *D. loddigesii*, *D. crepidatum*, *D. huoshanense*, *D. officinale* Kimura & Migo, and *D. chrysotoxum* Lindl. (Hei *et al.*, 2021; Lu *et al.*, 2014; Qian *et al.*, 2014; Xu *et al.*, 2019). In the present investigation, many species of *Dendrobium* orchids have never been reported for antidiabetic and inhibitory activity against AGEs formation. In this research, a preliminary of phytochemical constituents was indicated, as well. The methanolic extracts of 20 species of *Dendrobium* orchids were determined to show the presence of major phytoconstituents, like alkaloids, flavonoids, coumarins, tannins, terpenoids, and glycosides (Table 1).

Table 2. Quantitative estimation of phytochemicals.

Dendrobium species	Total phenolic contents (mg GAE/g of extract)	Total flavonoid contents (mg QE/g of extract)
Dendrobium albosanguineum Lindl.	80.41 ± 2.30	129.49 ± 6.39
Dendrobium bellatulum Rolfe.	119.92 ± 7.08	116.63 ± 6.88
Dendrobium brymerianum Rchb.f.	78.88 ± 4.71	237.57 ± 1.19
Dendrobium christyanum Rchb.f.	58.45 ± 7.33	133.86 ± 3.11
Dendrobium cretaceum Lindl.	57.72 ± 4.45	100.06 ± 9.47
Dendrobium delacourii Guill.	77.05 ± 6.97	111.89 ± 8.33
Dendrobium ellipsophyllum Tang & Wang.	31.73 ± 2.26	121.36 ± 7.07
Dendrobium findlayanum Par. & Rchb. f.	48.12 ± 1.58	73.00 ± 1.34
Dendrobium formosum Roxb. ex Lindl.	132.70 ± 3.41	179.51 ± 6.84
Dendrobium infundibulum Lindl.	77.90 ± 4.78	120.57 ± 7.66
Dendrobium lindleyi Steud.	73.38 ± 5.23	122.94 ± 4.92
Dendrobium moschatum (Buch.-Ham.) Sw.	55.27 ± 12.63	58.80 ± 4.73
Dendrobium ochreatum Lindl.	47.32 ± 4.04	197.73 ± 3.62
Dendrobium palpebrae Lindl.	58.70 ± 5.06	87.99 ± 5.02
Dendrobium parishii Rchb.f.	42.43 ± 6.10	117.57 ± 8.41
Dendrobium scabrilingue Lindl.	112.64 ± 4.42	214.93 ± 1.43
Dendrobium signatum Rchb. f.	87.51 ± 8.66	96.03 ± 6.19
Dendrobium tortile Lindl.	111.48 ± 7.52	228.82 ± 7.64
Dendrobium virgineum Rchb.f.	41.51 ± 1.16	144.59 ± 4.19
Dendrobium williamsonii Day & Rchb. f.	112.09 ± 4.30	187.40 ± 9.12

All the findings are displayed as mean ± SD (n = 3).

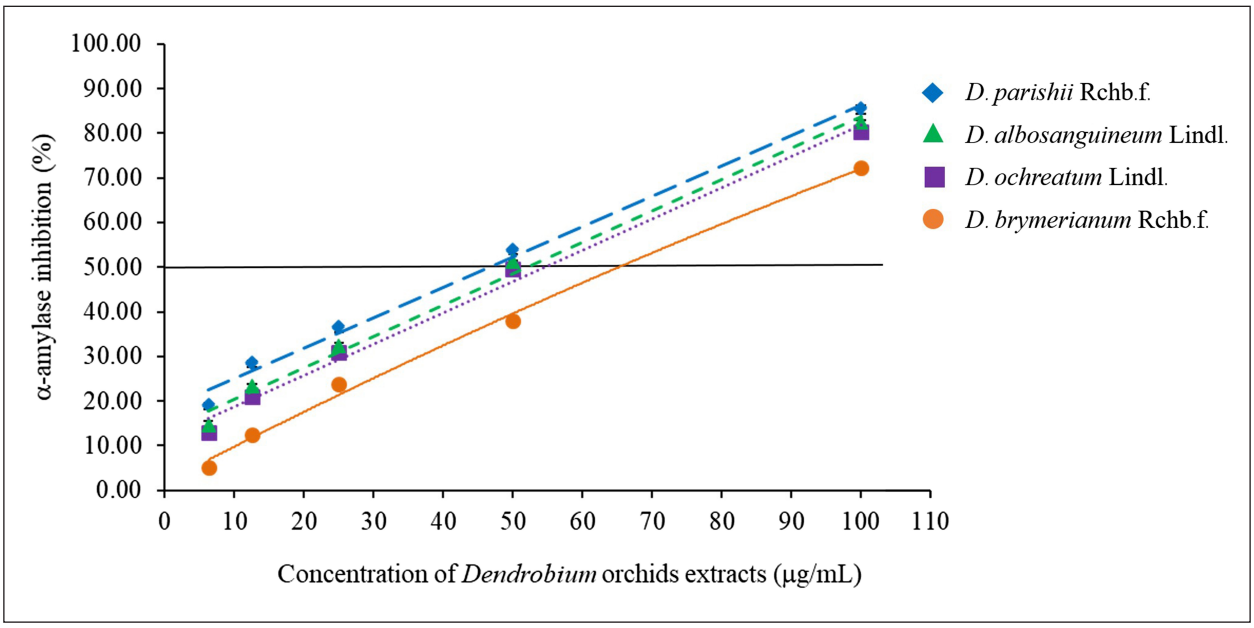

Figure 2. *Dendrobium* orchids extracts were detected for α-amylase inhibitory activity. *D. parishii* Rchb.f. (◆), *D. albosanguineum* Lindl. (▲), *D. ochreatum* Lindl. (■), and *D. brymerianum* Rchb.f. (●), whereas the other *Dendrobium* species were inactive at concentration up to 100 µg/ml.

Table 3. IC_{50} values of the methanolic extracts of 20 species of *Dendrobium* orchids for α-amylase and α-glucosidase inhibitory activities.

***Dendrobium* species**	**α-amylase inhibition**	**α-glucosidase inhibition**
	IC_{50} (µg/ml)	**IC_{50} (µg/ml)**
Dendrobium albosanguineum Lindl.	51.87 ± 0.61^{c}	>250
Dendrobium bellatulum Rolfe.	>100	>250
Dendrobium brymerianum Rchb.f.	67.12 ± 0.41^{e}	138.27 ± 1.60^{b}
Dendrobium christyanum Rchb.f.	>100	>250
Dendrobium cretaceum Lindl.	>100	218.97 ± 1.87^{e}
Dendrobium delacourii Guill.	>100	>250
Dendrobium ellipsophyllum Tang & Wang.	>100	128.69 ± 1.16^{a}
Dendrobium findlayanum Par. & Rchb. f.	>100	>250
Dendrobium formosum Roxb. ex Lindl.	>100	>250
Dendrobium infundibulum Lindl.	>100	>250
Dendrobium lindleyi Steud.	>100	>250
Dendrobium moschatum (Buch.-Ham.) Sw.	>100	>250
Dendrobium ochreatum Lindl.	54.45 ± 0.47^{d}	>250
Dendrobium palpebrae Lindl.	>100	>250
Dendrobium parishii Rchb.f.	46.57 ± 0.33^{b}	>250
Dendrobium scabrilingue Lindl.	>100	169.96 ± 0.83^{c}
Dendrobium signatum Rchb. f.	>100	166.86 ± 1.26^{c}
Dendrobium tortile Lindl.	>100	>250
Dendrobium virgineum Rchb.f.	>100	> 250
Dendrobium williamsonii Day & Rchb. f.	>100	209.57 ± 1.01^{d}
Acarbose	17.43 ± 0.67^{a}	166.83 ± 1.96^{c}

Data with a different letter are significantly different with a p value less than 0.05 ($p < 0.05$).
All the findings are displayed as mean ± SD ($n = 3$).

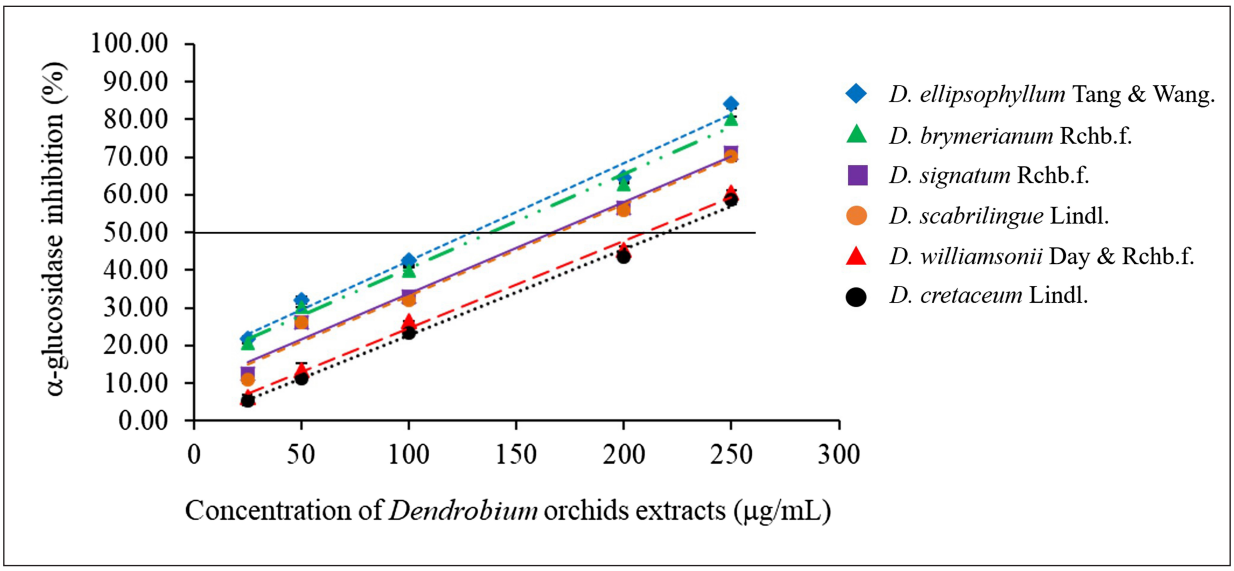

Figure 3. *Dendrobium* orchids extracts were detected for α-glucosidase inhibitory activity. *D. ellipsophyllum* Tang & Wang. (◆), *D. brymerianum* Rchb.f. (▲), *D. signatum* Rchb.f. (■), *D. scabrilingue* Lindl. (●), *D. williamsonii* Day & Rchb.f. (▲), and *D. cretaceum* Lindl. (●), whereas the other *Dendrobium* species were inactive at concentration up to 250 µg/ml.

Table 4. Inhibition of AGEs formation by the methanolic extracts of 20 species of *Dendrobium* orchids.

***Dendrobium* species**	**Inhibition of AGEs formation (IC_{50}) (µg/ml)**
Dendrobium albosanguineum Lindl.	55.00 ± 2.06[b]
Dendrobium bellatulum Rolfe.	129.20 ± 3.93[e]
Dendrobium brymerianum Rchb.f.	34.44 ± 1.29[a]
Dendrobium christyanum Rchb.f.	86.97 ± 2.42[d]
Dendrobium cretaceum Lindl.	>250
Dendrobium delacourii Guill.	185.77 ± 2.97[f]
Dendrobium ellipsophyllum Tang & Wang.	59.05 ± 1.62[c]
Dendrobium findlayanum Par. & Rchb. f.	203.28 ± 1.50[h]
Dendrobium formosum Roxb. ex Lindl.	>250
Dendrobium infundibulum Lindl.	51.50 ± 2.25[b]
Dendrobium lindleyi Steud.	204.10 ± 4.09[h]
Dendrobium moschatum (Buch.-Ham.) Sw.	87.46 ± 2.80[d]
Dendrobium ochreatum Lindl.	87.18 ± 2.58[d]
Dendrobium palpebrae Lindl.	>250
Dendrobium parishii Rchb.f.	194.54 ± 3.82[g]
Dendrobium scabrilingue Lindl.	46.95 ± 0.91[b]
Dendrobium signatum Rchb. f.	89.47 ± 1.29[d]
Dendrobium tortile Lindl.	>250
Dendrobium virgineum Rchb.f.	233.23 ± 5.52[i]
Dendrobium williamsonii Day & Rchb. f.	>250
Aminoguanidine	49.81 ± 4.50[b]

A *p*-value less than 0.05 ($p < 0.05$) indicates that data with a different letter are significantly different. All the findings are displayed as mean ± SD ($n = 3$).

Regarding the total phenolic and flavonoid contents (Table 2), it could be noticed that *D. formosum* Roxb. ex Lindl. contained the highest phenolic compounds at a value of 132.70 ± 3.41 mg GAE/g and the high value of total flavonoid content was recorded in *D. brymerianum* Rchb.f., *D. tortile* Lindl., and *D. scabrilingue* Lindl. at the values of 237.57 ± 1.19, 228.82 ± 7.64, and 214.93 ± 1.43 mg QE/g, respectively. The results support the previous studies; an extract rich in polyphenols from *Dendrobium* was used in mice to treat diabetes (Li *et al.*, 2018). 12 phenolic compounds were isolated from *D. formosum* Roxb. ex Lindl. and showed antidiabetic and antiobesity properties (Inthongkaew *et al.*, 2017). Therefore, phytochemical screening serves as the first initial step to predicting the kinds of potential active compounds from *Dendrobium* plants. Several studies showed that *Dendrobium* has antidiabetic activity and acts as a good inhibitor of crucial enzymes like α-amylase and α-glucosidase associated with type 2 diabetes (Cakova *et al.*, 2017; Hei *et al.*, 2021; Lam *et al.*, 2015). In this report, the methanolic extracts of *Dendrobium* orchids also reveal better *in vitro* enzyme inhibitory activity (α-amylase and α-glucosidase), which are involved in the regulation and absorption of carbohydrates (Table 3). In particular, *D. ellipsophyllum* Tang & Wang. and *D. brymerianum* Rchb.f. exhibited potent α-glucosidase inhibitory activities at the IC_{50} values of 128.69 ± 1.16 and 138.27 ± 1.60 µg/ml, respectively, compared to acarbose (IC_{50} 166.83 ± 1.96 µg/ml). In the cited reference, *D. tortile* Lindl., *D. christyanum* Rchb.f., *D. infundibulum* Lindl., and *D. formosum* Roxb. ex Lindl. exhibited strong α-glucosidase inhibitory activities, which is inconsistent with this study's results. The solvent EtOAc partition of separated extract may result in strong α-glucosidase inhibitory effects (Inthongkaew *et al.*, 2017; Limpanit *et al.*, 2016; Na Ranong *et al.*, 2019; San *et al.*, 2020). Besides, persistent hyperglycemia induces increased formation of AGEs, which play an important role in the pathogenesis of diabetic complications. At this point,

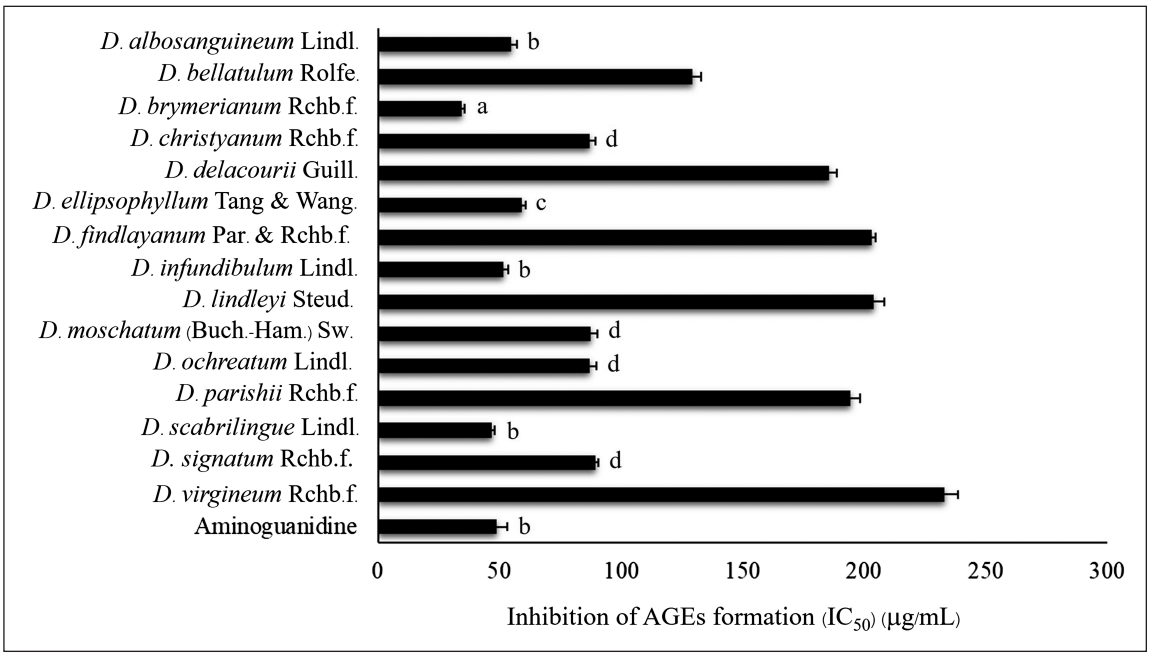

Figure 4. Inhibition of AGEs formation by the methanolic extracts of *Dendrobium* orchids**.** A bar graphical plot of the data expressed as the half maximal inhibitory concentration **(IC_{50}).** *p*- values less than 0.05 ($p < 0.05$) indicate significant differences among data with a different letter.

we investigated the AGEs formation inhibitory activities of 20 species of *Dendrobium* orchids; several species exhibited potent inhibitory activity against AGEs formation. It is interesting to note that *D. brymerianum* Rchb.f. (IC_{50} 34.44 ± 1.29 µg/ml) and *D. scabrilingue* Lindl. (IC_{50} 46.95 ± 0.91 µg/ml) showed higher AGEs inhibitory activities than aminoguanidine, as a reference compound (IC_{50} 49.81 ± 4.50 µg/ml). Furthermore, *D. infundibulum* Lindl., *D. albosanguineum* Lindl., and *D. ellipsophyllum* Tang & Wang. also showed strong AGEs inhibitory potential with IC_{50} values close to that of the standard substance (Table 4). The results from our investigation have established a basis for the future development of inhibition of diabetes and its complications.

CONCLUSIONS

According to the result, the methanol extracts of several *Dendrobium* species are effective against diabetes and AGEs formation. In particular, *D. brymerianum* Rchb.f. has potent inhibitory effects on α-amylase and α-glucosidase and anti-AGEs formation. A further benefit of this study is that it opened up the possibility of new drugs for diabetics with antidiabetic and AGEs inhibitory properties.

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AUTHOR CONTRIBUTION

Boonchoo Sritularak collected and identified plant materials. Boonchoo Sritularak performed the extraction of plant samples. Thaniwan Cheun-Arom initiated and designed the experiments, performed the experiments, and analyzed the data. Thaniwan Cheun-Arom revised the manuscript. The final version of the manuscript was approved by all authors.

CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

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ETHICAL APPROVAL

This study does not involve experiments on animals or human subjects.

DATA AVAILABILITY

All data generated and analyzed are included in this research article.

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