Songklanakarin J. Sci. Technol.
42 (6), 1304-1309, Nov. - Dec. 2020

http://www.sjst.psu.ac.th

Original Article

Microwave-assisted extraction of *Dendrobium nobile* Lindl. and its α-glucosidase inhibitory activity

Chattarika Pengdee[1, 2], Boonchoo Sritularak[3], and Waraporn Putalun[1, 2*]

[1] *Faculty of Pharmaceutical Sciences, Khon Kaen University, Mueang, Khon Kaen, 40002 Thailand*

[2] *Research Group for Pharmaceutical Activities of Natural Products using Pharmaceutical Biotechnology, Faculty of Pharmaceutical Sciences, Khon Kaen University, Mueang, Khon Kaen, 40002 Thailand*

[3] *Department of Pharmacognosy and Pharmaceutical Botany, Faculty of Pharmaceutical Sciences, Chulalongkorn University, Pathum Wan, Bangkok, 10330 Thailand*

Received: 4 August 2019; Revised: 13 September 2019; Accepted: 15 September 2019

Abstract

Diabetes mellitus (DM) is an important metabolic disease due to its worldwide increasing prevalence. *Dendrobium nobile* Lindl. is used in traditional Chinese medicine and has been reported to have antidiabetic activity. In this study, several extraction methods, including microwave-assisted extraction (MAE), were applied to *D. nobile* and the extracts were investigated for α-glucosidase inhibition potency. The optimal MAE conditions maximizing α-glucosidase inhibition were 540 W power, 60 sec extraction time, 40 mL/g solvent-to-feed ratio, and three extraction cycles. Our results show that MAE of *D. nobile* gave stronger inhibition of α-glucosidase with lower IC_{50} and faster extraction than the other extraction methods. IC_{50} of *D. nobile* extract was related to phenolic content with correlation -0.878, indicating that phenolics were the active compounds inhibiting α-glucosidase in these plant extracts.

Keywords: *Dendrobium nobile*, microwave-assisted extraction, Taguchi, α-glucosidase inhibitory activity

1. Introduction

Diabetes mellitus (DM) is a chronic non-communicable disease associated with abnormal metabolism unable to control blood glucose. Metabolic disorders cause complicated conditions such as cardiovascular disease, kidney disease, eye disease, and nerve damage. The World Health Organization reported that diabetic patients have increased from 180 million people in 1980 to 422 million people in 2014. It has been predicted that DM will increase to 642 million people by 2040, by the International Diabetes Federation, so diabetes mellitus is currently a most significant health emergency (International Diabetes Federation, 2015; Roglic & World Health Organization, 2016). The α-glucosidase inhibitors are a class of drugs prescribed in type 2 DM, such that inhibit intestinal α-glucosidase enzyme thereby reducing intestinal carbohydrate digestion and absorption, which lead to lesser glucose absorption into the blood. Acarbose, voglibose, and miglitol are α-glucosidase inhibitors available commercially. However, the drugs are poorly absorbed into the blood and have gastrointestinal side effects (Ajmal Shah, Khalil, Ul-Haq, & Panichayupakaranant, 2017; American Diabetes Association, 2017; Bhupathiraju & Hu, 2016). Therefore, combining an α-glucosidase inhibitor with other types of antidiabetic drugs is the preferred treatment, because this can increase the reducing of HbA1c and postprandial glucose from using α-glucosidase inhibitor alone (Schmeltz & Metzger, 2007; Sonia *et al*., 2014).

*Corresponding author
Email address: waraporn@kku.ac.th

Recently, functional foods that inhibit α-glucosidase have become an alternative dietary supplement for diabetes patients. The stems of various *Dendrobium* species have been used both in medicine and in foods, and they are known as "Shihu" (Lam *et al*., 2015; Teixeira da Silva & Ng, 2017). Shihu has benefits against diseases or symptoms associated with thirst, fever, diabetes, and atrophic gastritis. At present, the China Food and Drug Administration lists *Dendrobium* species as a nutrition food source. There are many functional food alternatives, such as wine, juice, tea, porridge, and soup (Xu & Wang, 2015). For antidiabetic effects, α-glucosidase inhibitory activity of compounds in various species of *Dendrobium* have been reported for phenolic compounds (loddigesiinols G–J, crepidatuol B from *D. loddigesii*, gigantol from *D. devoniannum*, 3,4-dihydroxy-3,4′-dimethoxybibenzyl, dendrofalconerol A from *D. tortile*, batatasin III, dendrosinen B from *D. infundibulum* and 5-Methoxy-7-hydroxy-9,10-dihydro-1,4-phenanthrenequinone from *D. formosum*), alkaloids (shihunine from *D. loddigesii* and anosmine from *D. nobile*), flavonoids (5-hydroxy-3-methoxyflavone-7-O-[β-D-apiosyl-(1→6)]-β-D-glucoside from *D. devoniannum*, (2S)-eriodictyol from *D. tortile*), polysaccharides from *D. devoniannum* and polar extract from *D. loddigesii*, *D. nobile* and *D. officinale* (Chen *et al*., 2018; Inthongkaew *et al*., 2017; Li *et al*., 2018; Limpanit *et al*., 2016; Lu *et al*., 2014; Na Ranong *et al*., 2018; Sun *et al*., 2014). Among these, *D. nobile* is one of the main sources of Shihu and has been listed in the Chinese Pharmacopoeia (Tang & Eisenbrand, 1992; Xu & Wang, 2015). Anosmine and polar extract of *D. nobile* have α-glucosidase inhibitory activity with IC_{50} 17.7 and 396.7 μg/mL, respectively (Chen *et al*., 2018).

The extraction process is an initial substantial step in a medicinal plant study, providing enriched crude extract. Traditional extraction methods such as maceration, water percolation, soxhlet extraction, etc. require large solvent volumes and long extraction times (Azmir *et al*., 2013; Nn, 2015). Microwave-assisted extraction (MAE) is a new extraction method that was developed for increasing extraction efficiency and decreasing the volume of solvent, while shortening the extraction time. In microwave extraction electromagnetic energy is transmitted to plant cells, and it is converted to heat energy by molecular interactions, destroying plant cells. This releases active compounds from the cells (Veggi, Martinez, & Meireles, 2012). MAE reportedly is effective for extracting active compounds, in comparison with other extraction methods. Microwave-assisted extraction gave a higher yield than other extraction methods of gymnemagenin from *Gymnema sylvestres* (Mandal, Dewanjee, & Mandal, 2009) and of triterpenoid saponins from *Ganoderma atrum* (Chen, Xie, & Gong, 2007).

In this study, the MAE of *D. nobile* was optimized, and α-glucosidase inhibitory activity of the obtained extract was studied. Correlations between the substance groups and α-glucosidase inhibition were also investigated.

2. Materials and Methods

2.1 Chemicals and materials

Gallic acid, quercetin, α-glucosidase from *Saccharomyces cerevisiae* (EC 3.2.1.20), and *p*-nitrophenyl-α-D-glucopyranoside (*p*NPG) were purchased from Sigma-Aldrich Chemicals Co. (MO, USA). Aluminium chloride ($AlCl_3$) was purchased from Ajax Finechem Pty Ltd (NSW, Australia). Folin-Ciocalteu reagent was obtained from Loba Chemie Pvt. Ltd. (Mumbai, India). Acarbose was obtained from LKT Laboratories, Inc. (St. Paul, MN, USA). Sodium carbonate (Na_2CO_3) was obtained from Nacalai Tesque, Inc. (Kyoto, Japan). All other solvents were analytical grade.

2.2 Plant material

Dried stems of *D. nobile* were obtained from an agricultural park (Khon Kaen University, Khon Kaen, Thailand). The plant samples were dried at 50°C in an oven for 48 hours. The dry samples were ground into powder and passed through a sieve before extraction.

2.3 Preparation of plant extracts

2.3.1 Microwave-assisted extraction (MAE)

The experiments were performed using a household microwave (MM717CKC, Media) with a frequency of 2,450 MHz and a 700 W maximum power. For MAE, 1 mL of absolute ethanol was added to a 25 mg of sample powder in a closed tube and irradiated with 540 W microwave power for 60 sec. The supernatant was collected. The extraction process was repeated for three cycles. Finally, the extract was allowed to dry under room temperature. The experiment was done in triplicate.

2.3.2 Ultrasonic-assisted extraction (UAE)

The extractions were performed in an ultrasonic bath at 35 kHz frequency. Twenty-five mg of sample powder and 1 mL of absolute ethanol were added into a closed tube, then sonicated for 15 minutes at room temperature. After extraction, the supernatant was collected. The extraction procedure was done in triplicate. The supernatant was dried under room temperature.

2.3.3 Heat extraction (HE)

Twenty-five mg of sample was extracted for 2 hours in a water bath at 50 °C with 1 mL of absolute ethanol. The supernatant was collected and allowed to dry. The extraction was repeated in triplicate.

2.3.4 Shaking extraction (SE)

Twenty-five mg of sample was extracted in a closed tube with 1 mL of absolute ethanol by shaking at room temperature for 6 hours using an orbital shaker at 100 rpm. The extraction was repeated in triplicate by the same method. After extraction, the supernatant was collected and evaporated until dry.

2.3.5 Maceration extraction (ME)

Twenty-five mg of sample was put into a tube, and 1 mL of absolute ethanol was added before closing the tube. The mixture was macerated at room temperature for 24 hours,

then supernatant was collected. The extraction was done for three cycles. The supernatant was allowed to dry under room temperature.

2.4 Experimental design

In MAE many factors affect the efficiency of extraction. The orthogonal array experimental design was chosen for investigating the optimal values of each factor, to achieve the most effective MAE. An L9 (3^4) orthogonal design was created for the experiment. For the various factors, i.e., microwave power, extraction time, solvent-to-feed ratio, and cycle count, three levels were assigned for each. These values of the factors are presented in Table 1. Minitab 18 software, English version (Minitab Inc., State College, PA, USA) was used to find the optimal conditions for extraction by use of the signal-to-noise ratio (S/N), which was calculated from mean and standard deviation. The best condition for extraction had the highest S/N.

Table 1. Manipulated factors and their levels in the experimental design.

Microwave power (w)	Extraction time (sec)	Solvent-to-feed ratio (mL/g)	Extraction cycle (cycle)
380	20	20	1
540	40	30	2
700	60	40	3

2.5 Determination of phenolic content

Phenolic content was determined using the Folin-Ciocalteu method as described previously with some modifications (Sembiring, Elya, & Sauriasari, 2018). Ten microliters of extract, 50 µL of Folin-Ciocalteu reagent, and 40 µL of Na_2CO_3 were mixed and allowed to stand at room temperature in the dark for 30 minutes. The absorbance was measured at 650 nm using a microplate reader (EZ read 400, Biochrom). The results are presented in gallic acid equivalents (GAE) with the unit milligrams per gram of sample by dry weight (mg GA/g DW).

2.6 Determination of flavonoid content

Flavonoid content was determined using the aluminum chloride colorimetric method with modifications (Sembiring *et al*., 2018). Ten microliters of extract, 70 µL of absolute ethanol and 20 µL of $AlCl_3$ were mixed and incubated at room temperature for 30 minutes. The absorbance was detected using a microplate reader (EZ read 400, Biochrom) at 405 nm. The results are presented in quercetin equivalents (Q) with the unit milligrams per gram of sample by dry weight (mg Q/g DW).

2.7 α-Glucosidase inhibitory activity assay

The assay was performed using the modified method of Inthongkaew *et al*. (2017). Briefly, ten microliters of the test sample, 30 µL of α-glucosidase (0.8 U/mL), and 140 µL of potassium phosphate buffer (0.1 M, pH 6.8) were mixed and incubated at 37°C for 15 min. After that, 40 µL of 2 mM *p*NPG was added and reacted for 20 min. Finally, 80 µL of 5 mM Na_2CO_3 was added to stop the reaction. The absorbance was measured at 405 nm using a microplate reader (EZ read 400, Biochrom). The percentage of inhibition was calculated as follow:

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

An enzyme kinetic study was performed by the Linewear-Burk plot to determine the type of inhibition (Inthongkaew *et al*., 2017; Mohamed Sham Shihabudeen, Hansi Priscilla, & Thirumurugan, 2011). The experiment had constant α-glucosidase level at 0.8 U/mL and varied *p*NPG concentration (0.25, 0.5, 1 and 2 mM) in the absence and presence of test samples (5 and 2.5 mg/mL of dry sample).

2.8 Statistical analysis

Statistical differences were analyzed by SPSS 22.0 software (SPSS, Armonk, NY, USA). All experiments were done in triplicates. All results are presented as mean ± SD. The differences between groups were investigated using one-way analysis of variance (ANOVA) with Duncan's multiple range test ($P < 0.05$). The correlation coefficient between the extracted compounds and α-glucosidase inhibitory activity was for Pearson correlation.

3. Results and Discussion

3.1 Optimization of MAE conditions

In microwave-assisted extraction, many factors affect the extraction, such as the choice of solvent system, solvent-to-feed ratio, extraction time, cycle count, microwave power, extraction temperature, contact surface, water content, and stirring (Veggi *et al*., 2012). These factors should be near optimal based on capabilities of the equipment and results of preliminary tests. Our preliminary results indicate that the highest extraction efficacy was obtained using absolute ethanol; therefore, this was selected for the extraction solvent throughout the studies. A nine run experimental design was performed (Table 2). The inhibition of α-glucosidase ranged from 63.67% to 89.70%.

The optimal conditions for extraction were analyzed using the signal-to-noise ratio (S/N). Regarding the inhibition of α-glucosidase, since high inhibition represents high antidiabetic activity, a larger-is-better analysis was chosen. According to the analysis model, the highest S/N indicated the optimal condition for each factor. Our results show that the highest efficacy was achieved with microwave power 540 W, extraction time 60 sec, 40 mL/g solvent-to feed ratio, and extraction for three cycles (Figure 1). Then MAE was run according to these optimal conditions and the inhibition was 98.70 ± 0.63% at a dry sample concentration of 25 mg/mL.

3.2 The mechanism of *D. nobile* extract

Acarbose is a market antidiabetic drug that inhibits the α-glucosidase enzyme by a competitive mechanism (Ag, 1994; Liu, Chen, & Shi, 2015). The mechanism of inhibition of *D. nobile* extract was assessed from the Lineweaver-Burk

Table 2. Design matrix based on the L9 array and the inhibition of α-glucosidase activity (*n*=3).

Run	Microwave power (w)	Extraction time (sec)	Solvent-to-feed ratio (mL/g)	Extraction cycle (cycle)	%Inhibition of dry sample (25 mg/mL)
1	380	20	20	1	63.67 ± 10.22
2	380	40	30	2	74.20 ± 3.80
3	380	60	40	3	86.74 ± 1.99
4	540	20	30	3	86.31 ± 0.23
5	540	60	40	1	85.76 ± 6.69
6	540	40	20	2	88.79 ± 0.53
7	700	20	40	2	82.03 ± 0.79
8	700	60	20	3	89.70 ± 1.15
9	700	40	30	1	84.26 ± 2.19

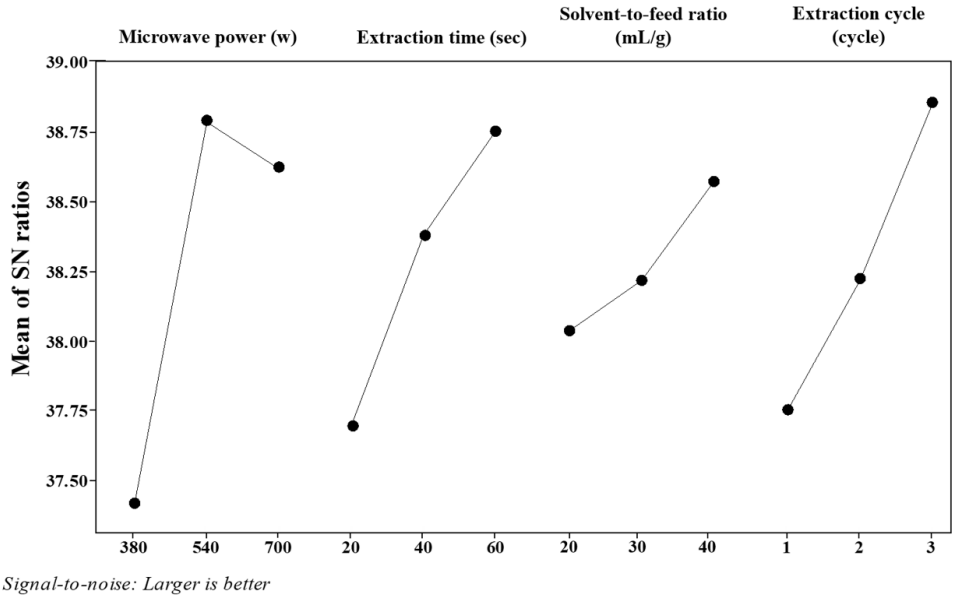

Figure 1. Factor effects shown as means of S/N ratios.

plot with two concentrations of the sample (2.5 and 5 mg/mL of dry sample) and four concentrations of *p*NPG (0.25-2 mM). The results indicate that *D. nobile* extract performs non-competitive inhibition of the enzyme activity (Figure 2). The concentration of *D. nobile* extract increased with decreasing V_{max} but did not affect K_m (Table 3). Hence, acarbose and *D. nobile* extract had different inhibition mechanisms. Therefore, a combination of these compounds might provide synergistic efficacy or allow reducing the dose of acarbose in a further study.

3.3 Comparison of MAE and other extraction methods

In order to assess the MAE method, also other extraction methods were tested to compare the IC_{50} as measure of extract efficacy. Our results indicate that IC_{50} obtained from MAE was below those of other extraction methods. Therefore, MAE provided higher efficacy in α-glucosidase inhibition than the other methods (Table 4). Besides, MAE is also simple and rapid extraction method. These results match the study of Mandal *et al*. (2009) in which MAE gave higher gymnermagenin concentration than the other extraction methods, as well as Chen *et al*. (2007) who found that extraction by MAE got a higher amount of triterpenoid saponins than other tested extraction methods.

3.4 Correlation between α-glucosidase inhibitor activity and phenolic and flavonoid contents

It has been reported that the inhibition of α-glucosidase activity stems from the polar components in *D. nobile* extract (Chen *et al*., 2018). Our preliminary study shows that when increasing the percentage of water in the solvent and extraction is done by microwaves, the α-glucosidase inhibition decreased. The inhibitory activity is expected to be in the semi-polar part; therefore, the correlations of α-glucosidase inhibition with phenolic and flavonoid contents were determined. The correlations of IC_{50} with phenolic and flavonoid contents were -0.878 and -0.785, respectively (Table 4), which are both high correlation levels but stronger for phenolics than for flavonoids. Previous studies have reported the most active α-glucosidase inhibitors in *Dendrobium* spp. as phenolic compounds, such as

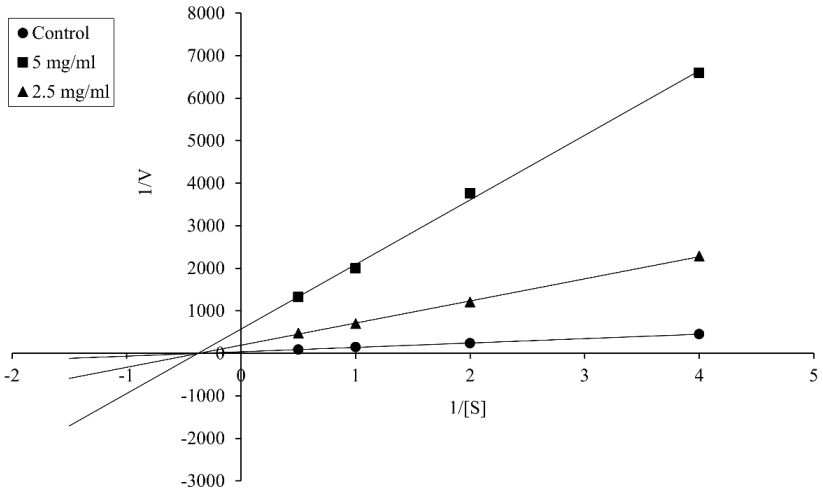

Figure 2. Lineweaver-Burk plot of *D. nobile* extract from the optimal MAE against α-glucosidase at different concentrations of *p*NPG.

Table 3. Kinetic parameters of α-glucosidase inhibition for *D. nobile* extract.

D. nobile extract (mg/mL of dry sample)	V_{max} (ΔA_{405}/min)	Km (mM)
0	0.026	2.7
2.5	0.005	2.7
5	0.002	2.7

Table 4. $IC_{50,}$ and phenolic and flavonoid contents in various extracts (n=5).

Method	IC_{50} of dry sample (mg/mL)	Phenolic content (mg GA/g sample)	Flavonoid content (mg Q/g sample)
MAE	0.36 ± 0.06^{a}	4.12 ± 0.33^{a}	1.17 ± 0.15^{a}
UAE	1.51 ± 0.18^{d}	2.08 ± 0.11^{e}	0.51 ± 0.08^{c}
ME	1.25 ± 0.12^{c}	3.15 ± 0.31^{c}	0.88 ± 0.09^{b}
SE	0.65 ± 0.03^{cd}	2.75 ± 0.08^{d}	0.80 ± 0.18^{b}
Heat	0.81 ± 0.13^{b}	3.78 ± 0.16^{b}	1.32 ± 0.08^{a}
Acarbose	0.3 ± 0.02	-	-
	Correlation coefficient	-0.878	-0.785

Mean ± SD followed by the same letter are not significantly different at P<0.05; Duncan's multiple range test. Correlation coefficients are for Pearson correlation.

loddigesiinols G–J, crepidatuol B from *D. loddigesii*, gigantol from *D. devoniannum*, 3,4-dihydroxy-3,4′-dimethoxybibenzyl, dendrofalconerol A from *D. tortile*, batatasin III, dendrosinen B from *D. infundibulum* and 5-Methoxy-7-hydroxy-9,10-dihydro-1,4-phenanthrenequinone from *D. formosum* (Inthongkaew *et al*., 2017; Limpanit *et al*., 2016; Lu *et al*., 2014; Na Ranong *et al*., 2018; Sun *et al*., 2014), therefore phenolics actively inhibit α-glucosidase activity in *D. nobile* extracts.

4. Conclusions

The optimal MAE conditions maximizing α-glucosidase inhibition were microwave power 540 W, extraction time 60 sec, 40 mL/g of solvent-to feed ratio and three cycles of extraction. The *D. nobile* extract from MAE had lower IC_{50} than extracts from other methods. Accordingly, MAE was the most efficient extraction method for collecting α-glucosidase inhibiting extract. The phenolic content showed a large negative correlation (-0.878) with IC_{50}, suggesting that phenolics are the active compounds inhibiting α-glucosidase in *D. nobile* extracts.

Acknowledgements

This research was supported by Faculty of Pharmaceutical Sciences, Khon Kaen University, Thailand, National Research Council of Thailand (NRCT), and the Thailand Research Fund (Grant no. IRN61W0005).

References

Ag, H. (1994). Pharmacology of α-glucosidase inhibition. *European Journal of Clinical Investigation, 24*, 3-10.

Ajmal Shah, M., Khalil, R., Ul-Haq, Z., & Panichayupakaranant, P. (2017). α-glucosidase inhibitory effect of rhinacanthins-rich extract from *Rhinacanthus nasutus* leaf and synergistic effect in combination with acarbose. *Journal of Functional Foods, 36*, 325–331.

American Diabetes Association. (2017). Pharmacologic approaches to glycemic treatment. *Diabetes Care, 40*(Suppl. 1), 64-74.

Azmir, J., Zaidul, I. S. M., Rahman, M. M., Sharif, K. M., Mohamed, A., Sahena, F., . . . Omar, A. K. M. (2013). Techniques for extraction of bioactive

compounds from plant materials: A review. *Journal of Food Engineering, 117*(4), 426–436.

Bhupathiraju, S. N., & Hu, F. B. (2016). Epidemiology of obesity and diabetes and their cardiovascular complications. *Circulation Research, 118*(11), 17 23–1735.

Chen, H., Li, X., Xu, Y., Lo, K., Zheng, H., Hu, H., . . . Lin, Y. (2018). Study on the polar extracts of *Dendrobium nobile*, *D. officinale*, *D. loddigesii*, and *Flickingeria fimbriata*: metabolite identification, content evaluation, and bioactivity assay. *Molecules, 23*(5), 1185.

Chen, Y., Xie, M. Y., & Gong, X. F. (2007). Microwave-assisted extraction used for the isolation of total triterpenoid saponins from *Ganoderma atrum*. *Journal of Food Engineering, 81*(1), 162–170.

International Diabetes Federation. (2015). *IDF diabetes atlas*. Brussels, Belgium: International Diabetes Federation.

Inthongkaew, P., Chatsumpun, N., Supasuteekul, C., Kitisripanya, T., Putalun, W., Likhitwitayawuid, K., & Sritularak, B. (2017). α-glucosidase and pancreatic lipase inhibitory activities and glucose uptake stimulatory effect of phenolic compounds from *Dendrobium formosum*. *Revista Brasileira de Farmacognosia, 27*(4), 480–487.

Lam, Y., Ng, T. B., Yao, R. M., Shi, J., Xu, K., Sze, S. C. W., & Zhang, K. Y. (2015). Evaluation of chemical constituents and important mechanism of pharmacological biology in *Dendrobium* plants. *Evidence-Based Complementary and Alternative Medicine, 2015*(1), 1–25.

Li, T., Li, Y., Peng, J., Xie, Q., Ruan, R., & Huang, X. (2018). Microwave puffing assisted extraction of polysaccharides from *Dendrobium devonianum*. *Journal of Food Processing and Preservation, 42*(2), 13490.

Limpanit, R., Chuanasa, T., Likhitwitayawuid, K., Jongbun prasert, V., & Sritularak, B. (2016). α-glucosidase inhibitors from *Dendrobium tortile*. *Records of Natural Products, 10*, 609-616.

Liu, D. M., Chen, J., & Shi, Y. P. (2015). An online immobilized α-glucosidase microreactor for enzyme kinetics and inhibition assays. *RSC Advances, 5*(70), 56841–56847.

Lu, Y., Kuang, M., Hu, G. P., Wu, R. B., Wang, J., Liu, L., & Lin, Y. C. (2014). Loddigesiinols G–J: α-glucosidase inhibitors from *Dendrobium loddigesii*. *Molecules, 19*(6), 8544–8555.

Mandal, V., Dewanjee, S., & Mandal, S. C. (2009). Microwave-assisted extraction of total bioactive saponin fraction from *Gymnema sylvestre* with reference to gymnemagenin: a potential biomarker. *Phytochemical Analysis, 20*(6), 491–497.

Mohamed Sham Shihabudeen, H., Hansi Priscilla, D., & Thirumurugan, K. (2011). Cinnamon extract inhibits α-glucosidase activity and dampens postprandial glucose excursion in diabetic rats. *Nutrition and Metabolism, 8*(1), 46.

Na Ranong, S., Likhitwitayawuid, K., Mekboonsonglarp, W., & Sritularak, B. (2018). New dihydrophenanthrenes from *Dendrobium infundibulum*. *Natural Product Research*, 1–7.

Nn, A. (2015). A review on the extraction methods use in medicinal plants, principle, strength and limitation. *Medicinal and Aromatic Plants, 4*(3), 1–6.

Roglic, G., & World Health Organization. (2016). *Global report on diabetes*. Geneva, Switzerland: World Health Organization.

Schmeltz, L., & Metzger, B. (2007). Diabetes/syndrome X. *Comprehensive Medicinal Chemistry II, 6*, 417–458.

Sembiring, E. N., Elya, B., & Sauriasari, R. (2018). Phytochemical screening, total flavonoid and total phenolic content and antioxidant activity of different parts of *Caesalpinia bonduc* (L.) Roxb. *Pharmacognosy Journal, 10*(1), 123-127.

Sonia, T. A., & Sharma, C. P. (2014). *Oral delivery of insulin*. Amsterdam, Netherlands: Elsevier.

Sun, J., Zhang, F., Yang, M., Zhang, J., Chen, L., Zhan, R., . . . Chen, Y. (2014). Isolation of α-glucosidase inhibitors including a new flavonol glycoside from *Dendrobium devonianum*. *Natural Product Research, 28*(21), 1900–1905.

Tang, W., & Eisenbrand, G. (1992). *Chinese drugs of plant origin*. Berlin, Germany: Springer.

Teixeira da Silva, J. A., & Ng, T. B. (2017). The medicinal and pharmaceutical importance of *Dendrobium* species. *Applied Microbiology and Biotechnology, 101*(6), 2227–2239.

Veggi, P. C., Martinez, J., & Meireles, M. A. A. (2012). Fundamentals of microwave extraction. In F. Chemat & G. Cravotto (Eds.), *Microwave-assisted Extraction for Bioactive Compounds* (pp. 15–52). Boston, MA: Springer US.

Xu, H., & Wang, Z. (2015). *Dietary Chinese herbs*. Vienna, Austria: Springer.