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Original Research Article

Phytochemical Screening, Antioxidant Activity and α-Glucosidase Inhibitability of *Bauhinia × blakeana* Dunn Leaf and Flower Extracts from Vietnam

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ABSTRACT

Bauhinia × blakeana Dunn is traditionally used as vegetables, medicinal herbs to treat diseases, and ornamental trees. The current study was performed to evaluate the phytochemicals, total phenolic (TPC) and total flavonoid (TFC) contents, antioxidant activity, and α-glucosidase inhibitability of *B× blakeana* leaf and flower from Vietnam. Qualitative phytochemical analysis showed positive results for carbohydrates, organic acids, carotenoids, steroids, cardiac glycosides, triterpenoids, saponins, flavonoids, and tannins. The TPC and TFC obtained from *B× blakeana* leaf were 99.81 ± 0.94 (mg GAE/g E) and 114.93 ± 1.06 (mg RE/g E), respectively, whereas they were 40.45 ± 0.50 (mg GAE/g E) and 59.61 ± 0.85 (mg RE/g E) from *B× blakeana* flower. The DPPH radical scavenging capacity assay of the crude ethanolic extracts of *B× blakeana* leaf (IC_{50} = 266.52 ± 1.03 µg/mL; R^2 = 0.9864) and flower (IC_{50} > 1000 µg/mL) was determined. The leaf and flower extracts showed significant α-glucosidase inhibitory activity with IC_{50} values of 61.88 ± 0.19 µg/mL (R^2 = 0.9838), 190.79 ± 0.18 µg/mL (R^2 = 0.9891), respectively. These results indicated that the extract of *B× blakeana* leaf and flower can be a promising candidate for alternative drug or functional food discovery, and nutritional recommendations for the control of oxidative stress and diabetes.

Keywords: antioxidant, *Bauhinia × blakeana*, phytoconstituents, α-glucosidase, polyphenols.

Introduction

Reactive oxygen species (ROS; e.g., $^{\bullet}O_2^-$, $HO_2^{\bullet}$, H_2O_2, $OH^{\bullet}$) and reactive nitrogen species (RNS; e.g., $NO^{\bullet}$, $NO_2^{\bullet}$) are free radicals that are produced by the human body when exposed to cellular stressors such as infections, diseases, toxins, or nutritional imbalances.[1] Normally, the formation of ROS/RNS is a physiological process that helps cells to reduce their oxygen demand.[2] Oxidative stress occurs when there is an excess production and/or an insufficient removal of these free radicals, and the consequence of this process is cellular damage, physiological dysfunction, and aging.[1,3] Among the disorders and diseases associated with oxidative stress, diabetes mellitus (DM) is gaining more interest because of its prevalence and complications that can cause disability and even death. DM is a group of common metabolic disorders whose main pathophysiological mechanism is hyperglycemia. There are two main types of DM, which are DM type 1, caused by the destruction of pancreatic β-cells, and DM type 2, characterized by the combination of peripheral insulin resistance and a gradual decrease in insulin secretion.[4]

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According to the International Diabetes Federation (IDF), about 10.5% of the global population (approximately 536.6 million people) aged 20–79 was suffering from DM in 2021, and the number was expected to rise to 12.2% (approximately 738.2 million people) in 2045.[5] Although mortality and disability-adjusted life-years (DALYs) rates for type 1 DM have been decreasing in recent years, these rates for type 2 DM have been increasing worldwide.[6]

The α-glucosidase inhibitors (αGIs), such as acarbose, miglitol, and voglibose, are among the pharmacological therapies used to treat DM. αGIs act as a competitive and reversible inhibitor of glucosidase in the small intestine, resulting in the blockage of degenerating polysaccharides into glucose and the prevention of glucose absorbance in the small intestine.[7,8] For about three decades, αGIs have been used to treat postprandial hyperglycemia, and they are now likely to be considered as the first-line treatment in type 2 DM.[8,9] However, the persistent use of αGIs may increase the risk of hypoglycemia, especially when combining with other antiglycemic agents, which are a common trend in the management of DM in clinical practice.[10] Additionally, αGIs can cause a reduction in skeletal muscle index, handgrip strength, and gait speed. Furthermore, there are more and more reports about αGIs-induced pneumatosis intestinalis.[11,12] Therefore, there is a need for finding a new αGI, especially one with natural origins that causes fewer or no adverse events and is affordable for long-term use. Recent phytochemical studies have revealed that a variety of medicinal plants possess antioxidant and α-glucosidase inhibiting properties. Among the phytochemical compounds involved in these activities, phenolic compounds, especially flavonoids (e.g., kaempferol, apigenin), and tannins, are predominant.[13,14]

The pantropical genus *Bauhinia* Plum. ex L., named after two Swiss botanists, Jean Bauhin and Gaspard Bauhin, is one of the largest genera in the family Fabaceae, with more than 200 species having been described morphologically. This genus contains trees, shrubs, herbs, or liana that are widely distributed in tropical regions, including

Vietnam.[15,16] Among the species of the genus, *Bauhinia ×blakeana* is a hybrid species between *B. purpurea* and *B. variegata*.[17] This hybrid plant is used in folk medicine to treat pain-related diseases, and previous studies indicated that *B× blakeana* possesses antidiabetic, antibacterial, anti-inflammatory, analgesic, haemostactic, and diuretic activities.[18] Like its parent species, *B× blakeana* is a rich source of bioflavonoids (e.g., kaempferol, apigenin) with antioxidant and *α*-glucosidase inhibiting properties.[19,20] Thus, *B× blakeana* can be a potential source of medicinal herbs for the prevention and treatment of DM.

Therefore, this work aimed to determine the antioxidant activity and *α*-glucosidase inhibitory effect of *B× blakeana* collected in Vietnam, thereby providing evidence for the antidiabetic potential of *B× blakeana* leaves and flowers.

Materials and Methods

Plant material

The *B× blakeana* leaves and flowers were collected from Ho Chi Minh City, Vietnam in October 2022. The sample was identified by Dr. Dinh Quang Diep (a botanist from Thu Dau Mot University). A voucher specimen (code: DQD-1022) was deposited at the Institute of Applied Technology, Thu Dau Mot University, Binh Duong Province, Vietnam. The morphological features of *B× blakeana* are shown in Figure 1.

Preliminary phytochemical screening evaluation

Phytochemical constituents including carbohydrates, essential oils, amino acids, fats, tannins, flavonoids, coumarins, alkaloids, steroids, terpenoids, cardiac glycosides, saponins, and polyuronides were analyzed qualitatively using the standard protocol as described by Reveny *et al*. [21] with slight improvement. Briefly, the leaf powder (50.0 g) and flower powder (50.0 g) were extracted by dissolving them in each 100 mL of 96% ethanol for 30 minutes. The leaf and flower extracts were then subjected to preliminary phytochemical screening under the same conditions.

Chemicals and reagents

Ethanol (OPC Company, Vietnam); methanol, dimethyl sulfoxide (DMSO), acarbose (Merck Co. Ltd, Germany); aluminium chloride ($AlCl_3$), ascorbic acid, 2.2-diphenyl-1-picrylhydrazyl (DPPH), sodium carbonate (Na_2CO_3), sodium phosphate (Na_3PO_4), rutin, folin-ciocalteu reagent (folin-ciocalteu's phenol reagent), *p*-nitrophenyl-*α*-D-glucopyranoside (*p*-NPG), *α*-glucosidase from *Saccharomyces cerevisiae* (Sigma-Aldrich, USA). All other chemical reagents and solvents were purchased from Sigma-Aldrich (USA) at analytical grade.

Preparation of crude extract

The *B× blakeana* leaves and flowers were dried at 55 ± 5°C in a drying oven (Xingchen 101-1AB, China) before being milled separately into a midding powder; and then each sample powder (300 g) was soaked in 96 % ethanol for three days, with the ratio of material in solvent (1:10 g/mL). These ethanol extracts were collected and and recovered at 50 ± 2°C by rotary evaporator to obtain the total extract, which was then stored at under 4°C for use in *in vitro* models.

Figure 1: *B× blakeana* Dunn; (**a**). Photographs of the aerial parts; (**b**). The leaves and flowers.

Screening of bioactive compounds of the crude extracts

Total phenolic content (TPC)

The total phenolic content was determined by using the method of optical density measurement, i.e., using 10% folin-ciocalteu's phenol as a reagent, and gallic acid as a standard, according to the slightly modified method of Falodun *et al*.[22] and Hossain *et al*.[23] Preparation of test samples: Dissolve exactly 0.01 g crude extract in methanol and dilute 50 times to give a solution with a concentration of 200 µg/mL. In brief, 1.0 mL of the test sample was mixed with 5.0 mL of 10% folin-ciocalteu's phenol. This mixture was incubated for 10 minutes. Then, this mixture was added with 4.0 mL of 10% Na_2CO_3 and incubated at room temperature for 120 minutes before measuring the UV absorbance at 744 nm (UVD-2960, Labomed Inc., USA). The results were indicated as mg/g of gallic acid equivalents (mg GAE/g E). The standard sample was done using the same procedure as the test sample.

Total flavonoid content (TFC)

The total flavonoid content was determined by UV spectroscopy after complexation with $AlCl_3$, following Hossain *et al*.[23] with improved adjustments. Preparation of test samples: Dissolve exactly 0.01 g crude extract in methanol and dilute 25 times to obtain a solution with a concentration of 400 µg/mL. Briefly, the test sample (1.0 mL) and 10% $NaNO_2$ (0.3 mL) were mixed together. This mixture was incubated for 10 minutes before being added with 0.3 mL of 10% $AlCl_3$. After that, 1.0 mL of 1.0 M NaOH and water (to dilute) were respectively added to obtain a mixture with a total volume of 10 mL. This mixture was incubated for 30 minutes at room temperature before measuring the UV absorbance at 510 nm (UVD-2960, Labomed Inc., USA). The results were indicated as mg/g of rutin equivalents (mg RE/g E). The standard sample was done using the same procedure as the test sample. The standard substance was used to establish the standard line of rutin.

DPPH free radical scavenging assay

The antioxidant assay of the crude extract was performed using the DPPH radical scavenging method described by Sohemat *et al*.[24] with minor improvement. The chemicals used in the assay included DPPH solution with the concentration of 0.6 mM, test samples with the concentrations of 25-1000 µg/mL, ascorbic acid (as a control substance) with the concentrations of 10; 20; 30; 40 µg/mL diluted with methanol. The test sample of 1.0 mL was added to a pre-existing 6.0 mL MeOH test tube before adding 1.0 mL of 0.6 mM DPPH solution. The test sample was replaced by MeOH for the control sample, the test tube of the blank sample contained MeOH only. The test tubes after reconstitution were incubated in the dark for 30 minutes at room temperature. The absorbance was then measured at 517 nm.

The antioxidant capacity was shown as a percentage of inhibition and was calculated using the following formula:

DPPH radical scavenging capacity (%) = $((Abs_c - Abs_t)/Abs_c) \times 100$

where: Abs_c and Abs_t are the spectral absorbance values of the control sample and test sample, respectively. From the calculated results and sample concentration, a linear equation between sample concentration and antioxidant activity was established to calculate the IC_{50} value.

The α-glucosidase inhibitory assay

The *α*-glucosidase inhibitory assay of the crude extract was performed using the method of Van Chen *et al*.[25] with some modifications. In brief, the absence or presence of 60 µL of the test samples solution (concentration of 25-250 µg/mL) in 96-well plates was mixed with 50 µL of 0.1 M sodium phosphate buffer (pH 6.8) containing the *α*-glucosidase (0.2 U/mL). The reaction mixture was incubated for 10 minutes at 37 °C. After pre-incubation, 50 µL of 5.0 mM *p*-NPG solution in the above buffer was added and continued incubation at 37 °C for 30 minutes. Finally, the mixture reaction was stopped by adding 40 µL of 0.1 M sodium phosphate buffer. A positive control, Acarbose (concentration of 50-250 µg/mL) was used in this study. The *α*-

glucosidase inhibitory effect was measured at 405 nm using a microplate reader (Powerwave HT, Biotek, USA).
The percentage of α-glucosidase inhibition was calculated as follows:

Inhibition activity (%) = [1 - (A_t - A_{ot})/(A_c - A_{oc})] x 100

where: A_t and A_{ot} are the absorbance of the test sample and the blank test sample, respectively. Similarly, A_c and A_{oc} are the absorbance of the control sample and the blank control sample respectively. The IC_{50} value (µg/mL) is the half inhibitory concentration of the extracts (i.e., 50% inhibition of maximal activity) on α-glucosidase activity.

Data analysis
The experiment of the phytochemical screening, total phenolic content, total flavonoid content, antioxidant, and α-glucosidase inhibitory studies were determined in triplicate. The results were analyzed and shown as mean values ± S.D (Standard Deviation) by using Microsoft Excel (Microsoft Corporation, 2023). Furthermore, the antioxidant activity and α-glucosidase inhibitory effect of the crude extract were presented as IC_{50} value (µg/mL) via Microsoft Excel software and linear regression.

Results and Discussion

Phytoconstituents evaluation
The preliminary phytochemical screening of *B× blakeana* leaf and flower extracts revealed the presence of carbohydrates, triterpenoids, organic acids, flavonoids, and tannins in both as shown in Table 1. However, carotenoids, steroids, cardiac glycosides, anthocyanidins, and saponins being absent in the leaves were found in flowers under the same analytical conditions.

Total phenolic content and total flavonoid content, and antioxidant activity
The total phenolic content (TPC) of the crude ethanolic extracts of *B× blakeana* leaves and flowers was performed by the Folin-Ciocalteu's phenol method and presented as mg gallic acid equivalents per gram of the sample crude extract (mg GAE/g E). Additionally, the total flavonoid content (TFC) of the *B× blakeana* leaf and flower extracts were expressed as rutin equivalents in mg per gram of the sample crude extract (mg RE/g E). The results of the TPC and TFC in various crude extracts were presented in Table 2. The highest amount of TPC and TFC were determined in *B× blakeana* leaves (99.81 ± 0.94 mg GAE/g and 114.93 ± 1.06 mg RE/g, respectively) followed by *B× blakeana* flowers (40.45 ± 0.50 mg GAE/g and 59.61 ± 0.85 mg RE/g, respectively). In other words, the TPC and TFC content of *B× blakeana* leaves were twice as high as that of flowers ones.
The DPPH free radical scavenging activity of crude ethanolic extracts of *B× blakeana* was determined. The leaf extract showed an IC_{50} value of 266.52 ± 1.03 µg/mL (R^2 = 0.9864), while the IC_{50} value of ascorbic acid (positive control) was 26.53 ± 0.42 µg/mL (R^2 = 0.9984). Surprisingly, the flower extract showed very weak DPPH radical scavenging with IC_{50} value > 1000 µg/mL. Based on the correlation of DPPH free radical scavenging antioxidant activity with total polyphenol content, the antioxidant results are moderate for leaves but very weak for *B× blakeana* flowers. It is possible that the main phytochemical compounds responsible for the antioxidant activity are polyphenol compounds. Therefore, the results of this work showed that the phenolic content as well as the flavonoid content of the leaves are twice as much as that of *B× blakeana* flowers. In other words, the antioxidant capacity of leaf is stronger than that of flower because of the higher polyphenol content in leaf.
Phenolic and flavonoid compounds are biologically active secondary metabolites. They act as protective compounds for the maintenance of good health when consumed daily in a diet rich in plants, vegetables, and fruits. In previous studies, these compounds showed beneficial pharmacological effects on human health against the development of several serious diseases such as diabetes, metabolic syndrome, obesity, hypertension, cancer, Alzheimer, and other diseases.[26-28] It is possible that these beneficial effects are mainly due to the antioxidant and free radical scavenging activities of polyphenolic compounds. Additionally, the ability of flavonoids to neutralize free radicals depends on molecular structure and the position of the hydroxyl groups in their chemical structure.[29,30] As a result, the oxidation of DNA, proteins, and lipids is delayed or inhibited.[31] This argument has been demonstrated through the efficacy of phenolic compounds and flavonoids as alternative pharmaceutical agents or in combination with active treatments of various diseases.[28]

Table 1: Phytochemical screening of *B× blakeana* leaf and flower extracts.

Chemical constituents	The test's name	The leaf and flower extracts of *B× blakeana*	
		Leaf extract	Flower extract
Fats	Staining test	-	-
Carbohydrates	Molisch, Fehling reagent	+	+
Carotenoids	H_2SO_4 test	-	+
Essential oils	Scenting test	-	-
Triterpenoids	Salkowski's test	+	+
Alkaloids	Bouchardat, Dragendoff reagent	-	-
Organic acids	Na_2CO_3 test	+	+
Steroids	Liebermann Burchard test	-	+
Cardiac glycosides	Raymond's test, Xanthydrol's test		
Saponins	Foam test	-	+
Coumarins	Lactone ring test	-	-
Flavonoids	Shinoda test	+	+
Anthocyanidins	NaOH 1%, HCl 1% reagent	-	+
Tannins	Gelatin, $FeCl_3$ reagent	+	+
Polyuronides		-	-

Note: the presence (+) and the absence (-).

The α-Glucosidase inhibitory effect of the leaf and flower extracts

In this study, the ethanolic extracts of two different parts of *B× blakeana* were investigated as potential α-glucosidase inhibitors. The α-glucosidase inhibitory assay results of *B× blakeana* leaves and flowers were summarized in Table 3. In brief, the leaf and flower of *B× blakeana* expressed significant α-glucosidase inhibitory effect with IC_{50} values of 61.88 ± 0.19 µg/mL (R^2 = 0.9838) and 190.79 ± 0.18 µg/mL (R^2 = 0.9891), respectively. Although such the α-glucosidase inhibitory effect was reported for some other species of *Bauhinia* like *B. forficata*,[32] *B. galpinii*, *B. variegata*, and *B. variegata* var. *candida*,[33] this is the first report on *B× blakeana* leaf and flower growing in Vietnam. Meanwhile, also in this assay acarbose (positive control, with an IC_{50} of 169.67 ± 0.39 µg/mL; R^2 = 0.9866) was less potent than the leaves extract (ca. 2.5 times) and equivalent to the flowers extract under the same conditions.

Phenolic compounds, a class of plant secondary metabolites, are an important type of natural products; particularly, they are well known for their beneficial effects on health. In the review report, Durazzo *et al.*[27] subdivided phenolic compounds into two main groups: flavonoids (e.g., eu-flavonoids, iso-flavonoids, neo-flavonoids) and non-flavonoid polyphenols (e.g., phenolic acids, xanthones, stilbenes, lignans, coumarins, and tannins).

In terms of the structure, flavonoids comprise fifteen carbon atoms (C_6-C_3-C_6) made up of two benzene rings (A and B rings) and a heterocyclic pyrane ring (C-ring), which are called derivative benzo-γ-pyrones.[28,34] Based on the binding site of the B-ring to the C-ring and the substitution pattern of the C-ring, flavonoids are divided into sub-groups: flavonols, flavones, flavanones, flavanonols or catechins, isoflavonoids, anthocyanins, and chalcones.[27,28,31] These flavonoid compounds are widely found in many edible vegetables, fruits, and medicinal herbs. They have been well investigated due to their beneficial bioactivities such as anti-cancer, antibacterial, anti-fungal, antimalarial, antioxidant, anti-inflammatory, anti-diabetic, neuroprotective, cardio-protective, anti-ulcer, and anti-edematogenic activities, etc.[22,35-37]

Tannins, belonging to the polyphenol group (i.e., non-flavonoid group) with a high molecular weight (500−30000 Da), are widely distributed all over the kingdom Plantae.[38] It can be found in fruits, vegetables, grains, and herbs with potential health benefit properties including anti-inflammatory, anti-cancer, anti-diabetic, antiallergic, antibacterial, antiviral, antioxidant, antitoxic, antiparasitic, and dysentery cure effects, etc.[29,38,39] Among the various natural sources of tannins, current research suggests that *B× blakeana* leaf and flower are also known as sources of tannins. Additionally, many organic acids, such as tartaric acid, malic acid, lactic acid, citric acid, and succinic acid in plants (e.g., fruits and vegetables, etc), have been reported to exert health benefits including antibacterial activity,[40] blood glucose control, and correction of lipid abnormalities by preventing oxidative stress-related damage.[41]

Generally, the phytoconstituents such as carbohydrates, amino acids, triterpenoids, tannins, and flavonoids present in *B× blakeana* leaves are also found in other *Bauhinia* species such as *B. variegata*,[15] *B. purpurea*,[42] *B. acuminata*,[43] *B. variegata* var. *candida alba*,[44] *B. forficata* subsp. *pruinosa*[45]. Similarly, carbohydrates (reducing sugars), organic acids, cardiac glycosides, flavonoids, tannins, triterpenoids, and saponins are the major phytochemicals in *B× blakeana* flowers, which are also discovered in the flowers of *B. alba*,[46] *B. purpurea*,[42] *B. variegata*,[15,46] etc.

The present study showed that *B× blakeana* leaf had higher TPC value than that of *B. scandens* leaf (47.33 ± 0.01 mg GAE/g E),[23] *B. forficata* leaf (59.47 ± 0.71 mg GAE/g E),[45] *B. rufescens* leaf (68.40 ± 0.02 mg GAE/g E),[47] and *B. nakhonphanomensis* leaf (48.69 ± 0.56 mg GAE/g E),[48] etc.

TPC includes both polyphenols and flavonoids. Thus, the TFC must be lower than the total phenolic content. However, in this study, higher amounts of flavonoids were observed when compared to total phenolics. That the lower TPC was lower than the TFC observed in this study could be explained as follows: (1) The TFC in the *B× blakeana* leaf and flower was actually higher than that of other polyphenols. (2) Specificity of the Folin-Ciocalteu's phenol and $AlCl_3$ methods used to quantify TPC and TFC. In brief, due to its lack of specificity, the Folin-Ciocalteu's phenol reagent will react not only with polyphenols but also with any reducing agent in the sample. Meanwhile, the $AlCl_3$ reagent will react specifically with functional groups in the flavonoid molecule such as the groups of keto, hydroxyl (forming acid-stable complexes), and *ortho*-dihydroxyl (forming acid-labile complexes). This argument was also explained and demonstrated in previous studies by Anh *et al*.[49] and Dedvisitsakul *et al*.[50]. A liquid chromatography–mass spectrometry (LC-MS) or LC-MS/MS analysis of the chemical composition of leaf and flower extracts of *B× blakeana* could be performed in a further study to confirm the results for flavonoid compounds and other polyphenols in this study. In addition, a high-performance liquid chromatography (HPLC) analysis of *B× blakeana* leaf and flower extracts with standard samples could be performed to qualitative and quantitative phenolic and flavonoid compounds in this species.

Table 2: Total phenolic, total flavonoid contents and antioxidant properties of *B× blakeana* leaf and flower extracts.

Plant material	TPC (mg GAE/g E)	TFC (mg RE/g E)	DPPH scavenging capacity IC_{50} (µg/mL); (y; R^2)
Leaves extract	99.81 ± 0.94	114.93 ± 1.06	266.52 ± 1.03; (y = 32.762lnx - 132.99; R^2 = 0.9864)
Flowers extract	40.45 ± 0.50	59.61 ± 0.85	> 1000
Ascorbic acid	-	-	26.53 ± 0.42; (y = 45.075lnx - 97.763; R^2 = 0.9984)

Note: GAE, RE, and E are gallic acid equivalent, rutin equivalent, and extract, respectively. Mean ± S.D, n = 3; (-) Not tested.

Table 3: The α-glucosidase inhibitory activity of the plant extract and acarbose.

Leaf extract		Flower extract		Acarbose	
Concentration (µg/mL)	Inhibition (%)	Concentration (µg/mL)	Inhibition (%)	Concentration (µg/mL)	Inhibition (%)
25	30.16 ± 1.34	50	12.50 ± 0.92	50	21.74 ± 0.76
50	49.21 ± 1.75	100	29.86 ± 1.20	100	35.51 ± 1.26
100	59.52 ± 1.38	150	40.97 ± 1.41	150	47.10 ± 1.23
150	65.87 ± 1.37	200	50.69 ± 1.22	200	52.17 ± 1.87
200	73.02 ± 1.64	250	60.42 ± 1.08	250	61.59 ± 1.30
y = 19.579lnx - 30.767; R^2 = 0.9838		y = 29.121lnx - 102.92; R^2 = 0.9891		y = 24.12lnx - 73.834; R^2 = 0.9866	
IC_{50} = 61.88 ± 0.19 (µg/mL)		IC_{50} = 190.79 ± 0.18 (µg/mL)		IC_{50} = 169.67 ± 0.39 (µg/mL)	

Polyphenols and flavonoids are powerful antioxidant compounds that can neutralize free radicals by donating their hydrogen atoms and electrons. The TPC and TFC in plant extract and their antioxidant capacity were positively correlated. This correlation was also noted in previous reports.[49,51] The TPC and TFC in the crude extracts *B× blakeana* were positively proportional to antioxidant activity measured by DPPH free radical scavenging capacity. In comparison with a similar study, the ethanolic extract of *B× blakeana* flower had lower TPC and TFC values than the ethanolic extract of *B. variegata* flower (which exhibited 134 ± 1.36 mg GAE/g E and 195 ± 2.03 mg RE/g E, respectively).[30] Moreover, the ethanol extracts of *B× blakeana* flower (IC_{50} > 1000 µg/mL) and *B. variegata* (IC_{50} = 2000 ± 0.01 µg/mL) both exhibited very weak antioxidant capacity.[30]

Previous studies reported that TPC and TFC are related to extraction solvents. In other words, chemical compounds have variable solubility in different solvents, and the properties of these compounds can contribute to changes in those contents.[30] In addition, the TPC and TFC may vary depending on the extraction process (e.g., concentration, time, and temperature), as well as the origin of the plant (e.g., plant genotype, part used, etc), collection seasons, ecological, and geological conditions.[30,45]

In summary, *B× blakeana* exhibited only low to moderate antioxidant activity in the DPPH scavenging assay, which could be linked to their total phenolic and total flavonoid contents. This result is similar to that of *B. purpurea* previously reported of Zakaria *et al*.[52]

The same scenario was also observed for the majority of *α*-glucosidase inhibitory effect of the plants because of phenolic constituents (e.g., flavonoids, tannins, and phenolic acids, etc) which generally play an important role in reducing oxidative stress as well as in the prevention and treatment of related diseases. For example, flavonoids are compounds beneficial for the treatment of diabetes due to their strong antioxidant activity.[53] Antioxidants are considered as a major alternative in the treatment of diabetes thanks to their ability to counteract the harmful effects of hyperglycemia, as well as improve glucose metabolism and absorption.[53,54] In addition to their antioxidant activities, flavonoids are also used effectively in the prevention and/or treatment of type-2 diabetes because they can act on the *α*-glycosidase.[53] Specifically for these structures, flavonoids like rutin, quercetin, apigenin, apigenin-7-*O*-glucoside, (2*S*)-5,7-dimethoxy-3′,4′-methylenedioxy flavanone, kaempferol-7,4′-dimethyl-ether-3-*O*-*β*-D-glucopyranoside, and kaempferol-3-*O*-*β*-D-glucopyranoside were found in different parts of *B. variegata* as evidence of the structure-effect relationship.[15] Additionally, regarding the relationship between the structure and action of flavonoids with *α*-glucosidase inhibitory effect, previous studies demonstrated that the hydroxyl group, the double bond at the C2=C3 position, and the 4-oxo (ketone) group were the main structural features of flavonoids relating to their antidiabetic effects.[53] In other words, the absence of a cathecholic system in the B ring (OH-C3′/C4′),[55] or saturation/ lack of the C2=C3 double bonds, together with the absence of an OH-C3/C5 group, and the ketone-C4 group at the C-ring reduced the antidiabetic effect.[53,56,57] In addition, the acetylation or alkylation of the OH groups in the A-ring (e.g., methyl and acetate groups) decreased or was unlikely to interact with the enzyme binding sites and scavenge the ROS radical.[53]

As the result obtained, the *α*-glucosidase inhibition described here for the first time may be one of the proven mechanisms regarding the lowering effect on blood glucose noted for *B×blakeana* leaf and flower. Overall, similar to some other *Bauhinia* species (e.g., *B. forficata*, *B. forficata* subsp. *pruinosa*, etc), *B× blakeana* showed a potent efficacy in inhibiting the *α*-glucosidase.

Conclusion

In conclusion, the antioxidant activity and *α*-glucosidase inhibitory effect of Vietnamese *B× blakeana* leaves and flowers were evaluated for the first time in this work. The leaf and flower of *B×blakeana* from Vietnam contained polyphenolic compounds. Carbohydrates, organic acids, carotenoids, steroids, cardiac glycosides, triterpenoids, saponins, flavonoids, and tannins were found in *B×blakeana* leaf and flower. The leaf extract showed stronger antioxidant activity than the flower extract. The high *α*-glucosidase inhibitory activity of *B× blakeana* extracts suggested that they could be utilized as ingredients for further studies to develop functional foods as well as drugs for the treatment of DM-related diseases.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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