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Antioxidant and *α*-Glucosidase Inhibitory Activities of *Dolichandrone serrulata* Extracts

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ABSTRACT

Dolichandrone serrulata (Bignoniaceae) is used as a vegetable and food ingredient by the local people in the northern and eastern parts of Thailand. The antioxidant and *α*-glucosidase inhibitory activities of *D. serrulata* extracts were investigated. Three parts of *D. serrulata* (the flowers, leaves and pods) were extracted using water and 95% ethanol. The antioxidant activity of the extracts was examined by DPPH, ABTS and FRAP assays. The total phenolic content (TPC) and total flavonoid content (TFC) were determined by the colorimetric method. The *α*-glucosidase inhibition assay was used to evaluate the *in vitro* anti-diabetic activity of the extracts. Results revealed that the extracts contained phenolic compounds and flavonoids. Aqueous pod extracts had the highest TPC and TFC (0.245 ± 0.020 mg GAE/g and 0.1102 ± 0.0033 mg QE/g, respectively), while aqueous leaf extracts had the highest antioxidant capacity against DPPH and ABTS (IC_{50} = 0.12 ± 0.020 and 0.05 ± 0.006 mg/mL, respectively). Ethanol leaf extracts had the highest FRAP value (0.988 ± 0.236 mg TE/g), while ethanol pod extracts had the highest *α*-glucosidase inhibitory activity (IC_{50} = 0.041 ± 0.002 mg/mL). In conclusion, aqueous pod extracts from *D. serrulata* displayed the highest TPC and TFC and antioxidant capacity against DPPH and ABTS, while ethanol leaf extracts had the highest antioxidant activity by FRAP assay. Fruit pod ethanol extracts exhibited *α*-glucosidase inhibitory activity, suggesting preliminary anti-diabetic activity possibly due to high phenolic and flavonoid contents.

Keywords: *Dolichandrone serrulata*, Total phenolic content, Total flavonoid content, Antioxidant activity, *α*-glucosidase inhibitory activity.

Introduction

Dolichandrone serrulata belongs to the family Bignoniaceae. The flower part of this plant is used as a vegetable and food ingredient.[1] The phytochemical studies of this plant revealed that the branches contained a new phenolic triglycoside, dolichandroside.[2] The flower part contained six compounds including hallerone, protocatechuic acid, rengyolone, cleroindicin B, ixoside and isomoltose.[3] Pharmacological studies showed that the root part of this plant is used in Thai folklore remedies, called Ben-Cha-Moon-Yai as an anti-pyretic medicine.[4] Leaf ethanolic extracts of *D. serrulata* had the highest DPPH free radical scavenging activity with IC_{50} of 500 µg/ml,[5] and also the highest antilipoxidase activity with IC_{50} of 1 mg/ml. However, the toxicity study revealed that the flower part of *D. serrulata* did not exhibit acute toxicity in mice.[6]

Diabetes mellitus is characterized by high blood glucose level and caused by insufficient insulin production. Ninety percent of all diabetes mellitus patients have type II diabetes mellitus, caused by the failure of *β*-cells in the pancreas to produce insulin. If insulin production is not functioning normally, the cells cannot take up glucose from the blood as a source of energy causing high blood glucose level. Enzymes related to lowering blood glucose levels include α-glucosidase or maltase found in the small intestine. Inhibition of α-glucosidase enzyme helps to reduce carbohydrate digestion and can reduce glucose absorption, causing blood glucose levels to decrease.[7]

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Therefore, this research studied the efficacy of α-glucosidase inhibition from various parts of *D. serrulata* and also the antioxidant activity. Results from this study suggest that *D. serrulata* aqueous fruit pod extracts could be used as an alternative medicine to help diabetic patients control blood glucose levels and treat type 2 diabetes mellitus by inhibiting free radicals. This will result in reducing the risk of various diseases.

Materials and Methods

Preparation and extraction of plant materials

Flower, leaf and fruit pod parts of *D. serrulata* were used in this study. Plant parts were collected between April and June 2019 from a cultivation area in Saraburi Province, Central Thailand. The plant materials were deposited at the Department of Biology, Faculty of Science, Mahasarakham University, Thailand, under voucher specimen number MSUBI-TK-001. Flowers, leaves and pods of the plant were washed with clean tap water. Plant materials were dried at 50°C in a hot air oven. The dry plant materials were then ground thoroughly to powder using an electrical grinder. Powders from the different plant parts were extracted by macerating 100 g dry plant powder with 400 mL distilled water and 95% ethanol, respectively at room temperature for 7 days. The extracts were then filtered using filter paper Whatman No. 1 and evaporated using a rotary evaporator. Finally, the crude extracts were freeze-dried as a fine powder and kept at -20°C until required for further experiments.

Determination of total phenolic content

Total phenolic content (TPC) was analyzed by the Folin-Ciocalteu method described by Singleton *et al*.[8] A quantity of 100 µL of gallic acid solution (standard) and *D. serrulata* crude extracts was prepared in 10 mL test tubes, and then 500 µL of Folin-Ciocalteu's reagent was added and mixed; 100 µL of distilled water was used as a blank. After that, 400 µL of 7.5% Na_2CO_3 solution was added in each tube and

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mixed well. The samples were incubated at 25°C for 30 min in a dark room. Measurements of the absorbance at 765 nm were performed using a UV-Vis spectrophotometer. Five replications were performed in each treatment. The TPC value was expressed as mg gallic acid equivalent (GAE) per g extract.

Determination of total flavonoid content
Total flavonoid content (TFC) was determined by the colorimetric method described by Zhishen *et al*.[9] Briefly, 100 µL of the quercetin solution and crude extract was prepared in a 10 mL test tube and then 400 µL of 2.5% $NaNO_2$ was added and mixed. Thereafter, 500 µL of 5% $AlCl_3$ solution and 2,000 µL of distilled water were added and the samples were allowed to react at 25 °C for 10 min. The distilled water was used as a blank. The absorbance at 415 nm was measured using a UV-Vis spectrophotometer. The TFC value was reported as mg quercetin equivalent (QE) per g extract.

DPPH free radical scavenging activity
DPPH assay was performed following the method described by Brand-Williams *et al*.[10] with some modifications. Briefly, 25 mg of extracts and 0.001g/10 mL Trolox were dissolved in a suitable solvent (DMSO) and then 100 µL of the extracts or standard were added to the test tubes. Aliquots of 900 µL of 1 mM DPPH solution were then added, mixed well and incubated in a dark room for 30 min. Absorbance of the solution was measured using a UV-Vis spectrophotometer at a wavelength of 515 nm. Percentage inhibition was calculated from the absorbance value, with activity reported as IC_{50} and compared with those of the standards.

ABTS assay
The ABTS assay was carried out following the procedure described by Re *et al*.[11] Briefly, 7 mM of ABTS was prepared in $K_2S_2O_8$ solution with pH 7.4 at a ratio of 1:0.5 and left in a dark room for 16 hours before use. ABTS solution was diluted at ratio of 1:50 and then 900 µL aliquots were added to 100 µL extracts or standards. The solutions were left in a dark room for 6 min. Then, the solutions were measured for absorbance at a wavelength of 734 nm using a UV-Vis spectrophotometer. The IC_{50} was then calculated.

Ferric reducing antioxidant power (FRAP) assay
The FRAP assay was performed and modified according to the method described by Benzie *et al*.[12] A FRAP solution containing 2.5 mL of a 10 mM TPTZ (2,4,6- tripyridy-s-triazine) solution was prepared in 40 mM HCl plus 2.5 mL of 20 mM $FeCl_3$ and 25 mL of 0.3 mM acetate buffer, pH 3.6. Then, 900 µL of FRAP solution was added to the 100 µL extracts or standard. The solutions were incubated at 37 °C for 4 min. The absorbance was measured using a UV-Vis spectrophotometer at a wavelength of 593 nm. Activities were reported as FRAP value (mg TE/g extract) and compared with those of the standard Trolox (Trolox equivalent).

Determination of α-glucosidase inhibitory activity
The *α*-glucosidase inhibition assay was conducted following the method of Ramadhan and Phuwapraisirisan[13] with some modifications. Briefly, 0.4 U/mL *α*-glucosidase and 1 mM p-nitrophenyl-*α*-D-glucopyranoside (substrate) were prepared in 0.1 M potassium phosphate buffer with pH 6.8. After pre-incubation of the extracts or standard with yeast *α*-glucosidase at 37°C for 20 min, the substrate solution (100 µL) was added to the reaction. The solution was then incubated at 37°C for 20 min and 100 of 1 M Na_2CO_3 was added to terminate the reaction. The absorbance of the solution was measured at 450 nm in order to quantify the inhibitory activity compared to those of positive control acarbose. The *α*-glucosidase inhibitory activity was expressed as IC_{50} values.

Statistical analysis
All data were expressed as mean ± S.E.M. The experiments were conducted in five replicates. Statistical analysis was tested using one-way analysis of variance (ANOVA) followed by Duncan's multiple range test (DMRT). Statistical significance was considered at $P < 0.05$. The SPSS program version 23.0 was used for data analysis.

Results and Discussion

Percentage yield of the extracts
Results revealed that aqueous pod extracts of *D. serrulata* (APDS) gave the highest percentage yield (19.25%) followed by aqueous flower extracts (AFDS) (15.3%) and aqueous leaf extracts (ALDS) (13.65%), while ethanol leaf extracts (ELDS) recorded (13.25%), followed by ethanol flower extracts (EFDS) (12.35%) and ethanol pod extracts (EPDS) (3.45%).

Total phenolic content
Total phenolic content was determined by the Folin-Ciocalteu colorimetric method. [19] Aqueous pod extracts of *D. serrulata* (APDS) had the highest total phenolic content (0.245 ± 0.020 mg GAE/g) in comparison to those of other extracts, while ethanol pod extracts of *D. serrulata* (EPDS) gave the lowest total phenolic content (0.021 ± 0.001 mg GAE/g) (Table 1).

Total flavonoid content
Aqueous pod extracts of *D. serrulata* (APDS) had the highest total flavonoid content (0.1102 ± 0.0033 mg QE/g), while ethanol flower extracts of *D. serrulata* (EFDS) recorded the lowest total phenolic content (0.0026 ± 0.0001 mg QE/g) (Table 2).

DPPH free radical scavenging activity
DPPH free radical scavenging activities of *D. serrulata* extracts were expressed as IC_{50} values as shown in Table 3. Results revealed that both aqueous (ALDS) and ethanol (ELDS) leaf extracts of *D. serrulata* had strong antioxidant activity at 0.12 ± 0.020 mg/mL and 0.19 ± 0.007 mg/mL, respectively against DPPH free radical in comparison to those of other extracts, while antioxidant activity of *D. serrulata* extracts were less potent than standard ascorbic acid and Trolox (Table 3).

ABTS assay
The study on inhibition of the 2,2-azinobis-(3-ethylbenzothaizoline-6-sulfate radical indicated that *D. serrulata* extracts had inhibitory activity against ABTS free radicals. Aqueous leaf extracts of *D. serrulata* (ALDS) had the strongest ABTS free radical scavenging activity (IC_{50} = 0.05 ± 0.006 mg/mL) in comparison to those of other extracts. However, these activities were less potent than those of ascorbic acid and Trolox (Table 4).

FRAP assay
To confirm the antioxidant capacity of *D. serrulata* extracts, ferric reducing antioxidant power (FRAP) assay was conducted. Results showed that ethanol leaf extracts of *D. serrulata* (ELDS) had the highest FRAP activity (FRAP value = 0.988 ± 0.236 mg TE/g) and similar to those of ALDS (FRAP value = 0.879 ± 0.114 mg TE/g). The lowest FRAP activity was found in EPDS (FRAP value = 0.225 ± 0.067 mg TE/g). However, the FRAP activities were less potent than those of the standard Trolox (Table 5).

α-glucosidase inhibitory activity
The *in vitro* anti-diabetic activity of *D. serrulata* extracts was evaluated by the *α*-glucosidase inhibition assay. Results revealed that *α*-glucosidase inhibitory activity was only found in ethanol pod extracts of *D. serrulata* (EPDS) with IC_{50} of 0.041 ± 0.002 mg/mL. However, *α*-glucosidase inhibitory activity of *D. serrulata* extracts was lower than acarbose (Table 6).
D. serrulata has been used as food and medicine in Thailand for a long time. The shoot-tip and flower parts of *D. serrulata* are used as vegetable food ingredients by Thai people in the northern and eastern parts of Thailand.[3] The safety assessment of this plant revealed that flower extracts from *D. serrulata* did not exhibit acute toxicity in mice, with maximum dose of 2,000 mg/kg. This finding suggested that consumption of *D. serrulata* flower as a vegetable may be safe for humans.[6] Pharmacological investigations revealed that the root parts of this plant are used in Thai folklore remedies as an antinociceptive and anti-inflammation agent.[14] *D. serrulata* also exhibited anti-bacterial activity.[15-17]

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Table 1: Total phenolic content of *D. serrulata* extracts

***D. serrulata* extracts**	**Total phenolic content (mg GAE/g)**
AFDS	0.109 ± 0.013 [b]
ALDS	0.146 ± 0.004 [b]
APDS	0.245 ± 0.020 [c]
EFDS	0.148 ± 0.008 [b]
ELDS	0.131 ± 0.009 [b]
EPDS	0.021 ± 0.001 [b]
Gallic acid	0.470 ± 0.005 [a]

[a, b, c] Different superscripts in the same column indicate statistical significance (P < 0.05). AFDS: aqueous flower extract of *D. serrulata*; ALDS: aqueous leaf extract of *D. serrulata*; APDS: aqueous pod extract of *D. serrulata*; EFDS: ethanol flower extract of *D. serrulata*; ELDS: ethanol leaf extract of *D. serrulata*; EPDS: ethanol pod extract of *D. serrulata*

Table 2: Total flavonoid content of *D. serrulata* extracts

***D. serrulata* extracts**	**Total flavonoid content (mg QE/g)**
AFDS	0.0387 + 0.0014 [b]
ALDS	0.0734 + 0.0028 [c]
APDS	0.1102 + 0.0033 [b]
EFDS	0.0026 + 0.0001 [a]
ELDS	0.0107 + 0.0003 [b]
EPDS	0.0528 + 0.0025 [b]
Quercetin	0.144 + 0.0030 [c]

[a, b, c] Different superscripts in the same column indicate statistical significance (P < 0.05).
AFDS: aqueous flower extract of *D. serrulata*; ALDS: aqueous leaf extract of *D. serrulata*; APDS: aqueous pod extract of *D. serrulata*; EFDS: ethanol flower extract of *D. serrulata*; ELDS: ethanol leaf extract of *D. serrulata*; EPDS: ethanol pod extract of *D. serrulata*

Table 3: DPPH free radical scavenging activity of *D. serrulata* extracts

***D. serrulata* extracts**	**IC_{50} (mg/mL)**
AFDS	0.55 ± 0.031 [c]
ALDS	0.12 ± 0.020 [b]
APDS	0.53 ± 0.024 [c]
EFDS	0.31 ± 0.005 [c]
ELDS	0.19 ± 0.007 [c]
EPDS	0.68 ± 0.035 [c]
Ascorbic acid	0.016 ± 0.0003 [a]
Trolox	0.044 ± 0.0008 [a]

[a, b, c] Different superscripts in the same column indicate statistical significance (P < 0.05).
AFDS: aqueous flower extract of *D. serrulata*; ALDS: aqueous leaf extract of *D. serrulata*; APDS: aqueous pod extract of *D. serrulata*; EFDS: ethanol flower extract of *D. serrulata*; ELDS: ethanol leaf extract of *D. serrulata*; EPDS: ethanol pod extract of *D. serrulata*

Table 4: ABTS radical scavenging activity of *D. serrulata* extracts

***D. serrulata* extracts**	**IC_{50} (mg/mL)**
AFDS	0.09 ± 0.006 [b]
ALDS	0.05 ± 0.006 [b]
APDS	0.63 ± 0.066 [c]
EFDS	0.21 ± 0.013 [c]
ELDS	0.07 ± 0.007 [b]
EPDS	0.45 ± 0.011 [c]
Ascorbic acid	0.0099 ± 0.0002 [a]
Trolox	0.0230 ± 0.0004 [a]

[a, b, c] Different superscripts in the same column indicate statistical significance (P < 0.05).
AFDS: aqueous flower extract of *D. serrulata*; ALDS: aqueous leaf extract of *D. serrulata*; APDS: aqueous pod extract of *D. serrulata*; EFDS: ethanol flower extract of *D. serrulata*; ELDS: ethanol leaf extract of *D. serrulata*; EPDS: ethanol pod extract of *D. serrulata*

Table 5: Ferric reducing antioxidant power (FRAP) capacity of *D. serrulata* extracts

***D. serrulata* extracts**	**FRAP value (mg TE/g)**
AFDS	0.511 ± 0.078 [b]
ALDS	0.879 ± 0.114 [c]
APDS	0.532 ± 0.073 [b]
EFDS	0.423 ± 0.077 [b]
ELDS	0.988 ± 0.236 [c]
EPDS	0.225 ± 0.067 [a]
Trolox	0.210 ± 0.021 [a]

[a, b, c] Different superscripts in the same column indicate statistical significance (P<0.05).
AFDS: aqueous flower extract of *D. serrulata*; ALDS: aqueous leaf extract of *D. serrulata*; APDS: aqueous pod extract of *D. serrulata*; EFDS: ethanol flower extract of *D. serrulata*; ELDS: ethanol leaf extract of *D. serrulata*; EPDS: ethanol pod extract of *D. serrulata*

Table 6: α-glucosidase inhibitory activity of *D. serrulata* extracts

***D. serrulata* extracts**	**IC_{50} (mg/mL)**
AFDS	ND
ALDS	ND
APDS	ND
EFDS	ND
ELDS	ND
EPDS	0.041 ± 0.002 [a]
Acarbose	0.207 ± 0.021 [b]

[a, b] Different superscripts in the same column indicate statistical significance (P < 0.05).
AFDS: aqueous flower extract of *D. serrulata*; ALDS: aqueous leaf extract of *D. serrulata*; APDS: aqueous pod extract of *D. serrulata*; EFDS: ethanol flower extract of *D. serrulata*; ELDS: ethanol leaf extract of *D. serrulata*; EPDS: ethanol pod extract of *D. serrulata*; ND: not determined

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The flower part of this plant is edible without toxicity and effective in food development.[18] *D. serrulata* extracts from all plant parts exhibited antioxidant activity against DPPH free radical.
However, the inhibition capacity of this plant against other free radicals and α-glucosidase enzyme has not yet been confirmed.
Results revealed that *D. serrulata* extracts contain secondary metabolites including phenolic compounds and flavonoids that are responsible for antioxidative effects and prevention of chronic diseases. This finding is in accordance with previous studies in which the branches contained a new phenolic triglycoside, dolichandroside. [2] The flower part contained six compounds including hallerone, protocatechuic acid, rengyolone, cleroindicin B, ixoside and isomoltose.[3] *D. serrulata* flower extracts comprised total phenolic contents and terpenoids that exhibited antioxidant efficacy without toxicity on the reproductive system in male rats.[19]
The antioxidant activity study revealed that *D. serrulata* extracts provided antioxidant activity against DPPH and ABTS free radicals as well as exhibiting ferric reducing antioxidant power (FRAP) capacity.
It is interesting that strong antioxidant activities were found in leaf extracts of this plant. Similar results reported by Sreeprasert[5] who found that the ethanolic leaf extracts of *D. serrulata* had the highest DPPH free radical scavenging activity. Phanthong *et al*.[3] found that protocatechuic acid extracted from flower parts of *D. serrulata* showed potent scavenging activity of DPPH and hydroxyl radicals. The hexane fraction of *D. serrulata* flower extracts contained total flavonoid contents suggesting antioxidant activity with anti-cancer capacity.[20] An *in vivo* study revealed that *D. serrulata* flower extracts decreased the free radical indicator malondialdehyde (MDA) in type 2 diabetic rats.[21] The antioxidant activity of this plant may be due to the presence of secondary metabolites such as phenolic compounds and flavonoids that are responsible for free radical scavenging effects. An *in vitro* study on the anti-diabetic activity of *D. serrulata* extracts was carried out using *α*-glucosidase inhibitory activity assay. This activity was only found in ethanol pod extracts, suggesting a preliminary anti-hyperglycemic effect of this plant.
Moreover, ethanol pod extracts from this plant had the highest *α*-glucosidase inhibitory activity, while extracts from other plant parts recorded no activity. However, an *in vivo* study suggested that *D. serrulata* flower extracts lowered blood glucose levels in type 2 diabetic rats.[21] These findings revealed that the ethanol pod extracts inhibited *α*-glucosidase activity, resulting in inhibition of the digestion of starch into glucose, suggesting that blood glucose level was not increased. Further studies on the anti-diabetic activity of different parts of this plant should be conducted to confirm the anti-diabetic potential.

Conclusion

Extracts from different parts of *D. serrulata* expressed antioxidant potential against DPPH, ABTS and FRAP capacity. Fruit pod extracts exhibited α-glucosidase inhibitory activity, suggesting preliminary anti-diabetic activity.

Conflicts of interest

The authors declare no conflict of interest.

Authors' Declaration

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

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