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Original Research Article

Antioxidant and α-glucosidase inhibitory activities of phytochemicals extracted from leaves, branches and roots of native and hybrid Thai mulberry (*Morus alba*, Linn.) cultivars

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Abstract

Purpose: *To determine the phytochemical compounds, in leaves, branches and roots of two mulberry cultivars, Monnoi and Buriram 60 (Br.60).*

Methods: *Both cultivars were grown under organic conditions. Extraction of plant parts was done using either distilled water (autoclave), 50 % ethanol or 95 % ethanol and the proximate chemical analysis including 1-Deoxynojirimycin (DNJ) content, total flavonoid (TFC) and total phenolic contents (TPC), antioxidant and α-glucosidase inhibitory properties of the plant extracts were analyzed.*

Results: *The proximate analysis showed that both cultivars had high protein content in leaves (21 – 25 %) compared to branches and roots. The carbohydrate content was high in all parts of both cultivars (32 – 50 %) and the DNJ content of all parts using 50 % ethanol extraction was higher compared to the other two methods, ranging from 10 – 17 mg/g Ext. Total phenolic content (TPC) in Monnoi branches extracted with distilled water (autoclave) was 13.45 ± 0.26 mg GE/g Ext while Br.60 roots with distilled water extraction (autoclave) showed the highest total flavonoid content (TFC) at 1.55 ± 0.02 mg QE/g Ext. Antioxidant activities of the extract on FRAP, DPPH radical and ABTS were high for both cultivars but the IC_{50} value was higher and significantly different ($p < 0.05$) compared to two standards used as controls (Trolox and Ascorbic acid). The α-glucosidase assay showed that the leaves and branches of Monnoi had IC_{50} values of 2.21 ± 0.09 and 2.45 ± 0.1, respectively, with the potential to reduce enzymatic activity but less than the standard substance Acarbose with an IC_{50} of 0.31 ± 0.01.*

Conclusion: *Two mulberry cultivars, Monnoi and Br.60, show high potential for diabetes treatment owing to their bioactive compounds. Extraction with 50 % ethanol gives the highest yield. Further isolation and characterization and possible mechanism of action of phytoactive constituents such as 1-deoxynojirimycin (DNJ) would be necessary.*

Keywords: *Mulberry leaves, Branches and roots, Deoxynojirimycin, Antioxidant activity, α-Glucosidase inhibition*

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INTRODUCTION

Mulberry (*Morus alba* L., Family: Moraceae) is an ancient local medicinal plant known as the "King of herbs", for silkworm rearing (*Bombyx mori*) and silk production for over 4,000 years [1]. It is one of the most valuable perennial plants and contains numerous active ingredients such as anthocyanins (purple substances in mulberry fruits), phenol, flavonoids and alkaloids [2]. Mulberry usage has been classified into three main forms nutrition and pharmacy (nutraceuticals), development into value-added food and beverage products, and cosmetics. Studies show that mulberry components have outstanding pharmacological qualities such as antibacterial, anti-inflammatory, antioxidant properties, and anti-tyrosinase enzymes that reduce skin pigmentation and reduce blood sugar and fat levels [3]. China, Korea and other Asian countries use various parts of the mulberry tree as important active substances in cosmetics [4] and also for diabetes treatment [5].

Diabetes is a condition in which the body has chronic high blood glucose levels, resulting in the deterioration of internal organs and other complications that cause death. By 2025, the number of people with diabetes is predicted to increase by 300 million [6]. Previous studies reported that using mulberry leaves grown in Thailand as a powder or extract reduced blood glucose levels. This is because the leaf extract contains flavonoids [7], chlorogenic acid, rutin, isoquercitrin loliolide, and 1-deoxynojirimycin, fagomine 2-O-alpha-D- galactopyranosyl-1-deoxynojirimycin [7,8]. Over 100 active compounds have been identified in mulberry; however, Thai mulberry branches and roots have not been examined for their diabetic treatment potential. Therefore, this study aimed to determine the phytochemical compounds, such as 1-deoxynojirimycin (DNJ) in leaves, branches and roots of two mulberry cultivars, Monnoi and Buriram 60 (Br.60).

EXPERIMENTAL

Preparation and identification of mulberry specimens

Two mulberry cultivars were used for this study namely the native Thai mulberry (Monnoi or little mulberry in Thai) and a hybrid Thai mulberry (Chinese X Thai; Monnoi) named Buriram 60 (Br.60). The mulberries were grown using organic fertilizer in the Center of Excellence for Silk Innovation, Mahasarakham University, Thailand. Samples of leaves, branches and roots were randomly collected in September 2023. The mulberry cultivars were identified as Monnoi (C. Butiman 2024-001) and Br.60 (C. Butiman 2024-002) by Dr. Chirapha Butiman at the Center of Excellence for Silk Innovation, Mahasarakham University.

Preparation of mulberry powder samples of leaves, branches and roots for proximate analysis

Preparation of dry powder from the two mulberry cultivars started immediately after picking the mulberry leaves. Leaves (300 g) were collected from the top of the 3rd – 10th mulberry tree. Branches and roots were harvested and the unwanted portions were removed. The material was washed with water until clean and cut into 0.5 – 1 cm pieces. Mulberry branches with a circumference of 4 – 5 cm and an average diameter of 1.1 - 1.4 cm were collected at 6 months from the time of planting. The remaining leaves on the branches were removed and the branches were cut into pieces 0.5 cm thick to make 300 g.

Mulberry roots from 6 months old or older plants with a circumference of 3 – 5 cm and a diameter of 0.7 – 0.9 cm were collected, washed, cleaned and cut into slices 0.5 cm thick to make 300 g. The cut mulberry leaves, branches and roots were dried at 60 °C, (3 hr for leaves and 3.5 hr for branches and roots). After drying, the plant parts were ground with an electric grinder (1.1 mm, mesh size). Thereafter, the leaf, branch and root samples of both cultivars were then subjected to proximate analysis to determine their nutritional values including moisture, protein, fat, ash, fiber and carbohydrates (AOAC method, 2000) with triplicate determinations for each sample.

Preparation of extract samples

Mulberry leaf samples of both cultivars were coarsely ground into powder after drying, whereas the fresh branches and roots were coarsely cut into 0.5 cm slices to make 100 g. The three parts of the mulberry plant were extracted using three different methods including distilled water (autoclave, 121 °C for 15 min), and 50 % or 90 % ethanol (incubated for 24 h after maceration). The three extracts were passed through filter paper (Whatman No. 2) using a vacuum pump to speed up the filtering process. Then, the filtrate was collected, solvents were removed using a Rotary Evaporator (Buchi) and the extracts were preserved in a freezer (-20 ºC) until further use.

1-Deoxynojirimycin (DNJ) content determination

To quantify DNJ in the mulberry extracts, the method of Kim *et al*. [9] was used with slight modifications. Briefly, 169 µL of 0.4 M potassium borate buffer (pH 8.5) was dispensed into 1.5 mL test tubes containing 35 µL each of the leaves, branches and roots extracts (0.5 g dissolved in 50 % ethanol) and 20 µL of 5 mM 9-fluorenyl methyl chloroformate (FMOC-Cl) in 50 % acetonitrile (CH_3CN). The mixture was incubated in a water bath at 20 ºC for 20 min before adding 25 µL of 1 M glycine and the FMOC-Cl-DNJ was determined by adding 0.1 % acetic acid (v/v). Finally, 707 µL of distilled water was added, thoroughly mixed and filtered into a glass bottle using a 0.45 µm nylon syringe before injection into the HPLC system: diode-array detector (Shimadzu, Kyoto Japan). The DNJ standard was purchased from the Tokyo Chemical Industry (TCI, Japan). Functional programs of HPLC were achieved using an Apollo C18 (ø 4.6 mm 250 mm, 5 µm) with the mobile phase consisting of a mixture of acetonitrile and 0.1 % acetic acid (11:16 v/v) at 0.6 mL/ min flow rate and 20 µL injection volume. The identity of DNJ and the quantity of the sample were determined by its specific retention time and compared to known DNJ concentration standards using triplicate determinations for each sample. The DNJ content was presented in mg/g Ext.

Screening of phytochemical compositions

Analysis of total flavonoid content (TFC)

The TFC assay was carried out using the aluminum chloride colorimetric method [10]. Each mulberry extract was mixed with 100 µL of 5 % aluminum chloride (w/v) and 400 µL of 2.5 % $NaNO_3$. After 5 min, 500 µL of $AlCl_3$ was added and thoroughly mixed. The resulting mixture was kept at room temperature for 10 min and then mixed with 2 mL of distilled water. The absorption was read at a wavelength of 415 nm with five replicates for each sample. The TFC value was calculated as quercetin equivalent and expressed as mg QE/g Ext.

Analysis of total phenolic content (TPC)

For the determination of TPC, the standard Folin-Ciocalteu method [10] was used with slight modifications. Briefly, 100 µL of each mulberry extract was mixed in a microtube with 0.2 N Folin-Ciocalteu's reagent (oxidative reaction). The reaction was neutralized by adding 400 µL of 7.5 % $NaCO_3$. The mixture was incubated at room temperature for 3 min and the absorption of five replicates for each extract was read at a wavelength of 765 nm. The results were calculated to quantify TPC and the final values were obtained using the standard graph of gallic acid equivalent and expressed as mg GE/g Ext.

Antioxidant assays

Ferric reducing antioxidant power (FRAP) assay

The FRAP assay was used to analyze the mulberry extract following the principle of absorption spectroscopy in a microplate reader according to a previously described method [10]. The FRAP reagent was prepared in a reaction tube by mixing 300 mM acetate buffer, 10 mL of 2,4,6-Tris (2-pyridyl)-s-triazine (TPTZ) in 40 mM HCl and 20 mM $FeCl_3.6H_2O$ (10:1:1 at 37 ºC). Then, an aliquot of 900 µL FRAP reagent was added and mixed well with 100 µL of each mulberry extract. The reaction mixture was incubated at room temperature for 5 min before the absorbance was read at 593 nm. Standard graph was prepared using Fe^{3+} as a control and the concentrations of each extract were extrapolated from the graph using the average of five replicates for each sample. Values were expressed as mg TE/g Ext.

The 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging assay

The DPPH assay was carried out by following the reaction color (reduction) between the DPPH solution (0.039 mg/mL in ethanol) and the mulberry extracts. Each extract was dissolved in 100 µL of distilled water at various concentrations, mixed with 900 µL of 0.1 mM DPPH solution and incubated in the dark for 30 min. Subsequently, absorption of the reaction mixture was determined at 515 nm using Trolox and Ascorbic acid as standard substances including the control by running blank samples in each assay. The DPPH radical capacity was presented as IC_{50} (mg/mL) and the inhibition percentage was calculated using Eq 1.

$$SA_{DPPH}\ (\%) = \{(A0 - A1)/A0\}\ 100 \quad \ldots\ldots\ldots\ldots\ldots\ (1)$$

where SA_{DPPH} is the DPPH scavenging activity, and A0 and A1 refer to absorbance of the control and sample, respectively.

2,2'-azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid ($ABTS^+$) radical scavenging assay

In the $ABTS^+$ assay, mulberry extracts react with ABTS by a model stable free radical obtained by

2,2-azinobis 3-ethylvenzothiazolin-6-sulphonic acid, ($ABTS^+$). The assay was accomplished according to method [11]. The $ABTS^+$ solution (900 µL) was mixed with each mulberry extract (100 µL). The test tube was then incubated at room temperature for 6 min before the absorbance was read at 734 nm. Trolox and Ascorbic acid (in 80 % ethanol) were used as the standard substances for analysis under the same conditions while an average of five replicates were used for each sample. The $ABTS^+$ scavenging ability was represented as IC_{50} (mg/mL) and the inhibition was calculated using Eq 2.

$$SA_{ABTS+} = \{(A0-A1)/A0\}100 \quad \ldots\ldots\ldots\ldots\ldots\ldots \quad (2)$$

where SA_{ABTS+} is the $ABTS^+$ scavenging activity, and A0 and A1 refer to absorbance of the control and sample, respectively.

α-Glucosidase inhibitory assay

Mulberry extracts were tested for *in vitro* α-glucosidase inhibitory activity to screen the promising findings. The rate of release of *p*-nitrophenol from *p*-nitrophenyl-α-D-glucopyranoside (PNPG), a substrate against α-glucosidase, was determined colorimetrically. In a test tube, each mulberry extract (180 µL) was mixed with 150 µL of 0.1M phosphate buffer (pH 6.8) containing α-glucosidase (0.2 U/mL) and incubated at 37 °C for 20 min. Then, 150 µL of 5 mM PNPG (in 0.1 M phosphate buffer, pH 6.8) was added and incubated again at 37 °C for 20 min. Each reaction was then quenched by adding 0.2 M $NaCO_3$ (480 µL).

The absorbance was monitored at 405 nm. A reaction without the α-glucosidase enzyme provided the blank (negative control) with Acarbose as positive control. The α-glucosidase inhibitory assay was performed using five replicates for each sample, with results presented as percent inhibition (I) and calculated using Eq 3.

$$I\ (\%) = \{(A0–A1)/A0\}100 \quad \ldots\ldots\ldots\ldots\ldots\ldots \quad (3)$$

where A0 and A1 refer to the absorbance of control and sample, respectively, and IC_{50} values were calculated using the graphical method.

Statistical analysis

Values were analyzed as mean ± standard deviation (SD) using IBM SPPSS ver. 26.0. The data were further analyzed using one-way ANOVA and DMRT to examine significant differences between means while $p < 0.05$ was considered statistically significant.

RESULTS

Proximate composition of the leaves, branches and roots of native and hybrid Thai mulberry cultivars

Six parameters including moisture content, protein, fat, ash, fiber and carbohydrate were investigated to determine the nutritional values of the two cultivars of the plant as shown in Table 1. Average means of moisture content ranged from 4.51 to 9.0 % with the leaves of Monnoi showing the highest moisture (9.00 ± 0.00 %). High protein content was found in mulberry leaves, with Br.60 (25.25 ± 0.35 %) and Monnoi (21.15 ± 0.02 %). The lowest protein content was found in Monnoi branches at 3.32 ± 0.05 % while the fat content was higher in leaves than in branches and roots in Br.60 and Monnoi (2.46 – 2.40 %). For ash content, the leaves of Monnoi had a higher value (8.41 ± 0.10 %) compared to Br.60 leaves (7.81 ± 0.10 %). The lowest ash content was found in branches in both cultivars (3.24 – 3.35 %). Also, the fiber content was high in branches in both cultivars – Br.60 (54.83 ± 0.97 %) and Monnoi (46.39 ± 0.36 %) – but lowest in mulberry leaves (9.80 – 11.58 %). Lastly, the highest carbohydrate content was found in roots of Br.60 (49.40 ± 0.11 %) and leaves of Monnoi (49.18 ± 0.11 %) with the lowest content in branches of Br.60 (32.56 ± 0.93 %).

DNJ content

In this study, the DNJ contents in the leaves, branches, and roots under the three extraction methods differed significantly for both cultivars. The leaves of Br.60 extracted with 50 % ethanol had the highest DNJ content at 17.03 ± 0.35 mg/g Ext, while its roots extracted with distilled water (autoclave) gave the lowest content (3.66 ± 0.15 mg/g Ext). Monnoi branches with 95 % ethanol extraction gave the highest DNJ content of 16.16 ± 0.75 mg/g Ext.

The leaves (autoclave) and 95 % ethanol extracts of Monnoi had the lowest DNJ contents (3.70 ± 0.26 and 3.43 ± 0.20 mg/g Ext, respectively) compared to its branches and roots extracted using other methods. Extraction of all three plant parts of mulberry with 50 % ethanol gave the greatest quantity of DNJ in both cultivars, except Monnoi, as shown in Table 2.

Table 1: Chemical composition of roots, leaves and branches of Monnoi and Br.60 cultivars

Sample	Parameter (%)					
	Moisture	Protein	Fat	Ash	Fiber	Carbohydrate
Monnoi: roots	4.72±0.05^{d}	5.09±0.06^{d}	1.37±0.06^{c}	7.03±0.05^{c}	36.14±0.22^{c}	45.65±0.01^{c}
Monnoi: leaves	9.00±0.00^{a}	21.15±0.02^{b}	2.46±0.02^{a}	8.41±0.10^{a}	9.80±0.03^{f}	49.18±0.11^{a}
Monnoi: branches	4.61±0.04^{e}	3.32±0.05^{f}	1.15±0.05^{d}	3.35±0.05^{d}	46.39±0.36^{b}	41.18±0.26^{d}
Br.60 roots	5.33±0.08^{c}	9.65±0.15^{c}	2.47±0.03^{a}	7.72±0.13^{b}	25.43±0.30^{d}	49.40±0.10^{a}
Br.60 leaves	6.93±0.01^{b}	25.25±0.35^{a}	1.66±0.08^{b}	7.81±0.10^{b}	11.58±0.17^{e}	46.77±0.50^{b}
Br.60 branches	4.51±0.09^{f}	4.09±0.02^{e}	0.99±0.07^{e}	3.24±0.07^{d}	54.83±0.97^{a}	32.56±0.93^{e}

Means with different superscripts within a column are significantly different (DMRT, $p < 0.05$).

Table 2: DNJ contents of Thai mulberry, Monnoi and Br.60 cultivars under the three extraction methods

Variety	Mulberry part	Extraction method	DNJ content (mg/g Ext)
Monnoi	Leaf (Crude powder)	DdH_2O: Autoclave	3.70 ± 0.26^{h}
		Ethanol:50%	10.00 ± 0.62^{f}
		Ethanol:95%	3.43 ± 0.20^{h}
	Branch (Fresh)	DdH_2O: Autoclave	14.13 ± 0.86^{d}
		Ethanol:50%	15.83 ± 0.57^{bc}
		Ethanol:95%	16.16 ± 0.75^{b}
	Root (Fresh)	DdH_2O: Autoclave	12.26 ± 0.60^{e}
		Ethanol:50%	12.20 ± 0.17^{e}
		Ethanol:95%	15.83 ± 0.55^{bc}
Br.60	Leaf (Crude powder)	DdH_2O: Autoclave	15.23 ± 0.20^{c}
		Ethanol:50%	17.03 ± 0.35^{a}
		Ethanol:95%	8.56 ± 0.32^{g}
	Branch (Fresh)	DdH_2O: Autoclave	8.46 ± 0.15^{g}
		Ethanol:50%	15.83 ± 0.57^{bc}
		Ethanol:95%	8.66 ± 0.20^{g}
	Root (Fresh)	DdH_2O: Autoclave	3.66 ± 0.15^{h}
		Ethanol:50%	11.73 ± 0.77^{e}
		Ethanol:95%	8.26 ± 0.20^{g}

Means with different superscripts within a column are significantly different (DMRT, $p < 0.05$). DdH_2O is distilled water.

Screening of phytochemical compositions, total phenolic content (TPC) and total flavonoid content (TFC)

Total phenolic contents obtained from mulberry plant part samples using different extraction solvents for both cultivars are shown in Table 3. Monnoi branches with autoclave extraction had the highest TPC value of 13.45 ± 0.26 g Ext ($p < 0.05$) but branches extracted with 95 % ethanol showed a lower value of 2.27 ± 0.05 g Ext. Br.60 branches with autoclave extraction had a TPC of 10.32 ± 0.11 g Ext, with the least content found in roots under autoclave at 2.23 ± 0.05 g Ext. The TPC contents in Monnoi were higher than in Br.60. For TFC content in Monnoi and Br.60, the Br.60 root (autoclave) had the highest value of 1.55 ± 0.02 g Ext, followed by branches extracted with 95 % ethanol (1.16 ± 0.02 g Ext). Furthermore, the TFC value in Monnoi leaves from autoclave extraction gave 1.19 ± 0.02 g Ext, while the branches extracted with 95% ethanol and autoclave gave 0.99 ± 0.03 and 0.77 ± 0.03 g Ext, respectively.

Antioxidant activity and α-glucosidase inhibition activity

Results shown in Table 4 reveal the antioxidant activities of the two mulberry samples under different extractions. The FRAP antioxidant activity of the mulberry extracts showed different efficacy. Promising antioxidant potential in Monnoi and Br.60 branches were recorded with 95 % ethanol extraction while the DPPH method showed that Monnoi branches, with 95 % ethanol extraction, had the potential to inhibit free radicals with IC_{50} of 4.99 ± 0.02 mg/mL, higher and significantly different ($p < 0.05$) from the two standard substances, Trolox and Ascorbic acid at 2.02 ± 0.04 and 2.58 ± 0.07 mg/mL, respectively. All extracts from the two cultivars showed diverse antioxidant inhibitory properties.

Table 3: Screening of phytochemical compositions (TPC and TFC) in Monnoi and Br.60

Mulberry sample	TPC (mg GE/g Ext)	TFC (mg QE/g Ext)
Monnoi Leaves: Autoclave	11.42±0.20^{b}	1.19±0.02^{b}
Monnoi Branch: Autoclave	13.45±0.26^{a}	0.77±0.03^{d}
Monnoi Branch: Eth95%	2.27±0.05^{e}	0.99±0.03^{c}
Monnoi: Root	N.D.	N.D.
Br.60: leaves	N.D.	N.D.
Br.60 Branch: Autoclave	10.32±0.11^{c}	0.72±0.02^{e}
Br.60 Branch Eth95%	5.12±0.11^{d}	1.16±0.02^{b}
Br.60 Root: Autoclave	2.23±0.05^{e}	1.55±0.02^{a}

N.D = Not determined. Means with different superscripts within a column are significantly different (DMRT, $p < 0.05$)

Table 4: Antioxidant activity and α-glucosidase inhibition activity from Monnoi and Br.60 plant part extracts

Mulberry sample	FRAP mg/gExt	DPPH IC_{50}(mg/mL)	ABTS IC_{50}(mg/mL)	α-Glucosidase IC_{50}(mg/mL)
Monnoi leaf: Autoclave	2.20±0.01^{a}	5.83±0.04^{c}	4.26±0.04^{a}	2.21±0.09^{f}
Monnoi branch: Autoclave	1.12±0.02^{c}	6.15±0.08^{b}	2.81±0.07^{d}	4.42±0.04^{a}
Monnoi branch: Eth 95%	0.33±0.01^{d}	4.99±0.02^{f}	2.57±0.13^{e}	2.45±0.11^{e}
Monnoi root	N.D.	N.D.	N.D.	N.D.
Br.60 leaf	N.D.	N.D.	N.D.	N.D.
Br.60 branch: Autoclave	1.15±0.02^{c}	6.30±0.04^{a}	3.66±0.03^{b}	4.10±0.08^{b}
Br.60 branch Eth95%	0.32±0.01^{d}	5.39±0.03^{e}	3.00±0.08^{c}	3.72±0.06^{c}
Br.60 root: Autoclave	1.45±0.04^{b}	5.51±0.04^{d}	0.66±0.04^{g}	2.77±0.13^{d}
Trolox	-	2.02±0.04^{h}	1.08±0.01^{f}	-
Ascorbic acid	-	2.58±0.07^{g}	1.08±0.02^{f}	-
Acarbose	-	-	-	0.31±0.01^{g}

N.D: Not determined. Means with different superscripts within a column are significantly different (DMRT, $p < 0.05$)

The $ABTS^{+}$ showed an IC_{50} of 0.66 ± 0.04 mg/mL for autoclaved Br.60 roots, followed by 2.57 ± 0.13 mg/mL for Monnoi branches with 95 % ethanol extraction. The extracts were effective in inhibiting antioxidant activity by the ABTS assay but exhibited lower potential compared to the two standard substances, Trolox and Ascorbic acid (1.08 ± 0.01 mg/mL), except for the autoclaved Br.60 roots which had a higher ability than the two standard substances (0.66 ± 0.04 mg/mL). The α -glucosidase assay showed that all mulberry extracts had the potential to inhibit the enzyme activity. The α-glucosidase inhibition was significantly different ($p < 0.05$), with the highest in autoclaved Monnoi leaves and branches with 95 % ethanol extraction (IC_{50} of 2.21 ± 0.09 and 2.45 ± 0.11 mg/mL, respectively). However, the enzyme inhibitory activities of all mulberry extracts were less effective than the standard substance, Acarbose, with IC_{50} of 0.31 ± 0.01 mg/mL.

DISCUSSION

This study determined the nutritional values of the leaves, branches, and roots of two Thai mulberry cultivars (Monnoi and Br.60). Cultivation of these two cultivars was manipulated via organic farming on a small scale. Results revealed that the mulberry leaves of both cultivars showed high nutritional values in protein, fat and carbohydrate. This is good for silk production and is used for green tea production for humans. Br.60 showed the highest value for protein (25.25 %), with Monnoi at 21.15 %. Br.60 is a Thai hybrid cultivar that has been improved to obtain better yield than native cultivars since 1988. Modern Thai sericulture farmers prefer to plant hybrid mulberry cultivars for raising silkworms rather than native varieties. The carbohydrate content showed high values in the leaves, roots and branches of both cultivars ranging from 32.56 to 49.40 %, indicating that carbohydrate is an important constituent in all parts of the mulberry tree. The fiber content was high in the roots and branches of both cultivars (25.43 – 54.83 %), with reduced content in the leaves. Mulberry leaves are enriched sources of nutritional contents including carbohydrates, protein, fat and vitamins [12]. Mulberry branches are also rich in active substances such as arabinose, glucose, fructose, maltose, stachyose and tannin but no data are available on the minerals, carbohydrates, lipids, or root bark protein contents [13]. The compound, 1-Deoxynojirimycin (DNJ), is an alkaloid with biological activity for diabetes treatment and effective α-glucosidase inhibitory activity. It is extracted from mulberry (*M. alba*), Asiatic dayflower (*Commelina communis*), Hyacin Tree

(*Hyacinthus orientalis*), insects such as silkworms (*Bombyx mori*) which eat mulberry leaves, fermentation by bacteria such as *Streptomyces subrutilus*, *Streptomyces spp. SID9135*, *Bacillus subtilis, Streptomyces lavendulae* and also synthesized in the laboratory [14]. In this study, the DNJ contents in the leaves, branches and roots of Monnoi and Br.60 cultivars under three different extractions were high, especially using 50 % ethanol (10 – 17 mg/g Ext). Distilled water extraction (autoclave) and 95 % ethanol methods showed fluctuations of DNJ values (3 – 15 mg/g Ext). The extraction method and raw material from different parts of the mulberry plant gave different yields of DNJ. A previous study reported the DNJ content as 100 mg/100 g (dry tea) while the effective dose of DNJ was 6 mg/60 kg human weight [15]. Interestingly, DNJ and chemical components are influenced by environmental conditions, temperature and seasons [16]. The quantity of phytochemicals in the two mulberry cultivars was determined by two extraction methods distilled water (autoclave) and 95 % ethanol. Different extraction methods gave diverse values of TPC (2.23 – 13.45 mg GE/g Ext) and different parts of the mulberry tree also exhibited different contained compounds. The TFC content of the roots, leaves and branches had values ranging from 0.72 to 1.55 mg QE/g Ext. A previous study reported that the method of extracting and harvesting mulberry leaves affected TPC and TFC [10]. The TPC of *M. alba* (leaves of a Sakon Nakhon cultivar) using distilled water and 50 % and 95 % ethanol extract showed a minimum value of TPC as 0.302 mg GE/g Ext for the 95 % ethanol extract (harvested 2 weeks after fertilization) and the maximum 124.44 mg GE/g Ext (harvested 4 weeks after fertilization) However, TFC gave the minimum value for distilled water extraction at 7.94 mg QE/g Ext. (harvested 4 weeks after fertilization) and maximum (95 % ethanol) value of 110.91 mg QE/g Ext (harvested 2 weeks after fertilization). The three antioxidant activity assays (FRAP, DPPH and ABTS) were used to confirm the antioxidant activities of Monnoi and Br.60 mulberry extracts. The antioxidant inhibitory activities of the extracts were higher than the standard substance values, Trolox and Ascorbic acid, similar to results reported by a previous publication [10]. Furthermore, TPC and TFC values in this study were correlated with the antioxidant effect. The α-glucosidase inhibitory assay was also assessed to verify the blood glucose-lowering potential of the mulberry extracts. Both cultivars showed a lower potency than the standard substance, Acarbose. The leaves and branch extracts of Monnoi had the highest potential among the extracts, related to many factors including mulberry varieties and extraction solvent concentrations. A previous report by Suriyaprom and his colleagues showed that mulberry leaves of the cultivar Sakon Nakhon extracted with ethanol (no specified concentration) showed greater antioxidant values than Br.60 and were associated with α-glucosidase inhibition [17]. The enzyme α-glucosidase plays an important role in carbohydrate digestion and absorption in the human small intestine and inhibition of α-glucosidase is a potential factor for type 2 diabetes treatment [18]. Furthermore, mulberry plant parts, mainly twigs, leaves, roots and fruit have been shown to possess antidiabetic potential [16]. Mulberry crude extracts showed potential for the treatment of diabetes and could be linked to the main substance, DNJ, together with other bioactive compounds.

CONCLUSIONS

The two mulberry cultivars, Monnoi and Br.60, show high potential for diabetes treatment due to the presence of bioactive compounds and extraction using 50 % ethanol gives the highest yield. Further isolation and characterization and possible mechanism of action of phytoactive constituents such as 1-deoxynojirimycin (DNJ) would be necessary.

DECLARATIONS

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Ethical approval

None provided.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Conflict of Interest

No conflict of interest associated with this work.

Contribution of Authors

The authors declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by them.

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