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ORIGINAL ARTICLE

Comparative Assessment of α-amylase and α-glucosidase Inhibition and *in vitro* Antioxidant Capacity of *Garcinia schomburgkiana* Bark, Fruit, and Leaf Extracts

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ABSTRACT

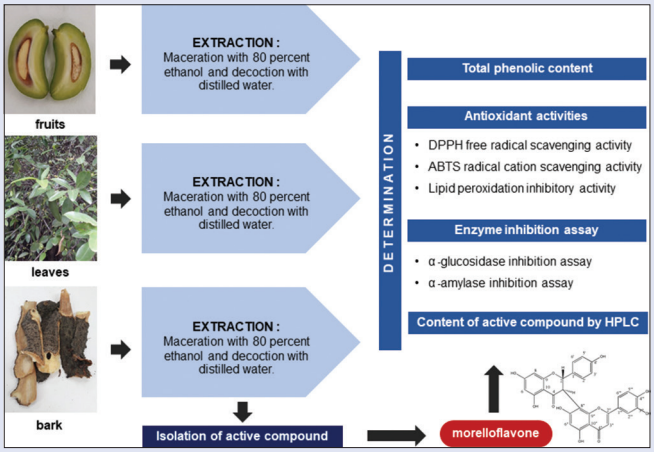
Background: *Garcinia schomburgkiana* is a conventionally used as an herb for the treatment of diabetes, coughs, and menstrual disturbances. **Objectives:** The study was to examine *in vitro* antioxidant potentials and inhibitory effect against α-amylase and α-glucosidase of the bark, fruit, and leaf extracts of *G. schomburgkiana* using different traditional extraction methods and investigate the bioactive compound using spectroscopic and chromatographic techniques. **Materials and Methods:** The extracts were prepared by maceration with 80% ethanol and decoction with distilled water. The anti-free radical activities of the extracts were tested through decolorization of 2,2-diphenyl-2-picrylhydrazyl (DPPH), 2,2′-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) (ABTS), and lipid peroxidation assays. The active compound was elucidated and quantified. **Results:** The ethanolic bark extract displayed the highest activities of DPPH, ABTS, lipid peroxidation, α-glucosidase, and α-amylase inhibition assays with EC_{50} values of 28.96 ± 1.62, 9.79 ± 0.14, 574.89 ± 14.68, 20.40 ± 1.33 and 2.81 ± 0.43 μg/mL, respectively. Thus, the ethanolic bark extract was selected to assess the bioactive compound by bioactivity-guided isolation. The active biflavonoid, named morelloflavone, was isolated and elucidated. Morelloflavone exhibited high activities comparable with positive controls (ascorbic acid and acarbose). Moreover, the content of morelloflavone from different extracts was analyzed by high-performance liquid chromatography. The bark maceration with ethanol yielded the highest contents of morelloflavone. **Conclusion:** The bark ethanolic extract of *G. schomburgkiana* has more potentials than other extracts. The isolated compound demonstrated the strong activities and could be the alternative source of natural antioxidants and α-amylase and α-glucosidase inhibitor.

Key words: Antioxidant, *Garcinia schomburgkiana*, morelloflavone, α-amylase, α-*glucosidase*

SUMMARY

- The optimum extraction method of *Garcinia schomburgkiana* extracts was determined
- Morelloflavone from *G. schomburgkiana* extract was identified as a bioactive compound
- Morelloflavone could be used as a marker for *G. schomburgkiana* extract standardization.

Abbreviations used: DPPH: decolorization of 2,2-diphenyl-2-picrylhydrazyl; ABTS: 2,2′-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid); FRAP: Ferric reducing antioxidant power; EC_{50}: 50% effective concentration; HPLC: High-performance liquid chromatography; UV: Ultraviolet–visible spectrophotometer; IR: Infrared spectroscopy; MS: mass spectrometer; NMR: Nuclear Magnetic Resonance; pNPG: p-nitrophenyl β-D-glucopyranoside; DAD: Diode array detector; LSD: Least significant difference; TPC: Total phenolic contents; EBM: Ethanolic bark extract by maceration; ELM: Ethanolic leaf extract by maceration; ABD: Aqueous bark extract by decoction; EFM: Ethanolic fruit extract by maceration; AFD: Aqueous fruit extract by decoction; ALD: Aqueous leaf extract by decoction; GAE: galic acid equivalent.



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INTRODUCTION

Diabetes mellitus is a complex chronic disease and a wild-growing medical problem over the globe in developing and developed countries. Diabetes type 2 diabetes is the most common type which is the seventh leading cause of death worldwide.[1] Increased insulin resistance and impaired insulin secretion are the key pathophysiological factors of type 2 diabetes that cause post-prandial hyperglycemia.[2] The long-term hyperglycemia increases oxidative stress and leads to numerous degenerative diseases such as cardiovascular disease, peripheral neuropathy, and nephropathy.[3] α-amylase and α-glucosidase are carbohydrate digestive enzymes in human body of which directly related to the postprandial glycemic level. The inhibition of these enzymes can

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reduce and delay the formation of glucose to be absorbed into blood circular and avoiding of hyperglycemia. Although the clinical glucosidase inhibitors such as voglibose, miglitol, and acarbose can decrease postprandial hyperglycemia, the adverse effects of these synthetic drugs such as flatulence, diarrhea, and abdominal discomfort can limit patient compliance. Natural products for dealing of postprandial hyperglycemia and antioxidant from plants have become an attractive approach due to their low cost, safety, and fewer side effects.[4,5]

The genus *Garcinia* in the family Clusiaceae native to America, Africa, and Asia has been recognized as a rich source of numerous biological active constituents.[6,7] *Garcinia schomburgkiana* Pierre is a medium evergreen tree locally known as Ma-dan, which is widely distributed in Southeast Asia. In folk medicine, this plant is used for the cure of cough, diabetes, and menstrual disturbances.[8] The pharmacological studies on *G. schomburgkiana* extracts have revealed the antimalarial activity and cytotoxicity against cancer cell lines. In addition, phytochemical constituents of *G. schomburgkiana* have displayed the presence of benzophenones, bioflavonoids, biphenyl derivatives, and xanthones.[9-11]

The aim of this study was to analyze α-glucosidase and α-amylase inhibitory activities including antioxidant activities performed by decolorization of 2,2-diphenyl-2-picrylhydrazyl (DPPH), 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) (ABTS), lipid peroxidation assay, and total phenolic contents (TPC) from bark, fruit, and leaf of *G. schomburgkiana* extracts using maceration with 80% ethanol and decoction with distilled water techniques. The extract owning the best α-glycosidase inhibition was further separated and purified through silica gel column and preparative thin-layer chromatography. The pure constituent was structurally characterized using infrared spectroscopy, mass spectrometry, and nuclear magnetic resonance to discover natural active compounds. The content of the bioactive component was also determined by high-performance liquid chromatography (HPLC).

MATERIALS AND METHODS

General equipment and chemicals

Analytical grade solvents for extraction and isolation were purchased from Labscan Asia Co., Thailand, and high-purity chemicals were purchased from Sigma/Aldrich, USA. Column chromatography isolation and purification were done using silica gel 60 (0.040–0.063 mm Scharlau, Spain). For compound detection, pre-coated silica gel plates were operated (Merck silica gel 60 GF254, Germany). Preparative thin-layer chromatography was carried on precoated silica gel G 1000 μm (Analtech Inc., Delaware, USA). Ultraviolet (UV), infrared spectroscopy (IR), mass spectrometer (MS), and nuclear magnetic resonance (NMR) spectra were obtained on Shimadzu photodiode array detector (Shimadzu, Japan), Frontier FT-IR spectrometer (Perkin Elmer Ltd., UK), MICROTOF benchtop ESI-TOF-MS (Bruker Daltonics, Germany), and Avance III HD 400 (Bruker, MA, USA), respectively.

Plant material

G. schomburgkiana (bark, leaves, and fruits) were collected from Phak Hai district, Ayutthaya Province, in November 2018. The species was identified by a taxonomist, Dr. Chakkrapong Rattamanee and the voucher specimen (GS20181101) was deposited at Faculty of Pharmaceutical Sciences, Burapha University. The bark, leaves, and fruits of *G. schomburgkiana* were air in a hot air oven (Memmert, Germany) at 50°C and then was powdered pass through a 0.5-mm sieve separately.

Extraction material

For maceration, the dried powder of bark, leaves, and fruits was set separately in an Erlenmeyer flask and extracted with 80% ethanol (1:20, w/v) for 72 h at 30°C with intermittent shaking. For decoction, the dried powder of bark, leaves, and fruits was also set in an Erlenmeyer flask and boiled with distilled water (1:20, w/v) at 80°C for 15 min and then filtered. The residue of each method was re-extracted again. After that, the pooled extract was separately filtered and dried using a vacuum evaporator (Buchi, Switzerland) under reduced pressure at 50°C.

Total phenolic content

Total phenolic content was evaluated by the Folin–Ciocalteu colorimetric method.[4] Total phenolics of triplicate analyses were showed as gallic acid equivalent (mg GAE)/g extract and present on mean ± standard deviation.

Antioxidant activities

Decolorization of 2,2-diphenyl-2-picryl hydrazyl free radical scavenging activity

The hydrogen donating capability of the crude extracts and pure compound from *G. schomburgkiana* were determined by DPPH.[12] Ascorbic acid and quercetin were used as standards at concentrations similar to those of morelloflavone (25, 12.5, 6.25, 3.125, 1.56, 0.78, and 0.39 μg/mL) and were treated under the same condition as the samples (625, 500, 250, 125, 62.5, 31.25, 15.63, 7.81, and 3.91 μg/mL). The absorbance was detected at 517 nm. The results were reported from EC_{50} value, which is the concentration of the antioxidant agent that is essential to scavenge 50%t of DDPH in the test sample.

2,2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) radical cation scavenging activity

The antioxidant capacity assay was carried out using the ABTS radical cation capturing method.[4] Ascorbic acid and quercetin were used as standards at concentrations similar to those of morelloflavone (25, 12.5, 6.25, 3.125, 1.56, 0.78, and 0.39 μg/mL) and were treated under the same condition as the samples (500, 250, 125, 62.5, 31.25, 15.63, 7.81, and 3.91 μg/mL). The absorbance was detected at 734 nm. The results were calculated as EC_{50} value which means the minimum concentration of antioxidant agent required to trap 50% of radicals.

Lipid peroxidation inhibitory activity

Lipid peroxidation assay was achieved according to the method of Srinivasan *et al.*[13] Briefly, the different concentrations of sample or control (100 μL) were added to 900 μL egg lectin mixture. Quercetin was used as the standard at concentrations similar to those of morelloflavone (125, 62.5, 31.25, 15.63, 7.81, and 3.91 μg/mL) and was treated under the same condition as the samples (1000, 500, 250, 125, 62.5, and 31.25 μg/mL). Lipid peroxidation was started by adding ferric chloride (400 mM, 40 μL) and ascorbic acid (200 mM, 40 μL) and incubate for 60 min at 37°C. After that, 2 mL of 0.25N HCl containing 0.375% thiobarbituric acid and 15% trichloroacetic acid was added to stop the reaction and boiled for 15 min. After chilling, the combination was centrifuged at 3000 g for 5 min and the absorbance of the supernatant was evaluated at 532 nm. The results were reported as EC_{50} value.

Enzyme inhibitory effects

α-glucosidase inhibitory activity

The activity was investigated using p-nitrophenyl β-D-glucopyranoside (pNPG) by the method of Vongsak *et al.*[14] In brief, 50 μL of different concentrations of samples and control was mixed with 50 μL of 0.1 M sodium phosphate buffer (pH 7.0) and 2 units/mL of 50 μL α-glucosidase and pre-incubated for 10 min at 37°C. Then, 50 μL of 20 mM pNPG was added to initiate the reaction. After incubation for 30 min at 37°C, the absorbance was determined at 405 nm and calculated as EC_{50} value.

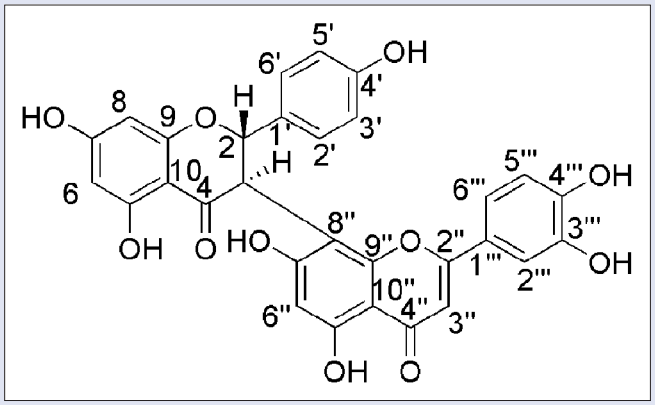

Figure 1: Structure of morelloflavone, an active compound of *Garcinia schomburgkiana*

α-amylase inhibitory activity

The inhibitory activity assay was carried out from the method of Thengyai *et al.*[5] In short, 0.2 mL of 50 mM Tris-HCl buffer (pH 6.9) consisting of 10 mM $CaCl_2$ was added starch azure (2 mg) and boiled for 10 min. Different concentrations of sample or control (0.2 mL) were added in 0.1 mL of α-amylase (2 units/mL) in 20 mM phosphate buffer (pH 6.9) containing 6.7 mM NaCl after pre-incubated at 37°C for 5 min. The reaction was kept at 37°C for 10 min and added 50% acetic acid (0.5 mL) to stop the reaction. After cooling, the combination was centrifuged at 3000 g for 5 min and the absorbance of the supernatant was evaluated at 595 nm. The results were reported as EC_{50} value.

Isolation of active compound

Crude extract exhibited the strongest antioxidant and enzyme inhibitory effects that were obtained by extracting dried powder of bark with 80% ethanol. The extract (10 g) was subjected to vacuum chromatography using silica gel as stationary phase and fractionated with hexane, dichloromethane, ethyl acetate, acetone, and methanol. The ethyl acetate fraction (2.50 g) displayed the highest antioxidant and enzyme inhibitory effects. This fraction was eluted with gradient hexane: ethyl acetate (50:50–0:100) by silica column chromatography (70 cm × 2.5 cm) to be collected and combined into 23 fractions. Subfraction 20 (300 mg) expressed the strongest activities and further separately purified through preparative thin-layer chromatography. Using a silica gel plate (Analtech Inc., Delaware, USA), the subfraction was eluted with hexane: ethyl acetate: methanol (3:6.5:0.5) to obtain pure compound 30 mg.

High-performance liquid chromatography

The pure compound, morelloflavone (purity ≥98%), was isolated from bark extract of *G. schomburgkiana*. Each standard solution was diluted into eight concentrations (200, 100, 50, 25 12.5, 6.25, 3.125, and 1.56 μg/mL) to achieve a calibration curve. Quantitative investigation of the active component was completed using the HPLC technique on a Shimadzu (Shimadzu, Kyoto, Japan) equipped with a SIL-20A HT autosampler, CT0-10ASvp column oven, LC-20AD pump, and SPD-M20A diode array detector (DAD). An ACE5 C_{18} column (150 mm × 4.6 mm i.d., 5 μm) with a C_{18} guard column (Phenomenex, California, USA) was also utilized. The elution was done using 0.5% formic acid in water (solvent A) and methanol (solvent B) as a mobile phase with the subsequent isocratic (50:50) for 20 min. The flow rate was fixed at 1.0 ml/mL with a measured temperature of 25°C. Each extract was dissolved in methanol to obtain a concentration of 1 mg/mL and filtered over a 0.2 mm nylon membrane filter. The DAD detector was examined at 290 nm and the injection amount for each sample and standard was 10 μL.

Statistic analysis

The results were described as mean ± standard deviation (n = 3). The average content of total phenolics and EC_{50} of the extracts carried on the different assays were statistically examined using one-way ANOVA with the least significant difference by SPSS (IBM Corp., NY, USA) for Windows 21.0. $P < 0.05$ was specified as a statistically significant difference.

RESULTS

Table 1 demonstrating the values of TPC and biological activities of six *G. schomburgkiana* extracts displays that the ethanolic bark extract by maceration (EBM) gave the highest amount of TPC with 222.18 ± 3.53 mg GAE/g extract. In addition, the ethanolic leaf extract by maceration (ELM) exhibited the second-high amount of TPC with 191.67 ± 5.95 mg GAE/g extract, while the aqueous decoction of bark, leaves, and fruits showed lower TPC with 133.76 ± 10.17, 102.26 ± 29.96, and 130.23 ± 34.92 mg GAE/g extract. For biological activities, the EBM also exhibited the strongest DPPH, ABTS, lipid peroxidation, α-glucosidase, and α-amylase inhibitory activities with value of EC_{50} 24.75 ± 1.69 μg/mL, 11.14 ± 0.22 μg/mL, 769.07 ± 36.01 μg/mL, 2.91 ± 0.09 μg/mL, and 8.17 ± 0.93 μg/mL, respectively. The EC_{50} values of DPPH, ABTS radical scavenging activities, α-glucosidase and α-amylase inhibitory activities of ELM extract were the second-high with values of 51.51 ± 2.15, 13.65 ± 0.04, 856.61 ± 8.31, 8.31 ± 0.36, and 22.10 ± 0.18, respectively. The aqueous leaf extract by decoction (ALD) provided the lowest DPPH, ABTS, lipid peroxidation, and α-glucosidase and α-amylase inhibitory activities with value of EC_{50} 535.99 ± 37.02, 225.63 ± 4.26 μg/mL, >1000, 48.21 ± 0.42, and >1000 μg/mL, respectively. In addition, the ethanolic extract by maceration method expressed the stronger biological activities than aqueous extract by decoction method for bark, fruits, and leaves of *G. schomburgkiana*.

Due to the maximum biological activities, EBM was selected to separate and elucidate the bioactive constituent by α-glucosidase inhibitory activity-guided isolation. The crude extract was subjected to column chromatographic separation and preparative thin layer chromatography to acquire morelloflavone [Figure 1].[15] The substance was obtained as a yellow solid, HREIMS: calcd for $C_{30}H_{20}O_{11}$, m/z 556.4732 [M] +, found 557.1083. UV (CH_3OH, 0.1%) $\lambda_{max/nm}$: 346, 289. IR: 3326, 1643, 1604, 1259, 1167, 837 cm^{-1}. ^1H NMR (CD_3OD, 400 MHz, δ-ppm): δ 5.76 (1H, d, J = 12 Hz, H-2), 4.83 (1H, d, J = 12 Hz, H-3), 5.98 (1H, d, J = 1.6 Hz, H-6), 6.00 (1H, d, J = 1.6, H-8), 7.12 (2H, d, J = 8.4 Hz, H-2'), 6.43 (2H, d, J = 8.4 Hz, H-3'), 6.43 (2H, d, J = 8.4 Hz, H-5'), 7.12 (2H, d, J = 8.4 Hz, H-6'), 6.41 (1H, s, H-3"), 6.27 (1H, s, H-6"), 7.35 (1H, d, J = 2.0, H-2'"), 6.90 (1H, d, J = 8.4, H-5'"), 7.29 (1H, dd, J = 8.4, 2.0, H-6'"). ^{13}C NMR (100 MHz, δ-ppm): δ 81.3 (C-2), 49.5 (C-3), 196.0 (C-4), 164.4 (C-5), 96.0 (C-6), 166.8 (C-7), 95.0 (C-8), 163.4 (C-9), 102.0 (C-10), 129.1 (C-1'), 127.9 (C-2'), 114.2 (C-3'), 157.5 (C-4'), 114.2 (C-5'), 127.9 (C-6'), 164.4 (C-2"), 102.0 (C-3"), 182.4 (C-4"), 161.2 (C-5"), 98.4 (C-6"), 161.9 (C-7"), 100.6 (C-8"), 157.2 (C-9"), 103.6 (C-10"), 122.1 (C-1'"), 112.8 (C-2'"), 145.4 (C-3'"), 149.6 (C-4'"), 115.5 (C-5'"), and 119.2 (C-6'") [Table 2]. The isolated compound, morelloflavone, demonstrated the strong DPPH, ABTS, lipid peroxidation, α-amylase, and α-glucosidase inhibitory activities comparable with positive controls (ascorbic acid, quercetin, and acarbose) with EC_{50} values of 3.22 ± 0.24, 6.46 ± 0.09, 9.54 ± 0.19, 6.57 ± 0.70, and 0.72 ± 0.07 μg/mL, respectively [Table 1]. The amount of morelloflavone from different extraction was in the range of not detected to 85.37 ± 2.96 mg/g extract. In the ethanol extract of bark, fruit and leaf, the content of morelloflavone was 85.37 ± 2.96, 5.13 ± 0.04, and undetectable, respectively [Figure 2], while the decoction method of bark, fruit, and leaf cannot detect morelloflavone content.

Table 1: Antioxidation activity and α-amylase and α-glucosidase inhibition activities of *Garcinia schomburgkiana* extracts using different plant part and extraction techniques and the active compounds

Samples/ standards	Total phenolic contents (mg GAE/g extract)	DPPHEC_{50} (μg/mL)	ABTSEC_{50} (μg/mL)	Lipid peroxidation EC_{50} (μg/mL)	α-glucosidase EC_{50} (μg/mL)	α-amylase EC_{50} (μg/mL)
EBM	222.18±3.53^{a}	24.75±1.69^{a}	11.14±0.22^{a}	769.07±36.01^{a}	2.91±0.09^{a}	8.17±0.93^{a}
ABD	133.76±10.17^{b}	94.64±5.30^{b}	58.76±0.66^{b}	797.17±9.22^{a}	13.19±0.41^{b}	158.47±0.48^{b}
EFM	36.86±1.94^{c}	107.75±4.12^{c}	48.79±2.04^{c}	>1000^{b}	14.38±0.25^{c}	843.08±14.98^{c}
AFD	102.26±29.96^{b}	435.16±15.34^{d}	135.05±3.12^{d}	834.39±19.0^{c}	40.87±0.38^{d}	>1000^{d}
ELM	191.67±5.95^{d}	51.51±2.15^{e}	13.65±0.04^{f}	856.61±8.31^{c}	8.31±0.36^{e}	22.10±0.18^{e}
ALD	130.23±34.92^{b}	535.99±37.02^{f}	225.63±4.26^{g}	>1000^{b}	48.21±0.42^{f}	>1000^{d}
Morelloflavone	-	3.22±0.24^{g}	6.46±0.09^{h}	9.54±0.19^{d}	0.72±0.07^{g}	6.57±0.70^{a}
Ascorbic acid	-	7.40±0.07^{h}	4.09±0.09^{i}	-	-	-
Quercetin	-	3.00±0.07^{g}	1.46±0.19^{j}	103.89±0.70^{e}	2.56±0.06^{h}	2.32±0.11^{f}
Acarbose	-	-	-	-	877.27±107.7^{i}	68.29±3.39^{g}

Values were represented as means±SD (n=3). Different alphabets represent a statistically significant difference at P<0.05 between the same column. GAE: Gallic acid equivalent; DPPH: 2-2'-Diphenyl-1-picrylhydrazyl; ABTS: 2,2'azinobis-(3-ethylbenzothiazoline-6-sulfonic acid); FRAP: Ferric reducing antioxidant power; EC_{50}: 50% effective concentration; EBM: Ethanolic bark extract by maceration; ABD: Aqueous bark extract by decoction; EFM: Ethanolic fruit extract by maceration; AFD: Aqueous fruit extract by decoction; ELM: Ethanolic leaf extract by maceration; ALD: Aqueous leaf extract by decoction, SD: Standard deviation

Table 2: ^{1}H (400 MHz) and ^{13}C (100 MHz) nuclear magnetic resonance spectroscopic data of isolated compound and reference compound

Position	δ_H (J in Hz)	δ_H (J in Hz) in CD_3OD[15]	δ_C	δ_C in CD3OD[15]	DEPT	HMBC
2	5.76 d 12.0	5.35 d 12	81.3	80.9	CH	3, 4, 1′, 2′, 6′
3	4.83 d 12.0	4.48 d 12	49.5	49.9	CH	2, 4, 10, 1′, 7′, 8′, 9″
4	-	-	196.0	196.3	C	-
5	-	-	164.4	163.7	C	-
6	5.98 d 1.6	5.89 d 2	96.0	96.2	CH	5, 7, 8, 10
7	-	-	166.8	166.4	C	-
8	6.00 d 1.6	5.77 d 2	95.0	95.2	CH	6, 7, 9, 10
9	-	-	163.4	162.1	C	-
10	-	-	102.0	101.5	C	-
1′	-	-	129.1	128.0	C	-
2′	7.12 d 8.4	7.11 d 8	127.9	128.4	CH	2, 4′, 6′
3′	6.43 d 8.4	6.65 d 8	114.2	114.4	CH	1′, 2′, 4′, 5′
4′	-	-	157.5	157.2	C	-
5′	6.43 d 8.4	6.65 d 8	114.2	114.4	CH	1′, 2′, 3′, 4′
6′	7.12 d 8.4	7.11 d 8	127.9	128.4	CH	2, 2′, 4′
2″	-	-	164.4	162.8	C	-
3″	6.41 s	6.58 s	102.0	102.4	CH	2″, 4″, 10″, 1‴
4″	-	-	182.4	179.5	C	-
5″	-	-	161.22	159.7	C	-
6″	6.27 s	5.99 s	98.4	97.9	CH	5″, 7″, 8″, 10″
7″	-	-	161.9	161.3	C	-
8″	-	-	100.6	100.0	C	-
9″	-	-	157.2	154.0	C	-
10″	-	-	103.6	103.0	C	-
1‴	-	-	122.1	121.6	C	-
2‴	7.35 d 2.0	6.85 s	112.8	117.6	CH	2″, 4‴, 6‴
3‴	-	-	145.4	145.0	C	-
4‴	-	-	149.6	147.6	C	-
5‴	6.90 d 8.4	6.81 d 8	115.5	116.2	CH	1‴, 3‴
6‴	7.29 dd 8.4, 2.0	6.79 d 8	119.2	120.3	CH	2″, 2‴, 4‴

DISCUSSION

The genus *Garcinia* contains a diversity of pharmacologically active natural constituents such as flavonoids, terpenoids, xanthones, and benzophenones.[16,17] In this study, the free radical-scavenging and antidiabetic activities of *G. schomburgkiana* were established. The results [Table 1] demonstrated that the bark of ethanolic extract exhibited the greatest ability to scavenge DPPH and ABTS radicals, having approximately fourfold greater activity than the aqueous extracts. The free radical-scavenging capacity of the extracts decreased in the order of EBM > aqueous bark extract by decoction > ELM > ethanolic fruit extract by maceration > aqueous fruit extract by decoction > ALD. These results illustrated the promising antioxidant effects of the plant, and these effects are influenced by the solubility and polarity of the active compound.[18] The slightly different results between TPC and antioxidant activity are probably attributable to synergistic effects and the complexity of the chemical structures. Thus, the activity of the phenolic compounds might depend on both the active constituents and other active phytochemicals.[19] In addition, Meechai *et al.* reported that the extracts of *G. schomburgkiana* obtained using organic solvents such as acetone, dichloromethane, and methanol have strong antioxidant activities and high TPC.[20] However, in traditional medicine, *G. schomburgkiana* was prepared via maceration with ethanol or decoction with water, as applied in this

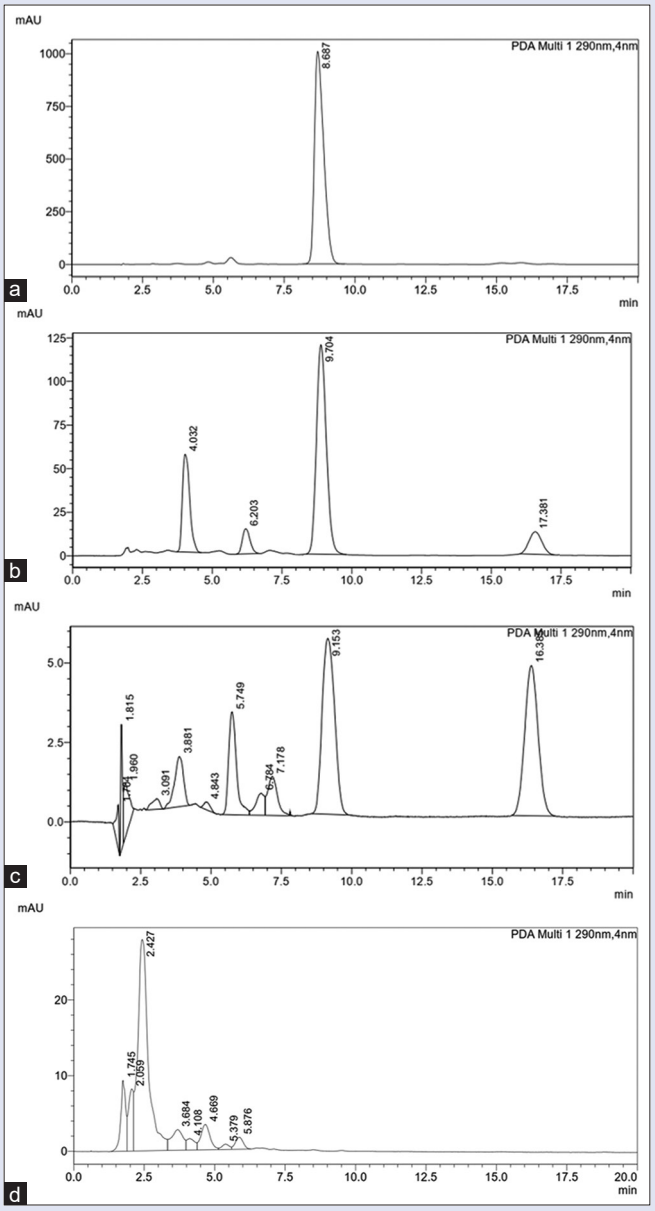

Figure 2: High-performance liquid chromatography chromatograms of morelloflavone (a) and ethanol extracts of bark (b), fruits (c) and leaf (d) of *Garcinia schomburgkiana* at 290 nm

study. The results illustrated that ethanolic extraction provided higher TPC than obtained using organic solvents.

Regarding enzyme inhibition, agents that inhibit α-amylase and α-glucosidase play important roles in deceasing hyperglycemia, which is one of the factors causing oxidative stress and type 2 diabetes.[5] *Garcinia* spp., such as *G. gracilis*, *G. mangostana*, and *G. oblongifolia*, also inhibited these enzymes, and the active compounds have been reported as phenolics and flavonoids.[21-24] All extracts exerted concentration-dependent inhibitory activity. In particular, the ethanolic extracts of bark, fruit, and leaves inhibited α-amylase and α-glucosidase activities at all analyzed concentrations. Meanwhile, the aqueous extracts did not inhibit enzyme activity at concentrations of below 1.56 μg/mL. Concentrations of higher than 1000 μg/mL were not considered because absorbance signals exceeded the linear range. These results indicated that the solvent used for extraction affects the enzyme-inhibitory activity of *G. schomburgkiana*, consistent with a previous report observing a comparable phenomenon for *Pluchea indica* extract.[25] Besides, the bark extract exhibited a stronger α-amylase and α-glucosidase inhibitory effect than the leaf and fruit extracts. Comparing the inhibition values, the extracts displayed weaker α-amylase inhibitory activity than α-glucosidase inhibitory activity. Researchers previously hypothesized that effective beneficial constituents for controlling postprandial hyperglycemia with fewer adverse effects than acarbose (an antidiabetic medicine) have weaker α-amylase inhibitory activity than α-glucosidase inhibitory activity.[26]

EBM most strongly inhibited α-amylase and α-glucosidase activities, and its EC_{50} values were relatively higher than those of acarbose. This phenomenon suggests that a large amount of the bioactive substance was present in this sample. Thus, this crude extract was chosen to clarify the active compound. Morelloflavone was identified as the active compound, and it displayed potent antioxidant and α-amylase and α-glucosidase inhibitory activities. Thus, morelloflavone could be used as a marker to standardize bioactivity. Using HPLC analysis, the highest content of morelloflavone was found in the ethanolic bark extract. In a previous study, several bioflavonoids and xanthones with antioxidant activity and cytotoxicity against cancer cell lines were isolated from *G. schomburgkiana*.[8,10,11,27] Nevertheless, no prior report described the active constituent of *G. schomburgkiana* extract or its antidiabetic activity. Plants from the same genus have been studied for α-amylase and α-glucosidase inhibitory activity. For example, cowanin and cowanol from the twig extract of *G. oblongifolia* inhibited α-glucosidase activities with IC_{50} values of 10.7 ± 1.7 and 28.0 ± 11.8 μM, respectively, whereas morelloflavone inhibited α-glucosidase activities with an IC_{50} of 1.29 ± 0.13 μM (48.21 ± 0.42 μg/mL).[23] Although some other phytochemicals of *G. schomburgkiana* such as kaempferol, gentisein, and norathyriol may have slightly influenced the observed biological activity in a previous report,[27] morelloflavone was a major active compound displaying strong activities using bioassay-guided isolation in this work. The gastrointestinal enzymes α-amylase and α-glucosidase were used to assess inhibitory activity *in vitro* as a comprehensive model for antidiabetic nutraceutical analysis to screen prospective inhibitors. Thus, EBM could represent a suitable alternative source of antidiabetic and antioxidant supplements, and morelloflavone could be the bioactive compound. *In vivo* studies will be essential for the clinical use of morelloflavone and EBM in the treatment of diabetes and associated maladies.

CONCLUSION

In this study, *in vitro* α-glucosidase and α-amylase inhibitory activity and antioxidant capacity of different plant parts and method of *G. schomburgkiana* that used as traditional antidiabetic treatment were compared. The EBM indicated the strongest activity and morelloflavone was isolated and identified as a bioactive substance by gastrointestinal enzymes inhibitory activity and antioxidant. Thus, the present work could endorse the traditional use of *G. schomburgkiana* to treat diabetes. EBM might be a more appropriate choice for development as a nutraceutical product and morelloflavone could be used as a marker for standardization.

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Conflicts of interest

There are no conflicts of interest.

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