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Original Article

In vitro alpha glucosidase inhibition and free-radical scavenging activity of propolis from Thai stingless bees in mangosteen orchard

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A B S T R A C T

The chemical component and biological activity of propolis depend on flora area of bee collection and bee species. In the study, the propolis from three stingless bee species, *Lepidotrigona ventralis* Smith, *Lepidotrigona terminata* Smith, and *Tetragonula pagdeni* Schwarz, was collected in the same region of mangosteen garden from Thailand. Total phenolic content, alpha glucosidase inhibitory effect, and free-radical scavenging activity using FRAP, ABTS, DPPH assays were determined. The most potent activity of propolis extract was investigated for bioactive compounds and their quantity. The ethanol extract of *T. pagdeni* propolis had the highest total phenolic content 12.83 ± 0.72 g of gallic acid equivalents in 100 g of the extract, and the strongest alpha glucosidase inhibitory effect with the IC_{50} of 70.79 ± 6.44 μg/ml. The free-radical scavenging activity evaluated by FRAP, ABTS, DPPH assays showed the FRAP value of 279.70 ± 20.55 μmol $FeSO_4$ equivalent/g extract and the IC_{50} of 59.52 ± 10.76 and 122.71 ± 11.76 μg/ml, respectively. Gamma- and alpha-mangostin from *T. pagdeni* propolis extract were isolated and determined for the biological activity. Gamma-mangostin exhibited the strongest activity for both alpha glucosidase inhibitory effect and free-radical scavenging activity. Using HPLC quantitative analysis method, the content of gamma- and alpha-mangostin in the extract was found to be 0.94 ± 0.01 and 2.77 ± 0.08% (w/w), respectively. These findings suggested that *T. pagdeni* propolis may be used as a more suitable raw material for nutraceutical and pharmaceutical products and these mangostin derivatives as markers.

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Introduction

Overproduction of free radical species can be damaging to host cells and lead to various ailments such as inflammatory, aging and diabetes (Zhou et al., 2015). In the case of diabetes, alpha glucosidase is a vital enzyme essential for cleavage of maltose to glucose for the absorption into the blood stream in the small intestine. Therefore, alpha glucosidase inhibitors could regulate abnormally high stages of plasma glucose after carbohydrate ingestion (Ryu et al., 2011). Nowadays, α-glucosidase inhibitors, such as acarbose, voglibose and miglitol, have been accepted for clinical use in the treatment of type 2 diabetes. Nonetheless, some synthetic alpha glucosidase inhibitors have undesirable side effects, such as abdominal cramping, flatulence and diarrhea (Zhang et al., 2015). As a result, many scientists have turned their attention to natural alpha glucosidase inhibitors including mangosteen and bee products, which are utilized to develop nutraceuticals or lead compounds for antidiabetic management (Matsui et al., 2004; Ryu et al., 2011; Juárez-Rojop et al., 2014).

Natural products from bees have been extensively employed since ancient time because of its broad pharmacological activity (Dantas et al., 2014; Kustiawan et al., 2014). Propolis is one of the bee products that exhibits numerous biological activities such as antioxidant, anti-inflammatory, antitumor, antiviral, antibacterial, antifungal, antidiabetic activities and is also listed in the London Pharmacopoeias and Chinese Pharmacopoeias (Sforcin and Bankova, 2011; Zhang et al., 2015). However, the chemical and biological activities of propolis vary depending on bee species and the flora at site of bee collection. For instance, propolis from Europe and North America regions' main compounds comprises mostly flavanones, flavones, cinnamic acids and their esters, while that from Brazil comprises mainly prenylated *p*-coumaric acids, diterpenic acids (Bankova, 2005; Sforcin, 2007). Also, different races of honeybees collected at the same area demonstrated varying potency.

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Apis mellifera caucasica showed a superior antibacterial activity to *Apis mellifera carnica* and *Apis mellifera anatolica*; stingless bees – *Trigona incisa*, *Timia apicalis*, *Trigona fusco-balteata* and *Trigona fuscibasis* – from Indonesia revealed different degrees of cytotoxicity (Silici and Kutluca, 2005; Kustiawan et al., 2014).

Stingless bees, whose propolis is utilized for medicinal and nutraceutical purposes, are a large group of eusocial insect that play a part in plant pollination in tropical regions (Choudhari et al., 2012; da Cunha et al., 2013). In Thailand and India, stingless bee propolis is popularly applied for the treatment of maladies such as acne, diabetes and inflammation (Umthong et al., 2011; Choudhari et al., 2012). Antimicrobial, antiproliferative and antioxidant activities of several species of stingless bee propolis were also investigated (da Cunha et al., 2013; Dutra et al., 2014). Nevertheless, the study of propolis from Thai stingless bees, (*Lepidotrigona ventralis* Smith, *Lepidotrigona terminata* Smith, and *Tetragonula pagdeni* Schwarz (Apidae)), which are commercially cultivated in artificial hives in fruit gardens and marketed in several preparations in Thailand, is limited. Thus, the objective of the present work was to compare alpha glucosidase inhibitory effect and free-radical scavenging activity of propolis from three stingless bee species in the same area of Thai mangosteen orchard. The active compounds from the species that demonstrated the strongest activity were identified and quantified.

Materials and methods

Chemical products

1,1-Diphenyl-2-picrylhydrazyl (DPPH) radical, 2,2′-azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS), 2,4,6-Tris(2-pyridyl)-s-triazine (TPTZ), α-glucosidase from *Saccharomyces cerevisiae*, acarbose, 4-nitrophenyl α-D-glucopyranoside (pNPG), iron(III)chloride hexahydrate, ferrous sulfate, iron(II) sulfate heptahydrate, were obtained from Sigma–Aldrich® (St. Louis, MO, USA), aluminum chloride, acetic acid, ascorbic acid, Folin–Ciocalteu reagent, gallic acid were purchased from Merck® (Darmstadt, Germany). Sodium bicarbonate and potassium persulfate were purchased from Ajax Finechem® (NSW, Australia). All reagents were of analytical grade. Deionized water was purified by Ultra Clear™ system (Siemen Water Technologies Corp®). Ethanol, ethyl acetate, dichloromethane, hexane, formic acid and HPLC grade methanol were purchased from Labscan® (Thailand). Standard gamma-mangostin (**1**) and alpha-mangostin (**2**) purity more than 98% were purchased from Chengdu Biopurify Phytochemicals Ltd, Sichuan, China.

Propolis sample and preparation

Propolis of *Lepidotrigona ventralis* Smith, *Lepidotrigona terminata* Smith, and *Tetragonula pagdeni* Schwarz were collected from an apiary in the mangosteen garden in December from Makham district, Chanthaburi province, eastern of Thailand in 2014, and kept in the dark at 4 °C until use. The stingless bees were identified by Dr. Chama Inson, Department of Entomology, Faculty of Agriculture, Kasetsart University. The voucher specimens (*Lepidotrigona ventralis* No. 1214001, *Lepidotrigona terminata* No. 1214003, and *Tetragonula pagdeni* No. 1214003) were deposited at Faculty of Pharmaceutical Sciences, Burapha University, Thailand.

Propolis from different bee species (10 g) was separately cleaned and cut into small pieces and was then sonicated with 80% ethyl alcohol (200 ml) at 40 °C for 30 min. The suspension was centrifuged at 3000 × *g* for 5 min at 20 °C. The supernatant was kept while the pellet was re-extracted using the same procedure. The supernatants were pooled together and evaporated in a rotary evaporator. Each extract was dewaxed by sonication with 100 ml of hexane at 40 °C for 20 min and centrifuged at 2000 × *g* for 5 min at 20 °C. The supernatant was discarded while the crude residue was kept and stored in the dark at 0 °C.

Separation of active compounds

The propolis extract that exhibited the strongest activity was selected to separate the bioactive compounds. The crude residue was subjected to column chromatography (3 cm × 20 cm, silica gel (0.063–0.200 mm, Merck 7734)) with 20% ethyl acetate in hexane as a mobile phase. The sub-fractions were pooled together to obtain two main fractions as monitored by thin-layer chromatography. The first fraction was subjected to preparative thin-layer chromatography (pTLC) with 50% ethyl acetate/hexane to obtain compound **1**. The second fraction was also applied to pTLC with dichloromethane (triple run) as a mobile phase to obtain compound **2**. Each pure compound was dissolved in 99.98% $CDCl_3$ or in 99.8% methanol-d_4 (*ca.* 5 mg in 0.7 ml) and transferred into 5 mm NMR sample tube (Promochem, Wesel, Germany). Spectra were recorded by the Bruker Topspin software on a Bruker AVANCE 400 spectrometer (Bruker, Rheinstetten, Germany).

Biological assay

Determination of contents of total phenolic compounds

Total phenolic content was determined using Folin–Ciocalteu reagent following method described by Vongsak et al. (2013b). Each sample (1000 μg/ml), 200 μl was mixed with 500 μl of the Folin–Ciocalteu reagent (diluted 1:10 with deionized water) and 800 μl of sodium bicarbonate solution (7.5%, w/v). The mixture was allowed to stand at room temperature for 30 min with intermittent shaking. The absorbance was measured at 765 nm using a UV–Visible spectrophotometer (Hitachi®, Japan). The same procedure was repeated for the gallic acid standard solution (500, 250, 125, 62.5, 31.25 and 0 μg/ml) and the quantification was made based on a standard curve generated with of gallic acid. The content of total phenolic compounds was calculated as mean ± SD (n = 3) and expressed as grams of gallic acid equivalents (GAE) in 100 g of the extract.

Alpha glucosidase inhibition assay

The α-glucosidase inhibitory activity was carried out spectrophotometrically using pNPG as substrate (Zhou et al., 2015). Samples, standard solutions (gamma- and alpha-mangostin) or positive control (acarbose) with different concentrations (50 μl, Tables 1 and 3), α-glucosidase (50 μl, 2 unit/ml) and phosphate buffer (50 μl, pH 7.0) were mixed and pre-incubated at 37 °C for 10 min, and then pNPG (50 μl, 20 mM) was added to start the reaction. After incubation at 37 °C for 30 min, the absorbance was measured at 405 nm using UV–Visible microplate reader (Metertech®, Taiwan). The percent inhibition of α-glucosidase activity was calculated as follows:

$$\text{Inhibition}\,(\%) = \frac{(A_1 - A_2)}{A_1} \times 100,$$

where A_1 is the absorbance of control, and A_2 is the absorbance of samples. The extent of inhibition was expressed as mean ± SD (n = 3) of the enzymatic activity (IC_{50}).

Ferric reducing antioxidant power (FRAP) assay

The FRAP assay was carried out by the method of Al-Mansoub et al. (2014). Briefly, 150 μl FRAP working solution (300 mM acetate buffer, pH 3.6, 10 mM TPTZ in 40 mM HCl and 20 mM $FeCl_3$ in a ratio of 10:1:1) was added to 50 μl test samples. The reaction mixtures were incubated at 37 °C for 8 min; the absorbance was measured at

600 nm using UV–Visible microplate reader (Metertech®, Taiwan). Ferrous sulfate ($FeSO_4 \cdot 7H_2O$, 2.0, 1.0, 0.5, 0.25, 0.125, 0.063 mM) was used as reference standard and the results were expressed as μmol Fe^{2+} equivalent/g extract. Standard solutions (gamma- and alpha-mangostin) and positive control (ascorbic acid) were treated under the same condition as the samples, and then mean ± SD ($n = 3$) was calculated.

ABTS radical scavenging assay

ABTS radical scavenging activity was measured by the method described by Al-Mansoub et al., 2014. ABTS radical cation ($ABTS^{\bullet +}$) solution was prepared by mixing 14 mM ABTS and 4.9 mM potassium persulfate solutions in equal volume. The solution was allowed to react in the dark at room temperature for 16–20 h before use. Then, 1 ml of the solution was diluted with 40 ml ethanol to yield working ABTS solution with an absorbance of 0.70 ± 0.02 at 734 nm. To 750 μl ABTS working solution, 750 μl test samples were added. The absorbance of each solution was determined at 734 nm using UV–Visible spectrophotometer (Hitachi®, Japan) after 6 min. The corresponding blank readings were also taken and percent inhibition was then calculated as follows:

$$\text{Inhibition}\,(\%) = \frac{(A_1 - A_2)}{A_1} \times 100,$$

where A_1 is the absorbance of control, and A_2 is the absorbance of samples. Each determination was done in triplicate, and the average IC_{50} value was calculated as mean ± SD. Standard solutions (gamma- and alpha-mangostin) and positive control (ascorbic acid) were treated under the same conditions as the samples.

DPPH-scavenging assay

The free radical scavenging activity of the extracts, standard solutions (gamma- and alpha-mangostin) and positive control (ascorbic acid) was investigated using 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging method (Vongsak et al., 2013b). A total of 750 μl of the extract or standard/positive control was added to 750 μl of DPPH in methanol solution (152 μM). After incubation at 37 °C for 20 min, the absorbance of each solution was determined at 517 nm using UV–Visible spectrophotometer (Hitachi®, Japan). The corresponding blank readings were also taken and percent inhibition was then calculated as follows:

$$\text{Inhibition}\,(\%) = \frac{(A_1 - A_2)}{A_1} \times 100,$$

where A_1 is the absorbance of control, and A_2 is the absorbance of samples. Each determination was done in triplicate, and the average IC_{50} value was calculated as mean ± SD.

Determination of bioactive constituent contents

The propolis extract with the highest activity was selected for investigating the quantity of bioactive compounds using validated HPLC method (Vongsak et al., 2014). The instrument used was an Agilent 1260 Series® (Agilent Technologies) equipped with a 1260 Quat pump VL quaternary pump, 1260 ALS autosampler, 1260 TCC column thermostat, and 1260 DAD VL diode array detector. The chromatographic separation was carried out on a Hypersil BDS® C_{18} column (4.6 mm × 100 mm i.d., 3.5 μm) with a C_{18} guard column. The mobile phases were (A) 0.2% formic acid in water and (B) methanol. Gradient elution was used: 75% B in A to 90% B in A for 10 min; 90% B in A to 100% B for 5 min; 100% B for 10 min. The column was re-equilibrated with 75% B in A for 10 min prior to each analysis. The flow rate was 1.0 ml/min at 25 °C. UV–Vis detector was monitored at the wavelength of 245 nm and injection volume was 5 μl for all samples and standards.

T. pagdeni sample was prepared by accurately weighing 30 mg of extract and dissolving in methanol (5 ml). To enable complete

Table 1
Total phenolic content, alpha glucosidase inhibitory effect and free radical scavenging activities on FRAP, ABTS and DPPH assays of different Thai stingless bees from mangosteen orchard.

Stingless bee species/standard	Total phenolics (g GAE/100 g extract)*	α-Glucosidase inhibitory assay IC_{50} (μg/ml)*	FRAP assay (μmol $FeSO_4$ equivalent/g extract)*	ABTS assay IC_{50} (μg/ml)*	DPPH assay IC_{50} (μg/ml)*
Tetragonula pagdeni	12.83 ± 0.72^a	70.79 ± 6.44^a	279.70 ± 20.55^a	59.52 ± 10.76^a	122.71 ± 11.76^a
Lepidotrigona ventralis	3.15 ± 0.25^b	387.97 ± 38.71^b	97.42 ± 24.78^b	576.40 ± 48.17^b	428.61 ± 26.24^b
Lepidotrigona terminata	2.16 ± 0.10^c	469.66 ± 76.55^b	61.14 ± 5.86^c	605.39 ± 56.32^b	1228.89 ± 67.29^c
Ascorbic acid	–	–	7698.02 ± 698.03^d	6.07 ± 1.69^d (34.48 ± 9.60 μM)	7.33 ± 0.85^d (41.62 ± 4.83 μM)
Acarbose	–	155.82 ± 6.69^c (241.36 ± 10.37 μM)	–	–	–

Dissimilar letters in the same column indicate significantly different for each parameter at $p < 0.05$ using one-way analysis of variance (ANOVA) with least significant difference (LSD).

* Expressed as mean ± SD ($n = 3$). The concentrations for calculation IC_{50} values of *T. pagdeni* were 250, 125, 62.5, 31.25 and 0 μg/ml, while *L. ventralis* and *L. terminata* were 2000, 1000, 500, 250, 125 and 0 μg/ml in alpha glucosidase inhibitory assay, ABTS assay and DPPH assay. The concentrations of acarbose were 250, 125, 62.5, 31.25 and 0 μg/ml in alpha glucosidase inhibitory assay. The concentrations for FRAP assay of *T. pagdeni*, *L. ventralis* and *L. terminata* were 1000 μg/ml.

Table 2
NMR data for compounds **1** and **2**.

Compounds	^{1}H NMR	^{13}C NMR
Gamma-mangostin (**1**)	(400 MHz, $CDCl_3$/methanol-d_4), δ (ppm) = 6.65 (s, 1H, Ar*H*), 6.20 (s, 1H, Ar*H*), 5.28–5.23 (m, 2H, vinylic-*H* × 2), 4.13 (d, *J* = 6.61 Hz, 2H, allylic-*H*), 3.34 (d, *J* = 7.06 Hz, 2H, allylic-*H*), 1.82 (s, 3H, C*H*$_3$), 1.78 (s, 3H, C*H*$_3$), 1.66 (s, 2 × 3H, 2 × C*H*$_3$)	(100 MHz, $CDCl_3$/methanol-d_4), δ (ppm) = 182.3 (*C*=O), 161.4 (Ar*C*-OAr), 160.3 (Ar*C*-OH), 154.9 (Ar*C*-OAr), 152.9 (Ar*C*-OH), 150.6 (Ar*C*-OH), 139.9 (Ar*C*-OH, 132.4 (Ar*C*), 132.3 [CH=*C*(CH$_3$)$_2$], 128.1 [CH=*C*(CH$_3$)$_2$], 122.8 (*C*H=C), 122.3 (CH=C), 111.6 (Ar*C*), 109.6 (Ar*C*), 103.4 (Ar*C*), 100.2 (Ar*C*H), 92.3 (Ar*C*H), 25.7 (2 × *C*H$_3$), 25.6 (*C*H$_2$), 21.3 (*C*H$_2$), 17.9 (*C*H$_3$), 17.6 (*C*H$_3$)
Alpha-mangostin (**2**)	(400 MHz, $CDCl_3$), δ (ppm) = 13.80 (s, 1H, O*H*), 6.86 (s, 1H, Ar*H*), 6.32 (s, 1H, Ar*H*), 6.28 (s, 1H, O*H*), 6.11 (s, 1H, O*H*), 5.34–5.28 (m, 2H, vinylic-*H* × 2), 4.12 (d, *J* = 6.20 Hz, 2H, allylic-*H*), 3.83 (s, 3H, OC*H*$_3$), 3.48 (d, *J* = 7.12 Hz, 2H, allylic-*H*), 1.87 (s, 3H, C*H*$_3$), 1.86 (s, 3H, C*H*$_3$), 1.80 (s, 3H, C*H*$_3$), 1.72 (s, 3H, C*H*$_3$)	(100 MHz, $CDCl_3$), δ (ppm) = 182.1 (*C*=O), 161.6 (Ar*C*-OAr), 160.7 (Ar*C*-OH), 155.8 (Ar*C*-OAr), 155.1 (Ar*C*-OH), 154.5 (Ar*C*-OH), 142.6 (Ar*C*-OCH$_3$), 137.1 (Ar*C*), 135.7 [CH=*C*(CH$_3$)$_2$], 132.1 [CH=*C*(CH$_3$)$_2$], 123.2 (*C*H=C), 121.4 (*C*H=C), 112.3 (Ar*C*), 108.4 (Ar*C*), 103.7 (Ar*C*), 101.5 (Ar*C*H), 93.3 (Ar*C*H), 62.0 (O*C*H$_3$), 26.6 (*C*H$_2$), 25.8 (*C*H$_3$), 25.7 (*C*H$_3$), 21.5 (*C*H$_2$), 18.2 (*C*H$_3$), 17.9 (*C*H$_3$)

dissolution, each sample was sonicated for 60 min. Prior to the injection, each solution was filtered through a 0.22 μm nylon membrane filter.

Stock solutions of standard compounds (**1**) and (**2**) were prepared by accurately weighing and dissolving the compounds in methanol to obtain the final concentration of 1000 μg/ml. Working solutions of standard compounds were obtained by diluting the stock standard solutions with methanol to achieve the desired concentrations. Calibration curves were constructed from the peak area *versus* the amount of the standards by least square regression across the range of 0.78–100 μg/ml for compounds (**1**) and 1.5–1000 μg/ml for compound (**2**).

Statistical analysis

The results were reported as mean ± standard deviation (SD) ($n = 3$). The average contents of total phenolics, FRAP values and IC_{50} of the different propolis bee species extract were statistically investigated using one-way analysis of variance (ANOVA) with least significant difference (LSD) by SPSS for Windows® 16.0. A statistical probability (p value) less than 0.05 indicated a statistically significant difference between groups.

Results and discussion

Phenolic compounds are the principal bioactive materials in propolis which have been reported to have numerous pharmacological effects, including anti-oxidation and anti-diabetes (Matsui et al., 2004). Total phenolic contents in various stingless bees propolis extracts are presented in Table 1. *T. pagdeni* propolis extract promoted significantly higher content of total phenolics than propolis extract of other species. Total phenolics in these different propolis extracts ranged from 2.16 to 12.83 g GAE/100 g extract. The results indicated the possible influences of diverse stingless bee species on different contents of phenolics in propolis. The alpha glucosidase inhibitory effect of various propolis extracts is displayed in Table 1. The IC_{50} values of propolis extracts ranged from 70.79 to 469.66 μg/ml against baker's yeast alpha glucosidase, and that of *T. pagdeni* propolis extract was significantly lower than 155.82 μg/ml of acarbose, a positive control. Thus, *T. pagdeni* propolis extract possessed the highest inhibitory effect on alpha glucosidase compared to the other species and positive reference. This effect was possibly due to phytochemicals, which include phenolic compounds such as xanthones and phenolic acids (Ryu et al., 2011).

Free radicals can be deleterious to cells and lead to various chronic diseases such as diabetes, arthritis, and asthma (Vongsak et al., 2013a). The free radical scavenging activity of stingless bees propolis extracts was determined on the basis of the scavenging activity of FRAP, ABTS and DPPH assays (Table 1). The results showed that the extracts possessed reducing activity in the range of 61.14–279.70 μmol $FeSO_4$ equivalent/g extract while that of ascorbic acid was 7698.02 μmol $FeSO_4$ equivalent/g extract. The extracts were able to scavenge ABTS and DPPH radical, especially that of *T. pagdeni* propolis. It promoted stronger ABTS and DPPH radical scavenging activity than the other extracts with IC_{50} of 59.52 and 122.71 μg/ml, respectively, while ascorbic acid, a positive reference, showed the activity at IC_{50} of 6.07 and 7.33 μg/ml, respectively. These experiments indicated that alpha glucosidase inhibition and free-radical scavenging activity of propolis extracts from *T. pagdeni* exhibited stronger activity than that of *L. ventralis* and *L. terminata*. Thus, *T. pagdeni* propolis extract was selected to identify and quantify the bioactive compounds.

Gamma-mangostin (**1**) and alpha-mangostin (**2**) were separated and identified as the active constituents in *T. pagdeni* propolis extract from mangosteen orchard (Table 2). In alpha glucosidase inhibition assay, each compound demonstrated noteworthy alpha glucosidase inhibitory effect (IC_{50} 4.22 and 29.27 μM) while acarbose displayed the IC_{50} of 241.36 μM (Table 3). These chemicals were previously reported in seedcases of *Garcinia mangostana* and had been reported to display alpha glucosidase inhibitory effect (Ryu et al., 2011). In regards to free radical scavenging activity,

Table 3
The contents of bioactive compounds and their biological activities in *Tetragonula pagdeni* propolis extract from mangosteen orchard.

Bioactive compounds	Contents of major compounds in 80% ethanolic extracts (% w/w)*	Alpha glucosidase inhibitory assay IC_{50} (μM)*	FRAP assay (μmol $FeSO_4$ equivalent/gram extract)*	ABTS assay IC_{50} (μM)*	DPPH assay IC_{50} (μM)*
Gamma-mangostin (**1**)	0.94 ± 0.01	4.22 ± 0.58[a]	5366.82 ± 54.71[a]	160.92 ± 1.23[a]	39.94 ± 1.16 [a]
alPha-mangostin (**2**)	2.77 ± 0.08	29.27 ± 7.02[c]	15.32 ± 3.89[c]	n.d.	n.d.

Dissimilar letters in the same column indicate significantly different for each parameter at $p < 0.05$ using one-way analysis of variance (ANOVA) with least significant difference (LSD).

* Expressed as mean ± SD ($n = 3$), n.d. = not detectable. In alpha glucosidase inhibitory assay, the concentrations of gamma-mangostin (**1**) were 12.5, 6.25, 3.125, 1.56, 0.77 and 0 μg/ml, while alpha-mangostin (**2**) were 100, 50, 25, 12.5, 6.25 and 0 μg/ml. In ABTS assay and DPPH assay the concentrations of gamma- and alpha-mangostin were 100, 50, 25, 12.5, 6.25 and 0 μg/ml. The concentrations for FRAP assay of gamma-mangostin and alpha-mangostin were 200 μg/ml.

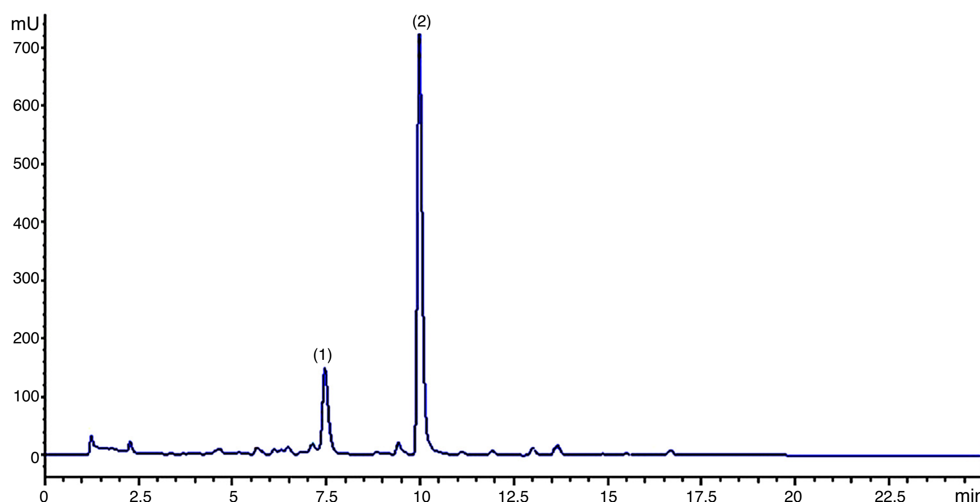
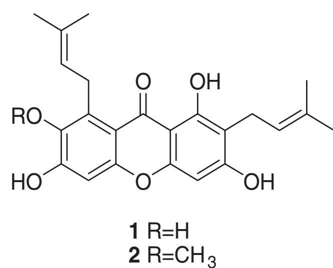


Fig. 1. HPLC fingerprints of *Tetragonula pagdeni* propolis extract from mangosteen orchard in Thailand, gamma-mangostin (**1**) and alpha-mangostin (**2**).

gamma-mangostin (**1**) exhibited a strong activity comparable to that of ascorbic acid in the DPPH assay while alpha-mangostin (**2**) showed weak to non-detectable activity in ABTS and DPPH assays (Table 3).



HPLC was used to quantify bioactive compounds, gamma- and alpha-mangostin in the *T. pagdeni* propolis extract because of the strongest activities. This method has been validated for its linearity, precision, and accuracy (Vongsak et al., 2014). The amounts of bioactive compounds, gamma-mangostin (**1**) and alpha-mangostin (**2**), in the *T. pagdeni* propolis extract were 0.94 and 2.77% (w/w), respectively (Table 3) at retention times of 7.46 and 9.98 min, respectively (Fig. 1), respectively, in the HPLC fingerprints. The presence of these mangostin derivatives is considered to be vital for the alpha glucosidase inhibitory effect of *T. pagdeni* propolis.

In conclusion, the study provides data to support the usage of different stingless bee propolis cultivated in mangosteen orchard as raw material for natural antioxidant and anti-diabetic agents. Based on this study, the propolis extract of *T. pagdeni* should be utilized as a better source of raw material for alpha glucosidase inhibitory effect and anti-oxidative purposes than others. The mangostin derivatives, especially gamma-mangostin, the most active agent, could possibly be applied as markers for standardization.

Authors' contributions

BV contribution included collecting samples, designing and performing laboratory work, analyzing the results, and preparing the paper. SK contribution included HPLC analysis. SJ contribution included the isolation and purification of the compounds. SM and CP contribution included collection of samples and supervision of the laboratory work. All the authors have read the final manuscript and approved the submission.

Conflicts of interest

The authors declare no conflicts of interest.

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References

Al-Mansoub, M.A., Asmawi, M.Z., Murugaiyah, V., 2014. Effect of extraction solvents and plant parts used on the antihyperlipidemic and antioxidant effects of *Garcinia atroviridis*: a comparative study. J. Sci. Food Agric. 94, 1552–1558.

Bankova, V., 2005. Chemical diversity of propolis and the problem of standardization. J. Ethnopharmacol. 100, 114–117.

Choudhari, M.K., Punekar, S.A., Ranade, R.V., Paknikar, K.M., 2012. Antimicrobial activity of stingless bee (*Trigona* sp.) propolis used in the folk medicine of Western Maharashtra, India. J. Ethnopharmacol. 141, 363–367.

da Cunha, M.G., Franchin, M., de Carvalho Galvao, L.C., de Ruiz, A.L., de Carvalho, J.E., Ikegaki, M., de Alencar, S.M., Koo, H., Rosalen, P.L., 2013. Antimicrobial and antiproliferative activities of stingless bee *Melipona scutellaris* geopropolis. BMC Complement Altern. Med. 13, 23.

Dantas, C.G., Nunes, T.L.G.M., Nunes, T.L.G.M., da Paixão, A.O., Reis, F.P., Júnior, W.d.L., Cardoso, J.C., Gomes, M.Z., Gramacho, K.P., 2014. Pharmacological evaluation of bee venom and melittin. Rev. Bras. Farmacogn. 24, 67–72.

Dutra, R.P., Abreu, B.V., Cunha, M.S., Batista, M.C., Torres, L.M., Nascimento, F.R., Ribeiro, M.N., Guerra, R.N., 2014. Phenolic acids, hydrolyzable tannins, and antioxidant activity of geopropolis from the stingless bee *Melipona fasciculata* Smith. J. Agric. Food Chem. 62, 2549–2557.

Juárez-Rojop, I.E., Tovilla-Zárate, C.A., Aguilar-Domínguez, D.E., Fuente, L.F.R.-d.l., Lobato-García, C.E., Blé-Castillo, J.L., López-Meraz, L., Díaz-Zagoya, J.C., Bermúdez-Ocaña, D.Y., 2014. Phytochemical screening and hypoglycemic activity of *Carica papaya* leaf in streptozotocin-induced diabetic rats. Rev. Bras. Farmacogn. 24, 341–347.

Kustiawan, P.M., Puthong, S., Arung, E.T., Chanchao, C., 2014. *In vitro* cytotoxicity of Indonesian stingless bee products against human cancer cell lines. Asian Pac. J. Trop. Biomed. 4, 549–556.

Matsui, T., Ebuchi, S., Fujise, T., Abesundara, K.J., Doi, S., Yamada, H., Matsumoto, K., 2004. Strong antihyperglycemic effects of water-soluble fraction of Brazilian propolis and its bioactive constituent, 3,4,5-tri-*O*-caffeoylquinic acid. Biol. Pharm. Bull. 27, 1797–1803.

Ryu, H.W., Cho, J.K., Curtis-Long, M.J., Yuk, H.J., Kim, Y.S., Jung, S., Kim, Y.S., Lee, B.W., Park, K.H., 2011. α-Glucosidase inhibition and antihyperglycemic activity of prenylated xanthones from *Garcinia mangostana*. Phytochemistry 72, 2148–2154.

Sforcin, J.M., Bankova, V., 2011. Propolis: is there a potential for the development of new drugs? J. Ethnopharmacol. 133, 253–260.

Sforcin, J.M., 2007. Propolis and the immune system: a review. J. Ethnopharmacol. 113, 1–14.

Silici, S., Kutluca, S., 2005. Chemical composition and antibacterial activity of propolis collected by three different races of honeybees in the same region. J. Ethnopharmacol. 99, 69–73.

Umthong, S., Phuwapraisirisan, P., Puthong, S., Chanchao, C., 2011. *In vitro* antiproliferative activity of partially purified *Trigona laeviceps* propolis from Thailand on human cancer cell lines. BMC Complement Altern. Med. 11, 37.

Vongsak, B., Gritsanapan, W., Wongkrajang, Y., Jantan, I., 2013a. *In vitro* inhibitory effects of *Moringa oleifera* leaf extract and its major components on chemiluminescence and chemotactic activity of phagocytes. Nat. Prod. Commun. 8, 1559–1561.

Vongsak, B., Sithisarn, P., Mangmool, S., Thongpraditchote, S., Wongkrajang, Y., Gritsanapan, W., 2013b. Maximizing total phenolics, total flavonoids contents and antioxidant activity of *Moringa oleifera* leaf extract by the appropriate extraction method. Ind. Crops Prod. 44, 566–571.

Vongsak, B., Kongkiatpaiboon, S., Machana, S., Weerakul, T., Pattarapanich, C., Laiwattanaphaial, J., Issaro, N., 2014. HPLC quantitative analysis of stingless bee propolis extracts. In: Burapha University International Conference 2014. Global Warming and Its Impacts, Chon Buri, Thailand.

Zhang, H., Wang, G., Beta, T., Dong, J., 2015. Inhibitory properties of aqueous ethanol extracts of propolis on alpha-glucosidase. Evid. Based Complement Altern. Med., http://dx.doi.org/10.1155/2015/587383, Article ID 587383.

Zhou, X., Liang, J., Zhang, Y., Zhao, H., Guo, Y., Shi, S., 2015. Separation and purification of α-glucosidase inhibitors from *Polygonatum odoratum* by stepwise high-speed counter-current chromatography combined with Sephadex LH-20 chromatography target-guided by ultrafiltration–HPLC screening. J. Chromatogr. B 985, 149–154.