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Contributed Paper

Chemical Profiles and Antimicrobial Activities of Thai Propolis Collected from *Apis mellifera*

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ABSTARCT

The aim of this study was to examine the antimicrobial properties and chemical composition of Thai *Apis mellifera* propolis from different locations. All propolis samples demonstrated significant activity (Minimal Inhibitory Concentration < 1000 μg/mL) against Gram-positive bacteria *Bacillus cereus*, *Staphylococcus aureus, S. epidermidis* and methicillin-resistant *Staphylococcus aureus* (MRSA) whereas the activities against Gram-negative bacteria and yeasts were lower. There were statistically significant differences ($p < 0.001$) between the Thai propolis extracts due to their DPPH free radical scavenging activity and total phenolic contents. The GC/MS chemical profiles of all the propolis samples demonstrated similar composition but different proportion of sugars and sugar derivatives, triterpenes and phenolic lipids. From the most active sample (Phayao), several triterpenes and three inseparable mixtures of phenolic lipids (cardols, cardanols, and anacardic acids) were isolated by chromatographic methods and they showed high antibacterial activities. This indicates that propolis from the studied regions belongs to the tropical propolis type, originating mainly from mango (*Mangifera indica*). Our results provide the information that is useful for future standardization of Thai propolis.

Keywords: antimicrobial, antioxidant, chemical composition, propolis

1. INTRODUCTION

Propolis is a natural substance that honeybees collect from buds and other parts of plants surrounding the hive. It is used by bees to block holes and cracks, repair combs, strengthen the thin borders of the comb, and make the entrance of the hive easier to defend [1]. Bees use propolis not only as a building material but also as a means for maintaining low levels of bacterial and fungal concentrations in the hive. Propolis has been used in folk medicines in many parts of the world since ancient times, both externally and internally. Recently, there have been reports of its biological and pharmacological activities such

as antibacterial activity [2,3], antioxidant activity [4], and other medicinal properties (e.g., anti-tumor effect) [5]. Due to its pharmacological properties, it has wide applications in medicine, food, and cosmetics industries. More than 300 different compounds have been identified in propolis, such as the following: polyphenols (e.g., flavonoids, phenolic acids, and their esters), terpenoids, steroids, and amino-acids [6]. The main constituents of propolis are wax, resin, and volatiles. Honeybees produce wax, while resin and volatiles are collected from plants. Propolis from Brazil contains flavonoids, prenylated derivatives of *p*-coumaric acids, lignans, and terpenoids [1]. Its chemical composition and pharmacological properties depend on the plants in the neighborhood of the hive and vary in different geographic regions [5,7].

Although the biological activities and the chemical composition of propolis have been studied worldwide, reports on *Apis mellifera* propolis in Thailand (Thai propolis) have been limited. The purpose of this work was to investigate biological properties, the chemical composition and the bioactive compounds of propolis collected from *Apis mellifera* colonies in five different regions of Thailand.

2. MATERIALS AND METHODS

2.1 Propolis Samples and Extract

The propolis samples were collected from *A. mellifera* colonies by using clean slide in northern Thailand (Chiang Rai, Lamphun, Nan, Phayao, and Phrae) during the period from January 2009 to January 2010. Moreover, propolis (500g) was pooled from 300-1000 colonies in each province. The propolis samples were dried in dark room at room temperature for at least two weeks then kept in the dark at -20 °C until used. Commercial sample of Brazilian propolis was compared to crude Thai propolis. Thirty grams of propolis powder was extracted with 300 mL of 70% ethanol at room temperature. After dissolving the propolis powder, the propolis samples were treated with ultrasound for 30 min [8,9], filtrated through Whatman No. 1 filter paper, and then lyophilized until dry.

2.2 Antimicrobial Activity of Ethanol Extract of Propolis (EEP)

Antimicrobial activity of EEP was investigated by the minimal inhibitory concentration (MIC) value in a 96-well microtiter plate [10]. Ten test microorganisms (*Klebsiella pneumoniae* DMST 8216, *Listeria monocytogenes* DMST 17303, *Micrococcus luteus* DMST 15503, *Proteus mirabilis* DMST 8212, *Pseudomonas aeruginosa* ATCC 9027, *Staphylococcus epidermidis* DMST 15505, *Streptococcus pyogenes* DMST 17020, methicillin-resistant *Staphylococcus aureus* [MRSA] DMST 20625, *Serratia marcescens* DMST 21632, and *Salmonella typhimurium* DMST 562) were purchased from the Department of Medical Sciences, Ministry of Public Health, Bangkok, Thailand (DMST). Five other test microorganisms (*Bacillus cereus* TISTR 687, *Escherichia coli* ATCC 25922, *S. aureus* TISTR 517, *Candida albicans* ATCC 10231, and *Saccharomyces cerevisiae* TISTR 5343) were obtained from The Thailand Institute of Scientific and Technological Research (TISTR), Thailand. The bacteria were all cultured in Mueller Hinton broth (MHB) and incubated at 37°C for 24 h, whereas the yeasts were cultured on yeast extract-malt extract broth (YMB) and incubated at 25°C for 48 h. The test strains were suspended in MHB and YMB by adjusting to 0.5 McFarland to give a final density of 10^8 cfu/mL. The dried ethanol extracts of propolis were dissolved using dimethylsulfoxide (DMSO) for the antimicrobial assays. Dilutions of propolis extracts, ranging from 0.06 mg/mL to 128 mg/mL, were prepared in a 96-well microtiter plate. The MIC was defined as the lowest concentration of propolis at which there was no visible microorganism growth. The minimum bactericidal (fungicidal) concentrations were determined by subculturing

10 μL of the inoculum from the MIC wells onto the Mueller Hinton and yeast extract-malt extract agar plates. The MBC and MFC values were defined as the lowest concentration that allows no visible growth of microorganisms on the agar.

2.3 Free Radical-Scavenging Activity on 2,2-Diphenyl-1-Picrylhydrazyl and Total Phenolic Contents

The free radical scavenging activity of the propolis extracts was measured using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay, as described by Yang et al. [11], on a 96-well microtiter plate. Ascorbic acid (1–6 mg/L) was used as the positive control. The free radical scavenging activity of the propolis extracts was calculated as follows:

$\%\text{inhibition} = [(A_c - A_s)/A_c] \times 100$, where A_c is the absorbance of the control and A_s is the absorbance of the sample. The results were expressed as IC_{50} value, which was calculated from the relationship curve of scavenging activity (%) versus concentration of the respective sample.

The total phenolic contents in the extracts were determined using the Folin–Ciocalteau colorimetric method, according to the description given in Mărghitaş et al. (2009) [12]. Gallic acid (0.01–0.25 mg/mL) was used as the calibration standard ($y = 6.1598x$; $R^2 = 0.999$). The total phenolic content was expressed as μg of the gallic acid equivalents per mg of the extract.

2.4 GC/MS Analysis

Crude propolis samples were extracted with 70% ethanol (1:10, w/v) at room temperature for 24 h (3 times). The ethanol extracts were evaporated to dryness under vacuum. About 5 mg of the residue were mixed with 50 μL of dry pyridine and 75 μL of BSTFA, and heated at 80°C for 20 min and analyzed by GC/MS. The GC/MS analysis was performed with a Hewlett Packard Gas Chromatograph 5890 Series II Plus linked to Hewlett Packard 5972 mass spectrometer system equipped with a 23 m long, 0.25 mm i.d., and 0.5 μm film thick HP5-MS capillary column. The temperature was programmed to rise from 100°C to 310°C at the rate of 5°C/min. Helium was used as the carrier gas, at a flow rate of 0.7 mL/min. The split ratio was 1:80, the injector temperature was 280°C, and the ionization voltage was 70 eV. The identification was accomplished using computer searches on a NIST98 MS data library and mass spectra and retention times of isolated reference compounds. The components of the ethanol extracts of propolis were determined by considering their areas as the percentage of the total ion current.

2.5 Extraction and Isolation of Individual Compounds

The propolis sample (240 g) obtained from Phayao, Thailand, was extracted with 70% ethanol (1:10, w/v) at room temperature for 24 h (3 times). The ethanol extract was extracted successively with petroleum ether (PE) (3 times), dichloromethane (3 times), and ethyl acetate (3 times). PE extract (20 g) was subjected to column chromatography on silica gel, mobile phase PE - CH_2Cl_2 - EtOAc gradient to give 22 fractions (A-V). Fraction N (0.8 g) was re-chromatographed on silica gel with PE – EtOAc gradient to give 17 fractions (N1-N17). Fractions N1 and N14 gave cycloartenol **1** (15.4 mg) and anacardic acid derivatives: major constituents being anacardic acids **2a** and **2b** (minor constituents, that is, under 1%: anacardic acids with side chains $C_{17:1}$ and $C_{19:0}$, identified in the mixture by GC/MS) (10 mg). Fraction P (0.5 g) was re-chromatographed on silica gel with the PE – EtOAc gradient system to give 13 fractions (P1-P13). Fraction P9 gave cardols (resorcinols) **3a**, **3b**, and **3c** (minor constituents, that is, under 1%: cardols with side chains $C_{17:0}$, $C_{17:1}$, and $C_{19:1}$, identified in the mixture by GC/MS)

(12.4 mg). Fraction G (40 mg) was subjected to preparative TLC (silica gel 60 F_{254} glass plates [Merck, 20 × 20 cm, 0.25 mm]), mobile phase PE – $CHCl_3$ 1:1; to obtain the cardanols **4a** and **4b** (14 mg). Fraction V (20 mg) was subjected to preparative TLC (mobile phase PE – EtOAc 7:3) to obtain isomangiferolic acid **5** (0.9 mg). Ethyl acetate extract (35 mg) was subjected to preparative TLC (mobile phase PE – EtOAc 7:2) to obtain three compounds, namely, mangiferolic acid **6** (1.1 mg), ambolic acid **7** (1.1 mg), and 27-hydroxyisomangiferolic acid **8** (5.9 mg).

2.6 Antibacterial Activity of Individual Compounds

Stock solutions of the pure compounds were prepared at different concentrations, ranging from 0.78 μg/mL to 200 μg/mL in DMSO. Seven Gram-positive bacteria, namely, *B. cereus*, *L. monocytogenes*, *M. luteus*, *S. aureus*, *S. epidermidis*, *S. pyogenes,* and MRSA as well as four Gram-negative bacteria, namely, *E. coli*, *P. aeruginosa*, *S. typhimurium*, and *S. marcescens* were used to test antibacterial activity. The MIC values of the individual compounds were determined in a 96-well microtiter plate and MBC values were tested by subculturing the microorganism from the MIC well on the agar.

2.7 Statistical Analysis

The results of the investigations carried out for the antimicrobial and antioxidant activities were checked for normal distribution, and the data were transformed to normal distribution. The statistical significance was evaluated using the one way analysis of variance (ANOVA) by SPSS version 16 (SPSS Inc.).

3. Results and Discussion

3.1 Antimicrobial Activity of EEP

The ethanol extracts of five propolis samples showed antimicrobial activity against the test microorganisms (Table 1). The observed minimal inhibitory concentrations (MICs) and minimum bactericidal concentrations (MBCs) were in the range of 0.06 to 32 mg/mL and 0.25 to 128 mg/mL, respectively whereas the minimum fungicidal concentrations (MFCs) were in range of 26.67 to 64 mg/mL. All the ethanol extracts of Thai propolis samples demonstrated significant activity (MIC < 1000 μg/mL) against the Gram-positive bacteria *Bacillus cereus*, *Staphylococcus aureus, S. epidermidis,* and MRSA. The activities against the Gram-negative bacteria and yeast were lower. This is in agreement with a number of previously published results showing that propolis has greater activity against Gram-positive bacteria than against Gram-negative bacteria [3,7]. The results demonstrated that *S. epidermidis* was the most sensitive, followed by *S. aureus* which are known to be the major causes of wound infections and skin diseases [13]. According to the results, ethanol extracts of propolis are able to inhibit bacterial growth, thus confirming the factual correctness of the traditional knowledge in Thai folk medicine regarding the topical application of the ethanol extracts of propolis to cure skin infections.

The most active sample (Phayao) had the lowest MIC and MBC against Gram-positive bacteria, compared to those of others. Significant differences (MIC [$F = 34.510$, df = 5, $p < 0.001$]; MBC [$F = 67.126$, df = 5, $p < 0.001$]) were found between the activities of the propolis extract from Phayao and those of others. All the same, Thailand has a diverse flora, which is somewhat different from the flora of the other parts of the world. Any information on the potential source of resin that is collected from *A. mellifera* available in Thailand and which can contribute to the bioactive properties is of great scientific and medicinal interest. This could promote the beekeeping industry in Thailand, which now has no interest in collecting propolis for commercial purpose.

Table 1. Minimal Inhibitory Concentration (MIC) and Minimum Bactericidal/Fungicidal Concentration (MBC/MFC) concentration (mg/mL) of the propolis extracts from several locations against microbial strains. (MIC [$F = 34.510$, df $= 5$, $p < 0.001$]; MBC [$F = 67.126$, df $= 5$, $p < 0.001$]).

Microorganism	Brazil[c]		Chiang Rai[a]		Lamphun[a]		Nan[a]		Phayao[b]		Phrae[a]		Gentamicin (mg/ml)	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
Gram positive bacteria														
Bacillus cereus TISTR 687	0.13	0.13	0.50	1.17 ± 0.76	1.67 ± 0.58	1.67 ± 0.58	2	2	0.13	0.25	1	1	0.02	0.52 ± 0.23
Listeria monocytogenes DMST 17303	2	16	4	26.67 ± 9.24	4	64	8	64	4	32	4	64	0.02	0.02
Micrococcus luteus DMST 15503	1	2	8	32	8	32	8	16	2	8	16	16	0.05	0.05
Staphylococcus aureus TISTR 517	0.06	2	0.25	16	0.25	16	0.13	16	0.25	8	0.13	16	0.05	0.05
Staphylococcus epidermidis DMST 15505	0.06	16	0.13	32	0.25	32	0.13	32	0.06	16	0.25	32	0.10	0.26 ± 0.11
Streptococcus pyogenes DMST 17020	0.25	4	4	128	4	128	16	128	1	64	8	128	0.10	0.20
MRSA DMST 20625	0.25	2	0.50	64	0.50	128	0.50	128	0.50	10.67 ± 4.62	0.50	128	0.10	0.10
Gram negative bacteria														
Escherichia coli ATCC 25922	1	64	2	128	2	128	2	128	2	64	2	128	0.20	0.39
Klebsiella pneumoniae DMST 8216	4	64	4	128	8	128	8	128	4	64	8	128	0.78	0.78
Pseudomonas aeruginosa ATCC 9027	8	32	16	64	16	64	16	64	5.33 ± 2.31	64	8	64	0.78	0.78
Proteus mirabilis DMST 8212	4	64	16	128	16	128	16	128	16	128	16	128	0.78	1.04 ± 0.45
Salmonella typhimurium DMST 562	4	64	8	128	8	128	8	128	4	64	4	128	0.10	0.78
Serratia marcescens DMST 21632	4	128	32	128	16	128	16	128	32	128	32	128	0.39	1.56
Yeast													**Nystatin**	
Candida albicans ATCC 10231	1	8	4	32	4	32	4	64	2	32	4	42.67 ± 18.47	0.20	0.39
Saccharomyces cerevisiae TISTR 5343	0.50	0.50	4	32	4	26.67 ± 9.24	8	48.00 ± 27.71	2	32	8	32	0.10	0.20

[a,b,c]Means with different letters are significant differences for locations.

3.2 Free Radical-Scavenging Activity on 2,2-Diphenyl-1-Picrylhydrazyl and Total Phenolic Contents

The result of 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging activity of various propolis samples showed that IC_{50} values were in range of 19.9 to 67.2 μg/mL (Table 2). According to the Folin–Ciocalteau assay determining the total phenolic contents, the amounts of total phenolic contents in Thai propolis samples were ranging from 13 to 33 μg/mg of propolis extract. There was negative correlation between total phenol content and DPPH activity ($R = -0.792$). The result explained that higher total phenolic content in propolis extracts would gave lower antioxidant capacities in DPPH. This result is in agreement with previous reports which pointed out that the total phenolic content and the ability of propolis to scavenge free radicals are closely related [2].

Table 2. DPPH free radical scavenging activity and total phenolic contents of propolis extracts from different locations. (IC_{50} [$F = 145.936$, df = 4, $p < 0.001$]; Total phenolic contents [$F = 20.876$, df = 4, $p < 0.001$]).

Province	DPPH IC_{50} (μg/mL)	Total phenolic contents (μg/mg of extract)
Chiang Rai	54.0 ± 1.6^{b}	20.0 ± 3.0^{bc}
Lamphun	67.2 ± 2.0^{a}	23.0 ± 3.0^{b}
Nan	59.9 ± 1.2^{ab}	13.0 ± 0.9^{c}
Phayao	19.9 ± 3.3^{c}	33.0 ± 5.0^{a}
Phrae	57.5 ± 0.1^{b}	16.1 ± 0.6^{bc}
Ascorbic acid	4.4 ± 0.3	-

[a,b,c] Means with different letters are significant differences for locations.

3.3 GC/MS Analysis

The chemical profiles of the studied Thai propolis samples were investigated by GC/MS in order to explore the composition–activity relationship. More than 50 individual compounds were identified. The results, represented as amounts of the major compound groups, are illustrated in Figure 1. Gas chromatograms showed that the major compounds of Thai *A. mellifera* propolis were composed of sugar and sugar derivatives (12-78%), triterpenes (8-37%) and phenolic lipids (4-47%). All propolis samples also showed similar compounds with some variations. The main compositions of propolis extracts from Chiang Rai and Lamphun were sugar and sugar derivatives whereas propolis extracts from Nan and Phrae were triterpenes. It is well known that because of the rich tropical flora, propolis from different tropical regions display different chemical profiles [14].

The chemical profile of the most active sample (Phayao) is characterized by the relatively high concentration of anacardic acids: in all the other samples, anacardic acids were found to be minor constituents. Anacardic acids are known and established antibacterial substances [15,16,17], and their side chain length and structure play significant roles in the magnitude of their activity [18,19]. It is important to note that the sample from Phayao is the one in which cardols, anacardic acids, and triterpenes are most evenly represented. It could be speculated that the combination of the numerous compounds with the different structures and the different mechanisms of action results in synergistic effects in its antimicrobial activity and, possibly, other bioactivities.

We also studied the action of our propolis samples against DPPH radicals. The highest value corresponded to the most bioactive propolis sample from Phayao, which was rich in anacardic acids. According to research literature data, anacardic acids together with cardols and cardanols contribute to the radical

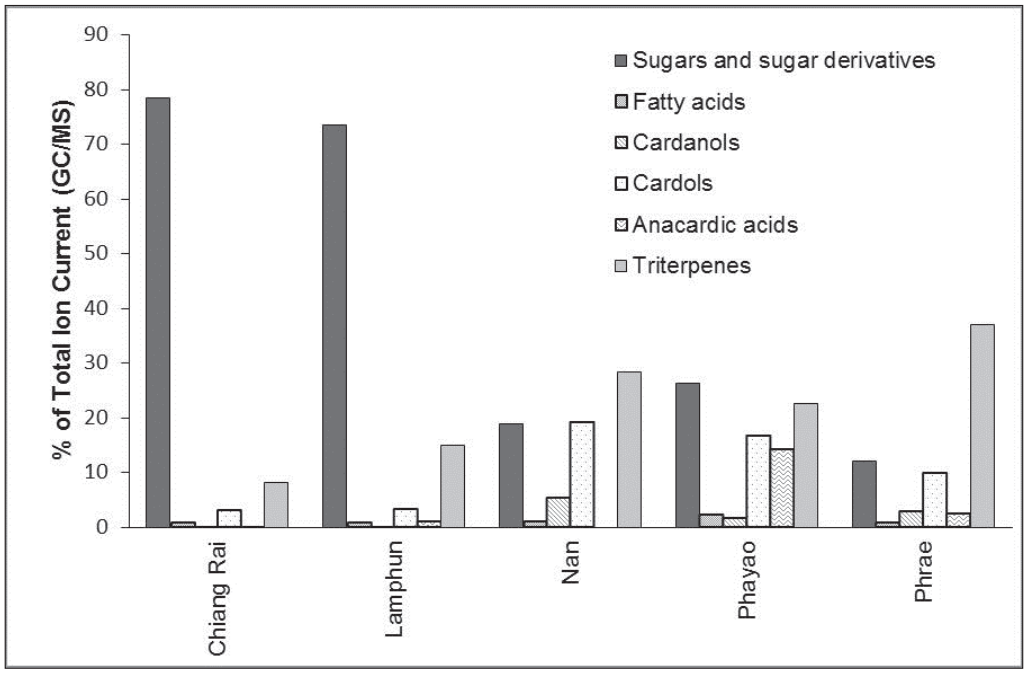

Figure 1. The chemical profiles of the Thai propolis extracts.

scavenging action against DPPH radicals [20]. The samples from Chiang Rai and Lamphun, although with higher values for total phenolics, did not demonstrate higher DPPH radical scavenging activity in comparison with the ones from Nan and Phrae. This observation could be explained by the high concentration of sugars in the Chiang Rai and Lamphun propolis: It is well known that reducing sugars interfere with the Folin–Ciocalteu reagent [21,22].

Our results demonstrated that chemical profile were familiar to the profiles of propolis from northeastern Brazil [17], Indonesia [23], and Oman [24]. Anacardic acids are commonly found in gum cashew trees *Anarcadium occidentale L*, as well as mango [25,26]. Moreover, the GC/MS results were indicated triterpenes and phenolic lipids are the main compounds in Thai propolis. It can be concluded that mango (*Mangifera indica*) was the source of the Thai propolis samples. Apart from this, it needs to be mentioned that the apiaries in the Phayao province, from which we collected the samples, surrounded by mango orchards. Mango is a significant fruit of South East Asian countries, including Thailand. The bark of mango has been used as traditional medicine all over the world [27]. Kaur et al. [28] has also reported the antibacterial activity of the mango seed kernel. The propolis sample obtained from Phayao has higher antibacterial and antioxidant activities which could be attributed to the constituents derived from mango. Mango resin may have potential properties in against microorganisms. Our findings may help beekeepers to choose the location for placing their bee colonies in order to obtain high bioactive propolis.

3.4 Antibacterial Activity of Individual Compounds

The most active sample from Phayao was further subjected to several chromatographic procedures in order to isolate the main constituents and tests their bioactivity. The structures of the isolated compounds were elucidated using different NMR experiments (1D

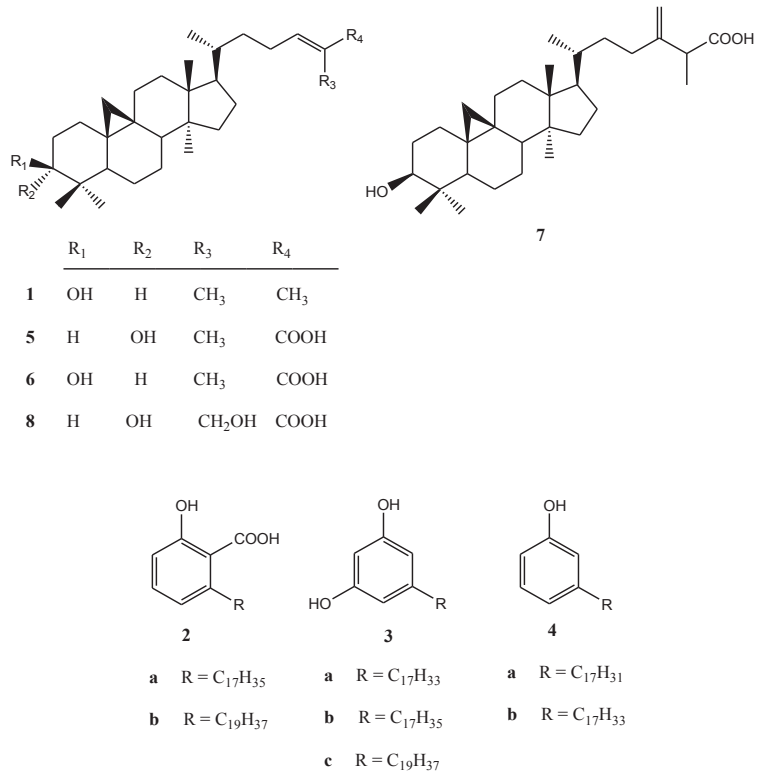

Figure 2. The structures of the isolated compounds.

and 2D), using the MS data, and by comparison with the literature data [17,29]. Cycloartenol **1,** anacardic acids **2a** and **2b,** cardols (resorcinols) **3a–3c**, cardanols **4a** and **4b,** isomangiferolic acid **5**, mangiferolic acid **6**, ambolic acid **7** and 27-hydroxyisomangiferolic acid **8** were isolated from this sample (Figure 2). All the isolated compounds, which were separated from Thai propolis, showed antibacterial activity against tested microorganisms. The MICs against seven Gram-positive bacteria and four Gram-negative bacteria were in range of 3.13 μg/mL to 50 μg/mL. Furthermore, the MBC values of the isolated compounds were in the range of 6.25 μg/mL to >200μg/mL (Table 3). Also, in most cases, the activities of the individual constituents were substantially higher (even by one order of magnitude) than the activity of the total extract. The lowest MICs were obtained for cardols (recorcinols) and cardanols, especially against *L. monocytogenes*. On the other hand, bacteria *S. pyogenes* were found to be the most sensitive to all the tested compounds. As far as the statistical analysis results are concerned, cardols (resorcinols) were observed to have statistically higher antibacterial activity than the other isolated compounds ($p < 0.001$). Phenolic lipids from the groups of cardol and cardanol, found in this study, have also been reported as bioactive constituents (having antibacterial, antiproliferative/cytotoxic properties) isolated from the propolis obtained from the Nan province in Thailand [30,31]. However, the other isolated compounds in our propolis sample have never been reported before as present in Thai propolis.

CONCLUSIONS

In this study, the antimicrobial and antioxidant activities of Thai *A. mellifera* propolis were

Table 3. Minimal Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) in μg/mL of pure compounds against pathogenic bacteria. (MIC [$F = 41.596$, df = 7, $p < 0.001$]; MBC [$F = 24.337$, df = 7, $p < 0.001$]).

Pure compound	Gram-positive bacteria						Gram-negative bacteria				
	B.c.	*L.m.*	*M.l.*	*S.a.*	*S.e.*	*S.p.*	MRSA	*E.c.*	*P.a.*	*S.t.*	*S.m.*
	MIC (μg/mL)										
Anacardic acid[a] (**2a** and **2b**)	50	50	50	50	50	6.25	50	50	50	50	50
Cardols[c] (**3a–3c**)	25	3.13	25	25	25	3.13	25	25	25	25	25
Cardanols[b] (**4a** and **4b**)	25	3.13	25	50	50	12.5	50	25	25	25	25
Cycloartenol[a] (**1**)	50	50	50	50	50	3.13	50	50	50	50	50
Mangiferolic acid[b] (**6**)	25	25	25	25	50	12.5	25	25	25	25	25
Isomangiferolic acid[bc] (**5**)	-	-	-	25	-	-	25	25	-	-	-
Ambolic acid[b] (**7**)	25	25	25	25	50	12.5	25	25	25	25	25
27-Hydroxyisomangiferolic acid[bc] (**8**)	25	25	25	25	50	6.25	25	25	25	25	25
	MBC (μg/mL)										
Anacardic acid[ab] (**2a** and **2b**)	50	100	100	100	200	6.25	200	200	100	200	200
Cardols[d] (**3a–3c**)	50	100	50	25	50	25	25	100	50	100	100
Cardanols[cd] (**4a** and **4b**)	50	100	50	50	100	25	100	100	50	100	100
Cycloartenol[a] (**1**)	200	200	50	100	200	25	200	166.67 ±57.74	100	200	200
Mangiferolic acid[bc] (**6**)	>200	100	100	100	100	25	100	100	83.33 ±28.87	100	100
Isomangiferolic acid[b] (**5**)	-	-	-	100	-	-	100	100	-	-	-
Ambolic acid[cd] (**7**)	50	100	50	100	100	12.5	100	100	50	100	100
27-Hydroxyisomangiferolic acid[bc] (**8**)	>200	100	50	100	100	16.67 ±7.22	100	100	100	100	100

Note: B.c. (*Bacillus cereus)*; *L.m.* (*Listeria monocytogenes)*; *M.l.* (*Micrococcus luteus)*; *S.a.* (*Staphylococcus aureus)*; *S.e.* (*Staphylococcus epidermidis)*; *S.p.* (*Streptococcus pyogenes)*; MRSA (methicillin-resistant *Staphylococcus aureus)*; *E.c.* (*Escherichia coli)*; *P.a.* (*Pseudomonas aeruginosa)*; *S.t.* (*Salmonella typhimurium)*; *S.m.* (*Serratia marcescens).*[a,b,c,d]Means with different letters are significant differences for pure compounds.

investigated. The results showed that Thai *A. mellifera* propolis sourced from the northern Thailand possessed significant antimicrobial properties, especially against *S. epidermidis*, the skin pathogen. Moreover, the chemical profile of propolis from Phayao, showing the highest antimicrobial activity, belonged to the tropical propolis type, originating mainly from mango (*Mangifera indica*). Our results provide useful information for the future standardization of Thai *A. mellifera* propolis and for reaffirming the traditional application of propolis in Thailand.

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