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Chemical profiling and antioxidant activity of Malaysian stingless bee propolis from ten different locations

Aulia Rani Annisava[1,2], Khamsah Suryati Mohd[1,*] Nor Elani Mat Nafi[1], Ammira Shafiqha Abdul Khadar[1], Nur Basyirah Md Zin[1], Norzilawati Pauzi[1], Ainur Awanis Mohd Badiazaman[1] and Abd Jamil Zakaria[1]

[1]School of Agriculture Sciences and Biotechnology, Faculty of Bioresources and Food Industry, Universiti Sultan Zainal Abidin, Besut Campus, 22200 Besut, Terengganu, **Malaysia**.
[2]Faculty of Agriculture and Animal Sciences, Universitas Islam Negeri Sultan Syarif Kasim Riau, **Indonesia**.

***Correspondence:** khamsahsuryati@unisza.edu.my **Revised: 10** Oct. 2019, **Accepted: 08 Oct. 2**019 e-**Published:** 11 Nov. 2019 **Reviewed by:** Dr. Nurul Aini Kamaruddin, Mr. Wan Mohd Fadli Wan Mokhtar

Propolis is a sticky material manufactured by stingless bees to build and protect their hive. This material is known to be a rich source of biologically active constituents. Chemical composition that is attribute to its biological activities is highly dependent on plant sources and geographical origin. Chemical profiling was performed on Malaysian stingless bee (*Heterotrigona itama*) propolis samples collected from ten localities in two Malaysian states of Kelantan and Terengganu. High performance thin layer chromatography (HPTLC) was employed in chemical profiling. Antioxidant activity, total phenolic content (TPC) and total flavonoid content (TFC) were also evaluated. Phytochemical profiling showed that all localities have different chemical composition in terms of number of bands and intensity of the bands, indicating the variation of chemical composition. Antioxidant activity varied significantly between the localities with sample of Besut, Terengganu (BST-1) possess the highest DPPH radical inhibition (88.86 µg/mL) with IC_{50} of 11 µg/mL. Likewise, all samples has varied total phenolic and total flavonoid content ranged from 15.89 – 115.98 mg GAE/g and 15.84 - 48.65 mg QE/g, respectively. BST-1 has the highest content of both group of compounds. Findings from this study show that Malaysian stingless bee propolis has great chemical diversity and can potentially be the source for important biological activity including antioxidant agent. This study also revealed that geographical origin play a significant role in quality of propolis.

Keywords: Propolis, stingless bee, *Heterotrigona itama*, antioxidant, total phenolic content, total flavonoid content

INTRODUCTION

Propolis is a resinous material that bees collect from various plants to protect their hive (Chang et al., 2017). Propolis has been used worldwide for years in folk medicine and currently marketed by the pharmaceutical industry (Rufatto et al., 2017). Based on Kapare et al., (2019), raw propolis contains resins (50%), waxes (30%), essential oils (10%), pollen (5%) and various organic compounds (5%). It has been reported to have various pharmacological activities such as antibacterial (Abo-Elyousr et al., 2017; do Nascimento et al., 2018; Fangio et al., 2019; Kasote et al., 2019; Popova et al., 2017; Thamopoulos et al. 2018), antiviral (Drescher et al. 2018), anti-inflammatory (Zaccaria et al. 2017), antifungal (Gucawa et al. 2018; Shokri et al. 2017; Silva-Castro et al., 2017), and anti-cancer (Thirugnanasampandan et al., 2012; Sanches et al., 2017; Seyhan et al., 2019; Vukovic et al., 2018).

In producing propolis, bees use materials from a variety of botanical sources in different parts of plants. These are substances actively secreted by plants as well as substances exuded from wounds in plants: lipophilic materials on leaves and leaf buds, gums, resins, lattices, etc. The plant origin of propolis determines its chemical diversity. Bee propolis' chemical composition depends on the specificity of the local flora at the site of collection and thus on the geographic and climatic characteristics of this site. Sanches et al., (2017) stated that phenolic and terpenoids are the main compounds of Brazilian propolis. It might be contributed by resin from *Clusia fluminensis, Eucalyptus* spp. and *Euphorbia milli* (Gastauer et al., 2011). Brazilian green propolis showed the higher flavonoid concentration that similar effect with *Baccharis dracunculifolia* as its resin sources (Roberto et al., 2016). Caffeoylquinic acids and arterpilin C might be the major effective components for quality control of Braziian green propolis (Zhang et al., 2017). Brazilian black propolis from Goias state, central Brazil has the highest amounts of total phenolic substances and flavonoids, that might be caused of *Macaranga* spp. (Righi et al., 2013).

Based on Salonen et al., (2012), 26 phenolic compounds were identified on Finland's boreal coniferous zone propolis (nine individual flavonoids, three chlorogenic acid derivatives, nine cinnamic acid derivatives, two caffeic acid derivatives and three other phenolic acids. *Pinus sylvestris* L., *Picea abies (*L.) H. Karst, *Salix* spp., *Betula* spp., *P. Tremula* and *Alnus incana* (L.) Moench are said to be the main plant that contributing to Finland's propolis composition. In another study, Molnar et al., (2017) stated that the major flavonoid components of propolis from Hungary were found to be chrysin and pinocembrin, mainly composed of the bud exudates of *Populus species* and their hybrids. Whereas, in Mediteranian and temperate division (Italian propolis), chrysin is the most abundant flavonoid followed by galangin and pinocembrin. Meanwhile, phenolic acid are characterized by high amount of ferulic and isoferulic acid (Gardini et al., 2018). This might due to the high presence of *Poplar* trees. While Mexican propolis resin from *Bursera simaruba* appeared to be pentacyclic triterpenoids, suc as α and β-amyrins derivatives and sterols (Boisard et al., 2015).

Study also showed that propolis from Poland contained mainly flavonones and dihydroflavonols, as well as series of esters. The main plants found in this location were *Populus alba, P. nigra, P. tremula, Betula verucosa, Acer pseudaplatanus, Pinus silvestris and Aesculus hippocastanum* (Popova et al., 2017). The sources of the major triterpenoids are from the regional Acacia waxes and gums (Rushdi et al., 2014). Work on propolis in Southeast Sulawesi, Indonesia, discovered that mangiferolic acid, cycloartenol, ambonic acid, mangiferonic acid and ambolic acid (cycloartane-type triterpenes) were the main compounds isolated from the ethanol extract. The plant source could be from *Mangifera indica* (Pujirahayu et al., 2019)

However, study on Malaysian stingless bee propolis is still lacking especially from local bee breaders. Previous study on Malaysian propolis of *Heterotrigona itama* (MHI) species showed antioxidant activity higher than Malaysian *Geniotrigona thoracica* (MGT) (Ibrahim et al., 2016a). Total phenolic content (TPC) and total flavonoid content (TFC) of MHI respectively higher than that of MGT (Ibrahim et al., 2016b). Very limited study on Malaysian propolis sourced from various location. Therefore, this study was carried out to evaluate and compare the phytochemical composition and antioxidant activity of ethanolic extracts of propolis produced by stingless bee *Heterotrigona itama* from ten locations in two Malaysian states, namely Kelantan and Terengganu.

MATERIALS AND METHODS

Sample collection and extraction

Propolis that was produced by stingless bees *Heterotrigona itama* was collected from ten localities in two states of Malaysia; Kelantan and Terengganu. List of the localities and their vegetation types shown in Table 1. Each sample was cleaned and froze in -20°C, before it was ground to powder. Twenty one grams crude propolis were macerated with 70 mL ethanol for three days. The extracts were filtered and reduced under vacuum using rotary evaporator. The extracts were kept in -20°C prior analysis.

Phytochemical screening

Conventional thin layer chromatography (TLC), High performance thin layer chromatography (HPTLC), total phenolic content and total flavonoid content were employed to evaluate phytochemical composition of propolis in this study.

Table 1: List of ten localities of propolis samples.

NO.	CODE	LOCALITIES	COORDINATE	TYPE OF VEGETATION
1	PC-1	Gajah Hulu Island Village, Pengkalan Chepa, Kelantan	N 06° 00' 50.1'' E 102° 23' 38.0''	Fragrant coconut, fruits plant, buttercup
2	JPL-1	Department of Agriculture, Lundang, Kelantan	N 06° 06' 12.3'' E 102° 16' 02.3''	Ornamental areca palm, floriculture
3	KTRH-1	Pasir Forest, Ketereh, Kelantan	N 05° 56' 29.0'' E 102° 14' 02.1''	Rubber plant, local fruits plant
4	TM-1	Panau Hill, Tanah Merah, Kelantan	N 05° 53' 23.2'' E 102° 09' 27.4''	Rubber plant, fruits plant
5	TM-2	Pondok Kelewek Village, Tanah Merah, Kelantan	N 05° 49' 08.1'' E 102° 06' 24.6''	Miracle fruit, floriculture, fruits plant
6	GM-3	Kesedar Putra, Gua Musang, Kelantan	N 04° 50' 38.2'' E 101° 57' 45.5''	Rambutan, manggo, betelnut
7	GM-4	Felda Chiku 1, Gua Musang, Kelantan	N 04° 54' 40.9'' E 102° 10' 23.8''	Salak farm, fruits plant
8	J-1	Relak Village, Kuala Balah, Jeli	N 04° 54' 40.9'' E 102° 10' 24.0''	Rambutan, mangosteen, manggo, betelnut
9	BST-1	UniSZA Apiary, Besut, Terengganu	N 05° 75' 96.5' E 102° 63' 84.4''	Broad-leaved paperbark, cajuput, Acacia, Baeckea frutescens
10	DGN-1	Padang Serai, Dungun, Terengganu	N 04° 71' 21.6'' E 103° 39' 71.9''	Acacia, broad-leaved paperbark, cajuput, fruits plant

TLC method was optimized according to Ibrahim et al., (2016a) with slight modification. Extracts were spotted on 20 cm x 10 cm silica gel aluminium plate $60F_{254}$ (Merck, Germany). Plate was developed using mobile system of toluene, ethyl acetate, acetic acid and methanol 8: 2: 0.1: 0.2 (v/v). Dried plates were visualized under UV 254 nm and 366 nm and sprayed with various derivatisation reagents of vanilin-sulphuric acid and iodine. Derivatised plates were visualized by naked eyes.

HPTLC method was perfomed according to Azemin et al., (2017). Extract were spotted using automated spotter (CAMAG, Switzerland) on 20 cm x 10 cm silica gel glass plate $60F_{254}$ (Merck, Germany). The development of the plate was carried out using mobile system T:EA:AA:MeOH 8:2:0.1:0.2 (v/v). Plates were visualized and scanned under UV 254 nm and 366 nm using CAMAG TLC Scanner 4 (CAMAG, Switzerland).

Total phenolic content were evaluated using modified Folin-Ciocalteu method according to Kasote et al. (2019). One hundred microlitres of each extract and 200 µL of Folin-Ciocalteu reagent were mixed. Then, 800 µL Na_2CO_3 was added to the solution and incubated at room temperature in dark condition for two hours. Accurately, 200 µL solution was transfered into 96 well plate and absorbance was measured at 765 nm by using microplate reader. Gallic acid was used to calculate the standard curve (60 - 180 µg/mL, r^2 = 0.997). Estimation of the phenolic content was carried out in triplicates. The results were expressed as mg of gallic acid equivalents (GAEs) per g propolis.

Total flavonoid content was determined by a modified colorimetric assay method from Bhaigyabati et al. (2014). A volume of 140 µL of each extract and 150 µL $AlCl_3$ were mixed. After that, 150 µL CH_3CO_2K was added to the solution and make up with 260 µL dH_2O. Then, the solution was incubated at room temperature in dark condition for 30 minutes. A volume of 200 µL solution was transfered into 96 well plate and absorbance was measured at 415 nm by using microplate reader. Quercetin was used to calculate the standard curve (0.78125 - 100 µg/mL, r^2 = 0.999). Estimation of the flavonoid content was carried out in triplicates. The results were expressed as mg of quercetin per g propolis.

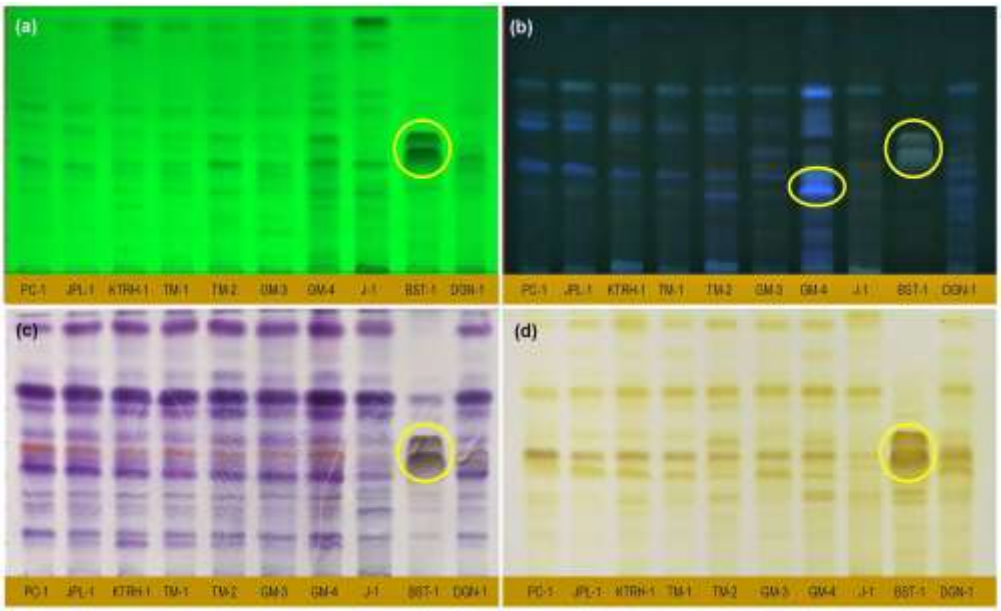

Figure 1: TLC chromatogram of 10 localities of propolis extracts by visualisation at: (a) UV 254 nm, (b) UV 366 nm, (c) post derivatization vanillin-sulphuric acid, (d) post derivatization iodine: 1) (PC-1) Gajah Hulu Island Village, Pengkalan Chepa; 2) (JPL-1) Department of Agriculture, Lundang; 3) (KTRH-1) Pasir Forest, Ketereh; 4) (TM-1) Panau Hill, Tanah Merah; 5) (TM-2) Pondok Kelewek Village, Tanah Merah; 6) (GM-3) Kesedar Putra, Gua Musang; 7) (GM-4) Felda Chiku 1, Gua Musang; 8) (J-1) Relak Village, Kuala Balah, Jeli; 9) (BST-1) UniSZA Apiary, Besut and 10) (DGN-1) Padang Serai, Dungun.

Antioxidant assay

Antioxidant assay was performed using DPPH scavenging assay. The principle of DPPH assay is the reduction of the purple stable free radical DPPH to the yellow diphenylpicrylhidrazine. DPPH radical scavenging activity was perfomed based on the Ibrahim et al. (2016a) with slight modification. One mM of DPPH was prepared by diluting 5 mg DPPH in 100 mL methanol and 25 µL of standard and extracts solution (7.8125 - 500 µg/mL) were added into 96 well plate. Then, 200 µL of 1 mM DPPH solution was mixed into each well and incubated at room temperature in dark condition for 30 minutes. The absorbance was measured at 517 nm by using microplate reader. A mixture of 50 µL DMSO and 200 µL of 1 mM DPPH used as blank. Quercetin and trolox were used as positive control. The ability of the extracts and positive controls to scavenge the DPPH free radical was calculated using the formula:

$$\text{Inhibition } \% = \left[\frac{(A\ Blank - A\ Sample)}{A\ Blank}\right] \times 100\%$$

Statistical Analysis

One-Way Anova (Duncan) analysis was performed using The SPSS for windows Ver. 21 software program was used to perform the statistical analyses. Data are expressed as mean ± SD and each value is representative of at least three independent experiments. Values of $\rho < 0.05$ were considered significant.

RESULTS AND DISCUSSION

Phytochemical Screening

Phytochemical composition was screened by thin layer chromatography (TLC), high performance thin layer chromatography (HPTLC), total phenolic content (TPC) and total flavonoid content (TFC). TLC profile is shown in Figure 1. The compounds were under UV light of short wavelength (254 nm) and long wavelength (366 nm) (Fig. 1a and 1b), as well as derivatised by vanillin-sulphuric acid and iodin vapour, respectively (Figure 1c and 1d). The chromatograms reveal different intensity of spot for different localities. BST-1 has the strongest intensity of the peak at R_f 0.53 and 0.48 under both wavelengths and derivatisation agents. Meanwhile, GM-4 shows the highest intensity at R_f 0.41 under UV 366 nm (Figure 1b).

The result showed the evidence of possible coumarins detected (light blue in 366 nm), saponin (violet blue by using vanillin-sulphuric acid reagent) and organic compound (brown by using iodine reagent). Based on Ibrahim et al. (2016a), vanillin-sulphuric acid used to detect amino acids, amines, saponin, phenols, essential oil and higher alcohols, meanwhile iodine used to detect organic compounds such as steroid, alkaloids, polycyclic aromatic compound, phenolic and ester. It might be caused of the local flora in each localities. Salonen et al., (2012) stated that bees seek different resin sources for propolis and collect the raw material for propolis from the vegetation near their hive.

HPTLC densitogram analysis of 10 localities of propolis extracts at 254 nm was summarised in Fig. 2 and Table 2. There were 13 spots occur in TM-2, 12 spots occur in PC-1 and GM-4, 11 spots occur in KTRH-1, 10 spots occur in JPL-1, TM-1 and DGN-1, 9 spots occur in J-1 and 8 spots occur in BST-1 when visualized under UV 254 nm. Only four spots at R_f 0.17, 0.29, 0.53 and 0.75 presence in all propolis extracts.

As mentioned in Figure 2, there was one highest peak at R_f 0.53 (BST-1). 3-D HPTLC densitogram under 366 nm was summarised in Fig. 3 and Table 3. There were 12 spots occur in TM-2 and GM-3, 11 spots occur in TM-1 and GM-4, 10 spots occur in KTRH-1, 8 spots occur in PC-1 and DGN-1, 7 spots occur in J-1 and BST-1 and 6 spots occur in JPL-1. There were only three spots at R_f 0.29, 0.48 and 0.76 presence in all propolis extracts. The highest peak under 366 nm occurs at R_f 0.48.

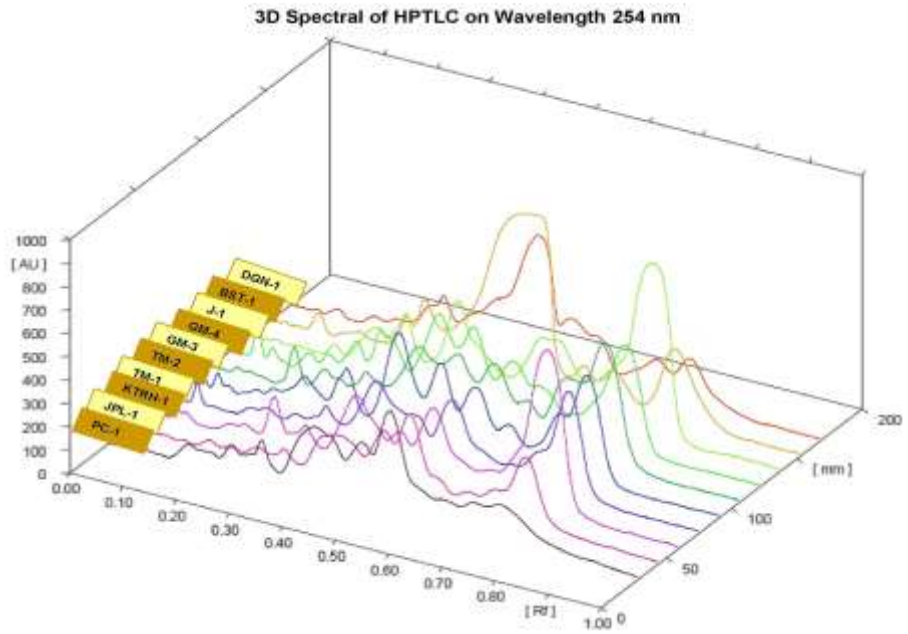

Figure 2: 3-D HPTLC densitogram analysis of 10 localities of propolis extracts at 254 nm, namely: 1) (PC-1) Gajah Hulu Island Village, Pengkalan Chepa; 2) (JPL-1) Department of Agriculture, Lundang; 3) (KTRH-1) Pasir Forest, Ketereh; 4) (TM-1) Panau Hill, Tanah Merah; 5) (TM-2) Pondok Kelewek Village, Tanah Merah; 6) (GM-3) Kesedar Putra, Gua Musang; 7) (GM-4) Felda Chiku 1, Gua Musang; 8) (J-1) Relak Village, Kuala Balah, Jeli; 9) (BST-1) UniSZA Apiary, Besut and 10) (DGN-1) Padang Serai, Dungun.

Table 2: The summary from UV spectra of 3-D HPTLC densitogram analysis of 10 localities of propolis extracts at 254 nm.

No	R*f*	TRACKS (254 nm)									
		PC-1	JPL-1	KTRH-1	TM-1	TM-2	GM-3	GM-4	J-1	BST-1	DGN-1
1	0.75	√	√	√	√	√	√	√	√	√	√
2	0.53	√	√	√	√	√	√	√	√	√	√
3	0.43	√	√	√	√	√	√	√	√		√
4	0.47	√	√	√	√	√	√	√			
5	0.67	√	√			√	√			√	
6	0.60	√				√	√	√	√	√	√
7	0.29	√	√	√	√	√	√	√	√	√	√
8	0.24	√	√	√	√	√	√	√	√	√	
9	0.17	√	√	√	√	√	√	√	√	√	√
10	0.66		√	√	√						√
11	0.05	√		√	√	√	√	√	√		√
12	0.19				√			√			√
13	0.24						√				
14	0.08			√		√	√	√		√	√
15	0.15	√	√								
16	0.13					√					
17	0.03			√		√					
18	0.92	√							√		

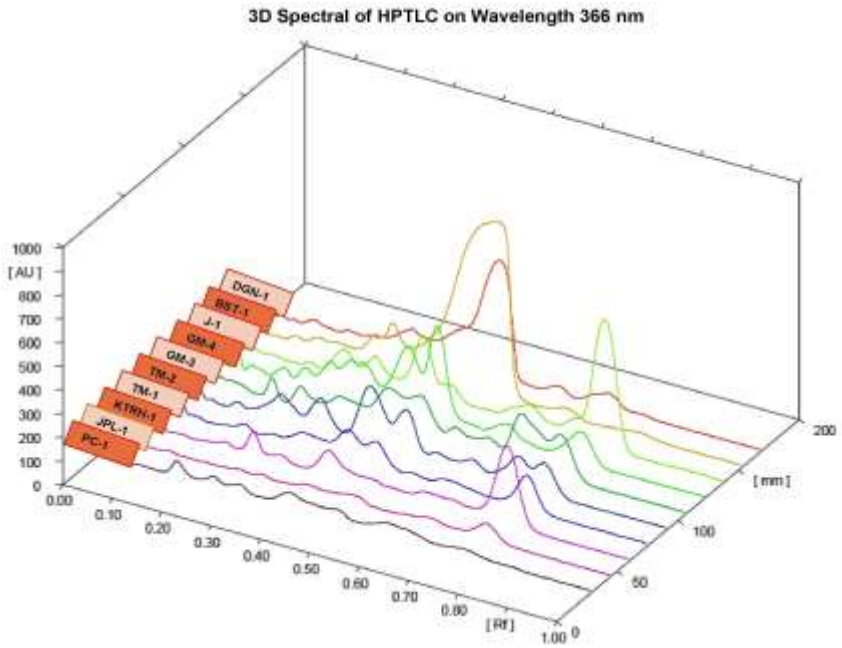

Figure 3: 3-D HPTLC densitogram analysis of 10 localities of propolis extracts at 366 nm, namely: 1) (PC-1) Gajah Hulu Island Village, Pengkalan Chepa; 2) (JPL-1) Department of Agriculture, Lundang; 3) (KTRH-1) Pasir Forest, Ketereh; 4) (TM-1) Panau Hill, Tanah Merah; 5) (TM-2) Pondok Kelewek Village, Tanah Merah; 6) (GM-3) Kesedar Putra, Gua Musang; 7) (GM-4) Felda Chiku 1, Gua Musang; 8) (J-1) Relak Village, Kuala Balah, Jeli; 9) (BST-1) UniSZA Apiary, Besut and 10) (DGN-1) Padang Serai, Dungun.

Table 3: The summary from UV spectra of 3-D HPTLC densitogram analysis of 10 localities of propolis extracts at 366 nm.

No	R*f*	TRACKS (366 nm)									
		PC-1	JPL-1	KTRH-1	TM-1	TM-2	GM-3	GM-4	J-1	BST-1	DGN-1
1	0.76	√	√	√	√	√	√	√	√	√	√
2	0.53				√	√	√				
3	0.39	√	√	√	√	√	√	√	√		
4	0.48	√	√	√	√	√	√	√	√	√	√
5	0.67					√	√			√	√
6	0.60	√									
7	0.29	√	√	√	√	√	√	√	√	√	√
8	0.24	√	√	√	√			√	√		
9	0.18	√		√	√	√	√	√	√	√	√
10	0.64			√							
11	0.05			√	√	√	√	√	√		
12	0.15						√	√			
13	0.26									√	
14	0.09				√	√	√	√		√	√
15	0.13					√					
16	0.03			√	√	√	√				√
17	0.31							√			
18	0.61	√	√	√	√	√	√	√	√		√

Figure 4 shows that BST-1 possess significant highest content of total phenolic (115.98 mg GAE/g) compared to other localities, followed by GM-4 (53.27 mg GAE/g), J-1 (50.32 mg GAE/g), TM-2 (47.36 mg GAE/g), GM-3 (44.91 mg GAE/g), DGN-1 (40.30 mg GAE/g), PC-1 (29.00 mg GAE/g), TM-1 (26.27 mg GAE/g), KTRH-1 (23.14 mg GAE/g) and JPL-1 (15.89 mg GAE/g). Galic acid was used as the standard. Previous study shows varied content of total phenolic in different location. Salonen et al. (2012) found that total phenolic compounds present in Finland's boreal coniferous zone propolis ranged from 79.8 to 156.3 mg GAE/g, the average being 119.5 mg GAE/g. TPC of Mandacaia propolis from state of Sao Paolo, Brazil is about 70.5 mg GAE/g. Molnar et al. (2017) stated that TPC of ethanolic extract of propolis from Hungary ranged between 104.60 and 286.90 mg GAE/g. Whereas, TPC of Brazilian green propolis ranged from 87.53 – 148.55 mg GAE/g (Zhang et al. 2017). Solorzano et al. (2012) also found that TPC of propolis from arid environments of North-Western Argentina ranged from 282.00 – 321.00 µg GAE/g. In another study, Kasote et al. (2019) revealed that TPC of Indian propolis ranged from 9.60 – 48.60 µg GAE/mg). Meanwhile, Fangio et al. (2019) reported that TPC of Argentina propolis ranged from 189.00 – 417.00 mg GAE/g.

Figure 5 shows that BST-1 possess significantly highest content of total flavonoid (48.65 mg QE/g) compare to other localities, followed by GM-3 (42.78 mg QE/g), GM-4 (33.68 mg QE/g), TM-2 (31.13 mg QE/g), J-1 (29.21 mg QE/g), DGN-1, KTRH-1, TM-1 (23.30, 22.74, 22.00 mg QE/g) and JPL-1, PC-1 (15.84, 15.47 mg QE/g). Quercetin was used as the standard. Previous study shows the variety of total flavonoid content in accordance to their locality. Pazin et al. (2017) found that TFC present in Brazilian green propolis from state of Minas Gerais, Brazil is about 47.5 mg QE/g. Meanwhile, Zhang et al. (2017) report that TFC of Brazilian green propolis ranged from 38.35 – 67.60 mg QE/g. Whereas, TFC of propolis from arid environments of North-Western Argentina ranged from 180.70 – 268.00 µg QE/g (Solorzano et al. 2012). Fangio et al. (2019) stated that TFC of Argentina propolis ranged from 46.00 – 191.00 mg QE/g.

Variation in the phenolic and flavonoid content of propolis is mainly affected by the differences of the local flora of each localities and stingless bees preference. Furthermore, our best finding on total phenolic content (BST-1) shows lower than TPC of propolis from arid environments of North-Western Argentina and Argentina propolis, still in ranged in TPC from Finland's boreal coniferous zone propolis, propolis from Hungary and Brazilian green propolis, but higher than TPC of Mandacaia propolis from state of Sao Paolo, Brazil and Indian propolis. Meanwhile, our best finding on total flavonoid content (BST-1) shows higher than Brazilian green propolis from state of Minas Gerais, Brazil, but still in ranged in TFC from Brazilian green propolis, arid environments of North-Western Argentina and Argentina propolis. Sanches et al. (2017) stated that the great diversity of plants visited by the stingless bees contribute to the varied chemical composition of propolis. BST-1 shows the significant highest of TPC and TFC might be caused of the natural plant around the apiary, such as broad-leaved paperbark, cajuput, *Baeckea frustescens* and Acacia. Tran et al. (2012) found that Australian propolis contain high flavonoid from an Australian endemic plant, *Acacia paradoxa*. Phenolics compounds are a large class of plant secondary metabolites, including flavonoids, tannins, phenolic acids, lignans, quinones, coumarins and others (Huang et al., 2010).

Antioxidant activity by DPPH scavenging assay showed in Figure 6 and Table 4. Corroborated with TPC and TFC, BST-1 have the highest DPPH scavenging capacity compare to other localities start from [7.812 – 500 µg/mL]. At concentration 250 µg/mL and 500 µg/mL BST-1 show similar inhibition percentage with that of standard Trolox and Quercetin (88.86, 91.23, 90.94 µg/mL and (88.81, 91.23, 90.94 µg/mL, respectively. Figure 7 show that scavenging activity of all localities are in following order; Trolox (3 µg/mL > Quercetin (6 µg/mL) > BST-1 (11 µg/mL) > DGN (110 µg/mL) > GM4 (164 µg/mL) > J-1 (253 µg/mL) > GM-3 (325 µg/mL) > TM-2 (362 µg/mL). Meanwhile, propolis from PC-1, JPL-1, KTRH-1 and TM-1 cannot reach IC_{50} (low antioxidant activity). IC_{50} of BST-1 almost the same with Mandacaia propolis from state of Sao Paolo, Brazil, 11.05 µg/mL (Pazin et al. 2017), but lower than Brazilian green propolis that ranged from 93.51 – 190.27 µg/mL (Zhang et al. 2017). The lower IC_{50} value means the higher scavenging activity of DPPH and stronger antioxidant activity (Ibrahim et al., 2016b).

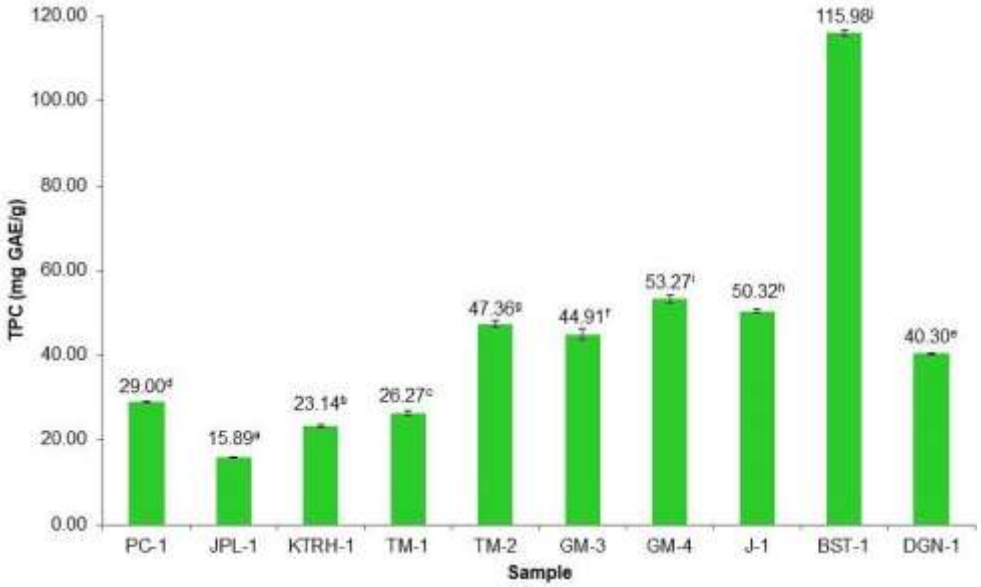

Figure 4: Total phenolic content of ten localities of propolis extracts analysed by using FC method, namely: 1) (PC-1) Gajah Hulu Island Village, Pengkalan Chepa; 2) (JPL-1) Department of Agriculture, Lundang; 3) (KTRH-1) Pasir Forest, Ketereh; 4) (TM-1) Panau Hill, Tanah Merah; 5) (TM-2) Pondok Kelewek Village, Tanah Merah; 6) (GM-3) Kesedar Putra, Gua Musang; 7) (GM-4) Felda Chiku 1, Gua Musang; 8) (J-1) Relak Village, Kuala Balah, Jeli; 9) (BST-1) UniSZA Apiary, Besut and 10) (DGN-1) Padang Serai, Dungun.

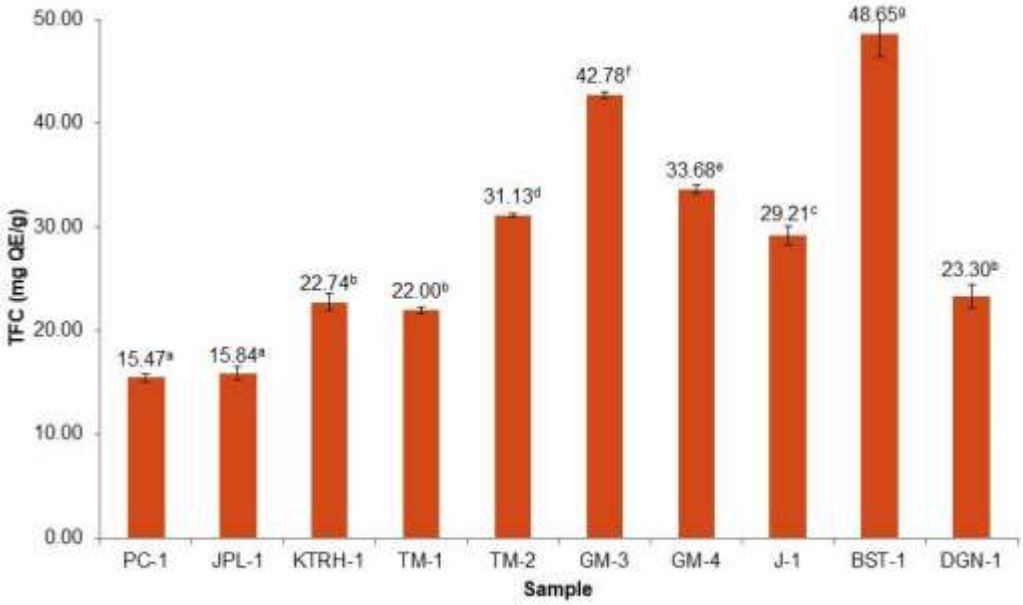

Figure 5. Total flavonoid content of ten localities of propolis extracts analysed by using a modified of colorimetric assay method, namely: 1) (PC-1) Gajah Hulu Island Village, Pengkalan Chepa; 2) (JPL-1) Department of Agriculture, Lundang; 3) (KTRH-1) Pasir Forest, Ketereh; 4) (TM-1) Panau Hill, Tanah Merah; 5) (TM-2) Pondok Kelewek Village, Tanah Merah; 6) (GM-3) Kesedar Putra, Gua Musang; 7) (GM-4) Felda Chiku 1, Gua Musang; 8) (J-1) Relak Village, Kuala Balah, Jeli; 9) (BST-1) UniSZA Apiary, Besut and 10) (DGN-1) Padang Serai, Dungun.

Antioxidant activity

In agreement with BST-1, the extract that has highest phenolic content has highest antioxidant activity than another extracts (Sari et al. 2019). Phenolic compounds can reduce the risk of developing degenerative diseases by reduce the oxidation levels of an organic matter by transferring a H atom from the OH group (Costa et al. 2017).

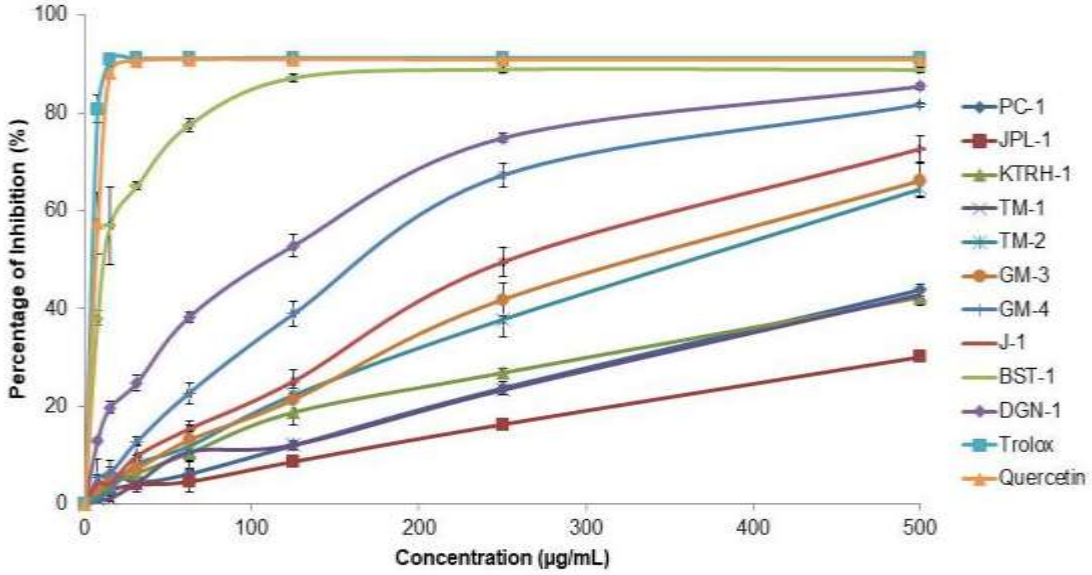

Figure 6: Percentage inhibition of ten localities of propolis extracts, trolox and quercetin evaluated through DPPH scavenging assay, namely: 1) (PC-1) Gajah Hulu Island Village, Pengkalan Chepa; 2) (JPL-1) Department of Agriculture, Lundang; 3) (KTRH-1) Pasir Forest, Ketereh; 4) (TM-1) Panau Hill, Tanah Merah; 5) (TM-2) Pondok Kelewek Village, Tanah Merah; 6) (GM-3) Kesedar Putra, Gua Musang; 7) (GM-4) Felda Chiku 1, Gua Musang; 8) (J-1) Relak Village, Kuala Balah, Jeli; 9) (BST-1) UniSZA Apiary, Besut; 10) (DGN-1) Padang Serai, Dungun; 11) Trolox and 12) Quercetin.

Table 4: Inhibition of DPPH at different concentrations and 10 localities of propolis extracts.

LOCALITIES	INHIBITION OF DPPH AT DIFFERENT CONCENTRATION (µg/mL)							
	0	7.8125	15.265	31.25	62.5	125	250	500
PC-1	0.00^{a}	1.48^{a}	6.23^{ab}	4.50^{a}	6.17^{a}	12.01^{b}	23.7^{bc}	43.80^{b}
JPL-1	0.00^{a}	1.26^{a}	2.89^{ab}	3.96^{a}	4.59^{a}	8.68^{a}	16.30^{a}	30.08^{a}
KTRH-1	0.00^{a}	2.22^{a}	4.13^{ab}	6.29^{ab}	10.43^{b}	18.75^{c}	26.84^{c}	42.26^{b}
TM-1	0.00^{a}	1.04^{a}	1.17^{a}	4.30^{a}	10.68^{b}	12.11^{b}	23.31^{bc}	42.84^{b}
TM-2	0.00^{a}	1.26^{a}	2.90^{ab}	8.14^{bc}	11.86^{bc}	22.27^{de}	37.73^{d}	64.42^{c}
GM-3	0.00^{a}	2.95^{a}	5.46^{ab}	7.26^{b}	13.10^{bc}	21.39^{cd}	41.81^{e}	66.15^{c}
GM-4	0.00^{a}	5.29^{a}	6.31^{b}	12.76^{d}	22.70^{d}	38.90^{f}	67.22^{g}	81.70^{e}
J-1	0.00^{a}	4.21^{a}	4.27^{ab}	9.82^{c}	15.37^{c}	25.13^{e}	49.49^{f}	72.64^{d}
BST-1	**0.00^{a}**	**38.06^{c}**	**56.89^{d}**	**65.04^{f}**	**77.50^{f}**	**87.14^{h}**	**88.86^{i}**	88.81^{g}
DGN-1	0.00^{a}	13.01^{b}	19.68^{c}	24.77^{e}	38.24^{e}	52.83^{g}	74.83^{h}	85.41^{f}
Trolox	**0.00^{a}**	**80.78^{e}**	**90.88^{e}**	**91.11^{g}**	**91.11^{g}**	**91.29^{i}**	**91.23^{i}**	91.23^{g}
Quercetin	**0.00^{a}**	**57.47^{d}**	**88.23^{e}**	**90.65^{g}**	**91.00^{g}**	**91.00^{i}**	**90.94^{i}**	90.94^{g}

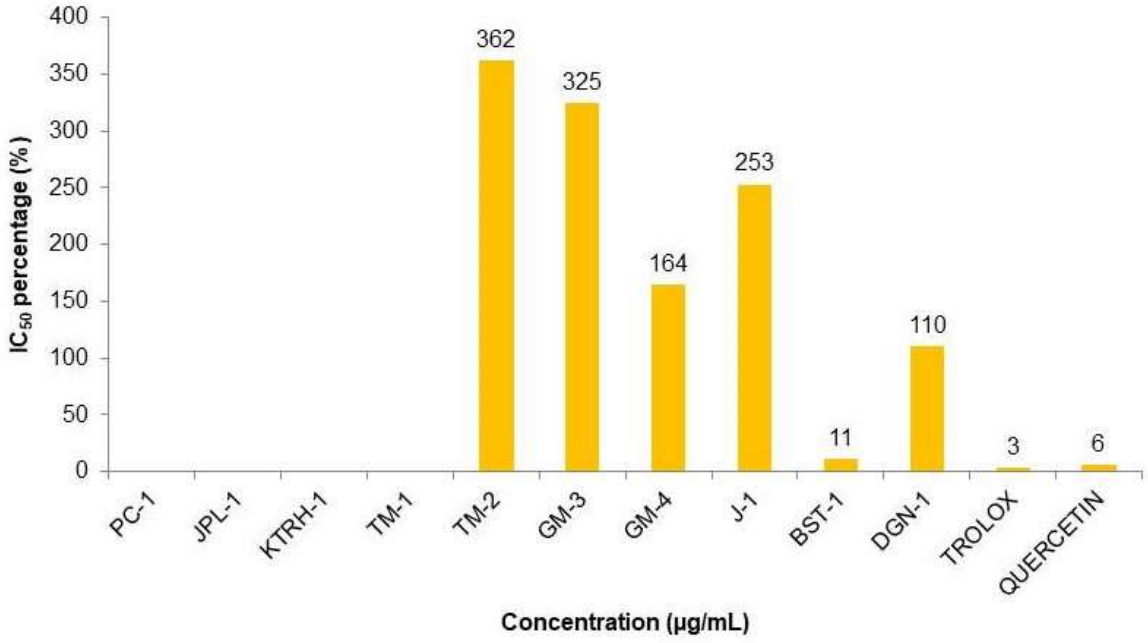

Figure 7: Fifty percent of inhibition concentration (IC_{50}) of ten localities of propolis extracts evaluated through DPPH scavenging assay, namely: 1) (PC-1) Gajah Hulu Island Village, Pengkalan Chepa; 2) (JPL-1) Department of Agriculture, Lundang; 3) (KTRH-1) Pasir Forest, Ketereh; 4) (TM-1) Panau Hill, Tanah Merah; 5) (TM-2) Pondok Kelewek Village, Tanah Merah; 6) (GM-3) Kesedar Putra, Gua Musang; 7) (GM-4) Felda Chiku 1, Gua Musang; 8) (J-1) Relak Village, Kuala Balah, Jeli; 9) (BST-1) UniSZA Apiary, Besut; 10) (DGN-1) Padang Serai, Dungun; 11) Trolox and 12) Quercetin.

Marventano et al., (2017) stated that the most substantial group of phenolic is flavonoid. Its usually classified into flavones, flavonols, flavanols, anthocyanins, isoflavones and flavanones (Kozlowska and Szostak-Wegierek, 2014). Based on Xiao et al., (2011), these components showed important antioxidant, antidiabetic, antiinflammatory, anticancer and cardioprotective effects.

CONCLUSION

Phytochemical screening showed that all localities have different chemical composition in terms of number of spots and intensity of the spots. Propolis from UniSZA Apiary, Besut (BST-1) gave the highest of total phenolic content, total flavonoid content and antioxidant activity. Data obtained from this study show that localities play a vital role in determination the quality of propolis. It is necessary to clarify the quality and quantity of the constituents in propolis in order to evaluate its biological activity.

CONFLICT OF INTEREST

There was not any conflict of interest in this study.

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AUTHOR CONTRIBUTION

ARA, AJZ and KSM contributed to the conception and design of the study. NEMF, ASAK and NP facilitated in cytotoxicity study. NBMZ and AAMB responsible for the designing analysis of metabolites and chemical profile. ARA was responsible for all the experiments. KSM was contributed in revising and approved this paper.

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