

Phytochemical screening and antioxidant properties of stingless bee *Geniotrigona thoracica* propolis

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Abstract

Propolis is a gum-like product that collected by bees from plants and it varies in colour range from light yellow to dark brown. The chemical composition of propolis depends on various factors such as vegetation, season and environmental conditions of the sample collection. The aim of the study was to determine the chemical profile and antioxidant properties of crude methanol extract of *Geniotrigona thoracica* propolis from five localities, namely Besut (BST), Dungun (DGN), Lundang (LDG), Tanah Merah (TM) and Gua Musang (GM). Phytochemical screening by thin layer chromatography (TLC) showed the presence of terpenoids, flavonoids, coumarins, essential oils and unsaturated and aromatic compounds. The extracts displayed different characteristics of chemical profile that varied from each other. The antioxidant properties of extracts were evaluated based on total phenolic content, total flavonoid content and 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical-scavenging activity. Propolis from Gua Musang (GM) exhibited the highest total phenolic content with 23.43 ± 0.50 mg GAE/g extract while propolis from Besut (BST) exhibited the highest total flavonoids content with 17.22 ± 0.16 mg QE/g extract and showed the lowest IC_{50} with 53 μ g/mL for DPPH radical scavenging activities. As a result, *Geniotrigona thoracica* from Besut (BST) could be considered as a good source of antioxidant due to its antioxidant properties.

Keywords: Stingless bee, propolis, *Geniotrigona thoracica*, TLC profiling, antioxidant

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INTRODUCTION

Propolis is a gum-like product that collected by bees from plants and it varies in color range from light yellow to dark brown (Shittu *et al.*, 2015). Bees apply propolis in a thin layer on the inner walls of their hives. It is used to block holes and cracks, repair combs, reinforce the thin edges of the comb and make the entrance of the hive to be weatherproof or easier to protect (Bankova *et al.*, 2000). Propolis acts as defensive antimicrobial substances in their hives and is used as a building material (Popova *et al.*, 2013). Propolis also acts as an “embalming substance” because it can cover the hive with invaders that have been killed by bees but cannot transport out of the hive (Ghisalberti, 1979). Propolis has a lipophilic properties that is hard and unbreakable when cold but soft, flexible and very sticky when warm (Umthong *et al.*, 2011; Marcucci, 1995; Fokt *et al.*, 2010). Generally, propolis contains 50-60% resins and balms, 30-40% waxes, 5-10% essential oils, 5% pollen grains, microelements and vitamins (Rufatto *et al.*, 2017).

The chemical composition of propolis is varied and depending on various factors such as vegetation, season and environmental conditions of the sample collection, leading to difficulty for standardization (Sforzin & Bankova, 2011; Barlak *et al.*, 2011; Bankova, 2005). Thus, constituents of propolis from different places may be different. For example in Europe, North America, New Zealand and temperate zones of Asia, propolis consists of flavonoids,

phenolic acids and their esters, meanwhile in Greece (Popova *et al.*, 2010; Celemlı *et al.*, 2013) and Switzerland (Bankova *et al.*, 2002), propolis is highly composed of diterpenoids. Propolis from Brazil, mainly in the south-eastern region is consisted with the presence of common and abundant chemical constituents prenylated phenylpropanoids, prenylated *p*-coumaric acids, acetophenones, diterpenic acids, and caffeoylquinic acids (Bankova, 2005; Bankova *et al.*, 2000; Falcão *et al.*, 2013). The flavonoids kaempferide, isosakuranetin and some of kaempferol are found in other Brazilian propolis (Park *et al.*, 2002). Meanwhile, the content of Cuban propolis is comprised of polyisoprenylated benzophenones, more specifically nemorosone, and a mixture of xanthochymol and guttiferone E (Bankova, 2005; Cuesta-Rubio *et al.*, 2002), making it to be chemically distinct from European and Brazilian propolis.

Propolis has numerous pharmacological activities such as antioxidant, antibacterial, anticancer, antifungal, anti-inflammatory, antiviral, and antidiabetic due to its various and complex chemical compositions (Bankova, 2009). Previous study by Ibrahim *et al.* (2016) reported that propolis produced from stingless bee *Heterotrigona itama* and *Geniotrigona thoracica* could inhibit the growth of *Staphylococcus aureus* better than Gram-negative (*Escherichia coli* and *Salmonella typhi*) bacteria. Propolis produced by stingless bee *Tetragonula carbonaria* from Australia could also inhibit the growth of *S. aureus* (Massaro *et al.*, 2014). Chemical compounds isolated from Brazilian propolis which are triterpenoids,

melliferone, moronic acid, anwuweizonic acid, betulinic acid and four known aromatic compounds have been tested for anti-HIV activity in H9 lymphocytes and the result showed that moronic acid has significant anti-HIV activity (Ito *et al.*, 2001). Amoros *et al.* (1992) revealed that major flavonoids of propolis like flavonols and flavones showed antiviral activity against HSV-1.

A free radical is any species that contains one or more unpaired electrons, in which an unpaired electron is in atomic or molecular orbitals (Halliwell *et al.*, 1995). Free radicals and reactive oxygen species (ROS) such as hydrogen peroxide (H₂O₂), superoxide anion (O₂⁻), and hydroxyl ion (HO⁻), as well as reactive nitrogen species (RNS), especially nitric oxide (NO) can be generated through the process of cellular metabolism and the exposure to the exogenous agents like UV, toxins and drugs (Sosa *et al.*, 2013; Salmon *et al.*, 2004; Viuda-Martos *et al.*, 2008). Numerous systemic and cellular dysfunctions such as hyperglycaemia, dyslipidemia, advanced glycation end products, endoplasmic reticulum (ER) stress, nitric oxide synthase and lipid peroxides are responsible for over production of reactive oxygen species (ROS) that will induce the oxidative stress in the cells and plasma (Henriksen *et al.*, 2011). Oxidative stress can potentially distribute in causing various diseases in human such as neurodegenerative or cardiovascular disease, diabetes, cancer and atherosclerosis (Silva-Carvalho *et al.*, 2015). An antioxidant can be defined as any substance that inhibits or delays oxidative damage to a target molecule (Yamagishi & Matsui, 2011). Antioxidant compounds such as polyphenols, phenolic acid and flavonoids can scavenge free radicals like peroxide, hydroperoxide or lipid peroxy, causing inhibition of oxidative mechanism which leads to degenerative mechanism (Wu *et al.*, 2011). Portuguese propolis has the potential to protect human erythrocytes from free radical damages by decreasing the lipid peroxidation due to its powerful antioxidant agent that can be used to prevent oxidative stress and thus, maintaining the structural and functional integrity of the cells (Valente *et al.*, 2011). Red propolis from China contains high amounts of galangin and phenethyl caffeate which lead to higher antioxidant activity (Kumazawa *et al.*, 2004). Galangin and phenethyl caffeate are reported to be responsible for antioxidant activity (Hatano *et al.*, 2012). Hence, the present study was designed to screen the phytochemicals as well as to evaluate the antioxidants activity, total phenolic contents and total flavonoid contents in stingless bee *Geniotrigona thoracica* propolis from different localities namely as Besut (BST), Dungun (DGN), Lundang (LDG), Tanah Merah (TM), and Gua Musang (GM).

EXPERIMENTAL

Materials

Gallic acid, quercetin, trolox, Folin-Ciocalteu phenol reagent, aluminium chloride, potassium acetate, sodium carbonate solution, 1,1-diphenyl-2-picrylhydrazyl (DPPH), dimethyl sulfoxide (DMSO), methanol, sulphuric acid, ethanol, glacial acetic acid, toluene, ethyl acetate, and TLC silica gel 60 F₂₅₄ plate of 0.05 mm thickness were purchased from Merck.

Propolis samples

Propolis samples from stingless bee were collected from hives of five different localities which were Besut (N 5° 45' 34.6" N 102° 38' 18.5"E), Dungun (N 04° 42' 43.7" N 103° 23' 49.3"E), Lundang (N 06° 06' 12.3" E 102° 16' 02.3"), Tanah Merah (N 05° 49' 08.1" E 102° 06' 24.6") and Gua Musang (N 05° 09' 12.5"E 101°58' 33.8").

Preparation of extract

The 30 grams of powdered propolis was extracted using maceration process with 70 mL of methanol for at least 3 days. The extract was filtered by using Whatman No.1 filter paper. The extract was dried under pressure using rotatory evaporator and kept in -20°C prior for further analysis. The propolis extract was coded as following; Besut (BST), Dungun (DGN), Lundang (LDG), Tanah Merah (TM) and Gua Musang (GM).

Phytochemical screening by thin layer chromatography

G. thoracica propolis from different localities were screened to identify the presence of various classes of compound according to the standard screening method by Trease and Evans (1983) with slight modification using thin layer chromatography (TLC) silica gel 60 F₂₅₄ plate of 0.05 mm thickness. The crude extracts were accurately weighed at 30 mg and dissolved in 1 mL of methanol, followed by sonication for 30 min and centrifugation for 10 min at 140 rpm. The development of plate was done in a glass twin-through chamber using toluene: ethyl acetate: acetic acid: methanol (8: 2: 0.1: 0.2 v/v/v/v) as a mobile phase. After development, the plate was dried with heat gun and visualized at 254 nm and 366 nm, and then sprayed with various chemical reagents such as vanillin-sulphuric acid and iodine for detection of the respective classes of compound.

Determination of total phenolic content (TPC)

Total phenolic content was determined using the Folin-Ciocalteu reagent according to the method developed by Singleton and Rossi (1965) with slight modification. Briefly, 100 µL of the each sample was added into microtubes and followed by adding 200 µL of Folin-Ciocalteu reagent and was vortex thoroughly. Next, 800 µL of 700 mM Na₂CO₃ was added into the mixture and incubated at room temperature for 2 hrs. After incubation, 200 µL of sample was transferred into the 96-well microplate and the absorbance was read at 765 nm using Elisa reader spectrophotometer. Gallic acid was used as a positive control with concentration that ranged from 60 to 200 mg/mL. The total phenolics content was measured in triplicate. The results were expressed as mg of gallic acid equivalents (GAE) per g of extract.

Determination of total flavonoid content (TFC)

Total flavonoids content was determined by aluminium chloride using method described by Woisky and Salatino (1998) with slight modification. Briefly, 140 µL of each sample, 150 µL of aluminium chloride solution, 150 µL potassium acetate solution and 260 µL of distilled water were added and mixed well. After incubation at room temperature for 30 min, the samples were transferred into the 96-well plate. The absorbance was read at 415 nm with Elisa reader spectrophotometer. The calibration curve was constructed with serial dilution of quercetin in concentration that ranged from 0.7815 to 100 mg/mL. The results were expressed as mg of quercetin equivalents (QE) per g of extract.

DPPH free radical scavenging activity

The antioxidant activity of the extracts was determined using the DPPH free radical scavenging assay described by Brand-Williams *et al.* (1995) with some modification. Quercetin and trolox were used as a positive control. Briefly, 5 mg of quercetin, trolox standard and extracts were dissolved in 1 mL of dimethyl sulfoxide (DMSO). About 25 µL of standard and extracts were added into the 96-well plate. Serial dilution was performed from stock solution of standard and extracts to produce final concentrations of 7.1825, 15.625, 31.125, 62.5, 125, 250 and 500 µg/mL. Next, 200 µL of 1 mM DPPH in methanol solution was added into each well and left at room temperature in the dark condition for 30 min to complete the reaction. After incubation period, the absorbance was measured at 517 nm using Elisa reader spectrophotometer. The blank sample used was the solution comprised of 50 µL DMSO and 200 µL of 1 mM DPPH. The percentage of inhibition was calculated using the equation (1).

$$\text{Inhibition \%} = [(A_{\text{Blank}} - A_{\text{Sample}}) / A_{\text{Blank}}] \times 100\% \quad (1)$$

A_{Blank} is the absorbance of DMSO with 1 mM of DPPH in methanol solution while A_{Sample} is the absorbance of the extracts and positive control solution. The lower absorbance and the decrease intensity of the purple to yellow colour were indicated for a higher scavenging activity. The radical scavenging activities of crude extracts were interpreted by IC₅₀ values. The IC₅₀ is a concentration that has the ability to scavenge the 50% of DPPH free radical.

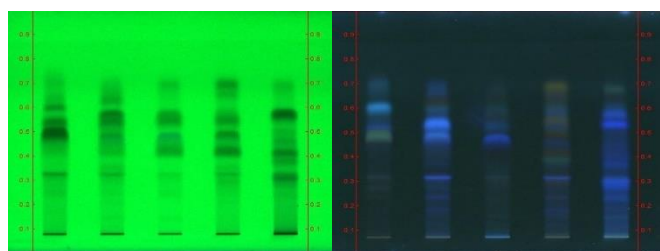
Statistical analysis

Assays were performed in triplicate and the results were expressed as mean values with standard deviations (SD). The significant differences, represented by letters, were obtained by a one-way analysis of variance (ANOVA), followed by Tukey's honestly significant difference (HSD) post hoc test ($p < 0.05$) using Statistical Package for Social Science (SPSS) version 24.0 (Armonk, NY, USA).

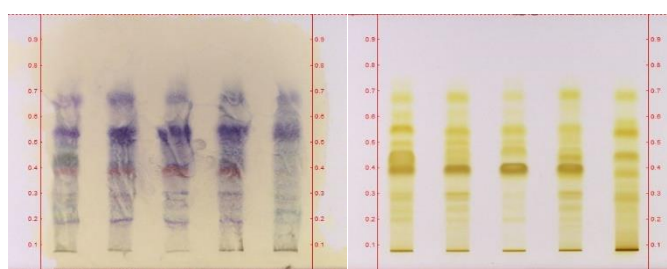
RESULTS AND DISCUSSION

Phytochemical screening by thin layer chromatography analysis

Phytochemicals are non-nutritive chemical compounds derived from plants which have some disease preventative properties. They are non-essential nutrients which are not necessarily required by the human body for sustaining life (Kalaiselvi et al., 2016). Phytochemical screening is the earlier step in finding out the chemical constituents presented in the plants, which are helpful for determination of quantitative of those chemical constituents. In the present study, the phytochemical screening of the stingless bee *G. thoracica* propolis from different locations was carried out using thin layer chromatography (TLC) analysis. TLC analysis is helpful for detection of various classes of chemical compound that presented in the samples. There were two spray reagents used for detection of compound which were vanillin-sulphuric acid and iodine. Vanillin-sulphuric acid is a universal reagent that used to detect compound from group of amines, amino acids, higher alcohols, phenols and essential oils. Meanwhile, iodine is used for detection of many organic compounds because iodine has a high affinity for both unsaturated and aromatic compounds like phenolic steroid, ester, alkaloids and polycyclic compounds.



(a) BST DGN LDG TM GM (b) BST DGN LDG TM GM



(c) BST DGN LDG TM GM (d) BST DGN LDG TM GM

Fig. 1 TLC photodocumentation of methanolic extract of *G. thoracica* propolis from different locations by visualization (a) UV 254 nm (b) UV 366 nm (c) Post derivatisation vanillin-sulphuric acid (d) Post derivatisation iodine vapour.

Based on Fig. 1 (a) and (b), the compounds were visible when TLC plate was visualized under short and long waves of UV light. Coumarins (light blue) were presented in all propolis samples when being visualized under 366 nm UV light. When the TLC plate was sprayed with vanillin as shown in Fig. 1 (c), terpenoids (purple) were presented in all propolis samples while flavonoids (pink) were only absent in sample from GM and essential oils (brown) were only presented in sample from BST. In Fig. 1 (d), the unsaturated and aromatic compounds (yellow brown) were detected in all propolis samples when the plate was stained with iodine vapour. Overall,

propolis from BST showed the presence of all compounds and contained more chemical compositions compared to propolis from other locations. This result of present study was coincided with the study reported by Ibrahim et al. (2016) that conducted on the phytochemical screening of propolis produced by stingless bee *H. itama* and *G. thoracica*. Propolis from *G. thoracica* showed the presence of terpenoids, flavonoids, essential oil, unsaturated and aromatic compounds. Previous study reported that phytochemical screening of propolis from *G. thoracica* revealed the presence of chemical constituents like terpenoids, flavonoids and stilbenes (Ibrahim et al., 2016).

The differences in phytochemical screening of compound in *G. thoracica* propolis might due to the different geographical and locations of the collected samples. A previous study by Milojković Opsenica et al. (2016) stated that chemical composition of propolis was depended on the geographical, plant source in the area from which it was collected, collecting seasons and climate factors, leading to difficulty in standardization. For example, propolis from Europe and China possessed many types of flavonoids and phenolic acid ester (Bankova et al., 2000) while in Brazilian propolis, the major components were terpenoids and prenylated derivatives of *p*-coumaric acids (Marcucci & Bankova, 1999; Tazawa et al., 1998; Tazawa et al., 1999).

Table 1 Phytochemical test of the methanolic extract of *G. thoracica* propolis from different locations of Besut (BST), Dungun (DGN), Lundang (LDG), Tanah Merah (TM) and Gua Musang (GM).

Constituents	Colour detected	BST	DGN	LDG	TM	GM	Chemical /Spray reagent
Terpenoids	Purple	+	+	+	+	+	Vanillin-sulphuric acid
Flavonoids	Pink	+	+	+	+	-	Vanillin-sulphuric acid
Coumarins	Light blue	+	+	+	+	+	UV 366 nm
Essential oils	Brown	+	-	-	-	-	Vanillin-sulphuric acid
Unsaturated and aromatic compounds	Yellow brown	+	+	+	+	+	Iodine

Remarks: +, detected; -, not detected

Total phenolic content

Polyphenols gave benefit on human health in various ways. It has been reported to exhibit anti-carcinogenic, anti-ulcer, anti-thrombic, anti-atherogenic, anti-inflammatory, anti-microbial, immune modulating, vasolidatory and analgesic effects (Wollgast & Anklam, 2000). The beneficial effects of polyphenols on human health might due to their free radical scavenging activity which could block the deleterious action of these molecules on human cells (Bobo-García et al., 2015). The total phenolics content in *G. thoracica* propolis extracts was based on Folin-Ciocalteu method. The FC reagent was consisted of phosphomolybdic/ phosphotungstic acid complexes. The method worked based on the transfer of electron from phenolic compounds in alkaline medium to form a blue chromophore consisting of a phosphotungstic/ phosphomolybdenum complex, in which the maximum absorption was depended on the concentration of the phenolic compounds (Singleton & Rossi, 1965). The total phenolics content in *G. thoracica* propolis extracts were different according to the location of collected sample (Table 2). The total phenolics content of *G. thoracica* propolis were varied from 9.23 ± 0.37 to 23.43 ± 0.50 mg GAE/g extract. Propolis from GM showed the high amount of this compound with 23.43 ± 0.50 mg GAE/g extract while propolis from LDG showed the lowest value of total phenolics content with 9.23 ± 0.37 mg GAE/g extract. There were significant differences in total phenolics content when compared

propolis samples from different locations (one-way ANOVA; $p < 0.05$). Study by Moreira *et al.* (2008) previously reported that propolis from Borneo exerted the highest total phenolics content with 329.00 ± 0.01 mg GAE/g, twice the value in Fundão propolis (151.00 ± 0.01 mg GAE/g). Another study by Kumazawa *et al.* (2004) stated that polyphenol content from Europe propolis (Bulgaria and Hungary) and China (Hebei, Hubei and Zhejiang) had the value that ranged from 200-300 mg/g of extract while Thailand propolis exerted the lowest phenolics content by 31.2 ± 0.7 mg/g of extract. The variations in value of polyphenol content in propolis might be due to the origin of the raw material (Machado *et al.*, 2016).

Total flavonoid content

Propolis has a wide variety of phenolic compounds, mainly of flavonoids. Aluminium nitrate colorimetric method was used to study the total flavonoids content in *G. thoracica* propolis. Aluminium nitrate would form acid stable complex when reacted with C-4 keto groups and either the C-3 or C-5 hydroxyl group of flavones and flavonols. It would also form acid labile complexes when reacted with the ortho-dihydroxyl groups in the A- or B- ring of flavonoid (Bhaigyabati *et al.*, 2014). Quercetin was used as a positive control due to the fact that it was a common compound of flavonoid group found in propolis. Due to the presence of its functional hydroxyl group, quercetin had strong absorbance at 415 nm even though the concentrations were lower than 100 ppm (Mohammadzadeh *et al.*, 2007). Based on Table 2, total flavonoids content of *G. thoracica* propolis were varied from 9.52 ± 0.54 to 17.22 ± 0.16 mg QE/g extract with the lowest value of flavonoids content was found in propolis from DGN and the highest one for propolis from BST. There were significant differences in total flavonoids content between propolis from LDG and GM when compared with BST, DGN and TM (one-way ANOVA; $p < 0.05$).

Table 2 Total phenolic and flavonoid contents determined by Folin-Ciocalteu and aluminium nitrate colorimetric methods.

Localities of <i>G. thoracica</i> propolis	Total phenolic contents mean \pm SD (mg GAE/g extract)	Total flavonoid contents mean \pm SD (mg QE/g extract)
BST	13.07 ± 0.62^b	17.22 ± 0.16^d
DGN	15.27 ± 0.12^c	9.52 ± 0.54^a
LDG	9.23 ± 0.37^a	13.34 ± 0.20^c
TM	17.96 ± 0.64^d	11.33 ± 0.03^b
GM	23.43 ± 0.50^e	14.06 ± 0.36^c

Means were compared by using one-way ANOVA with post hoc multiple comparisons. In each column, values with different letters (superscripts) were indicated for significant differences ($p < 0.05$).

Previous study by Ahn *et al.* (2007) reported that propolis from different locations in China showed variations in the total flavonoids content. Propolis from Gansu had the highest flavonoids content by 188 ± 6.6 mg/g of extract compared to propolis from Yunnan which had the lowest flavonoids content with 8.3 ± 3.7 mg/g of extract. Another study by Kumazawa *et al.* (2004) stated that propolis from Hungary showed the highest flavonoids content with value 176 ± 1.7 mg/g of extract while propolis from Thailand had the lowest flavonoids content with 2.5 ± 0.8 mg/g extract. The variations in total flavonoids content in propolis might be due to the differences in the preferred regional plants collected by bees. Flavonoids and other phenolic substances might act as preventive role in the development of cancer and heart disease (Kahkonen *et al.*, 1999).

DPPH free radical scavenging activity

Propolis was considered to be an abundant source of antioxidant activity due to the presence of phenolics and flavonoids compounds. The divergent varieties of propolis found throughout the world showed the variations in their antioxidant activity. Numerous studies have been conducted to determine the antioxidant activity in propolis due to its antioxidant potential. The antioxidant activity in *G. thoracica* propolis in the present study was determined by using DPPH scavenging method. The method was relied on the electron

transfer as it could assesses the scavenging potential of a given substances (Garcia *et al.*, 2012). The method would give an intense violet solution which was stable at room temperature. The colour of the purple solution would change to colorless or light yellow when DPPH solution was mixed with the tested compounds. The changes of the colour would indicate the free radical that has been scavenged (Kedare & Singh, 2011). To evaluate the antioxidant activity, the concentration of propolis used in this study was varied from 7.8125 to 500 μ g/mL with quercetin and trolox that were served as standards. From Fig. 2, propolis from BST exhibited the highest percentage of inhibition, followed closely by TM while LDG exerted the lowest percentage of inhibition.

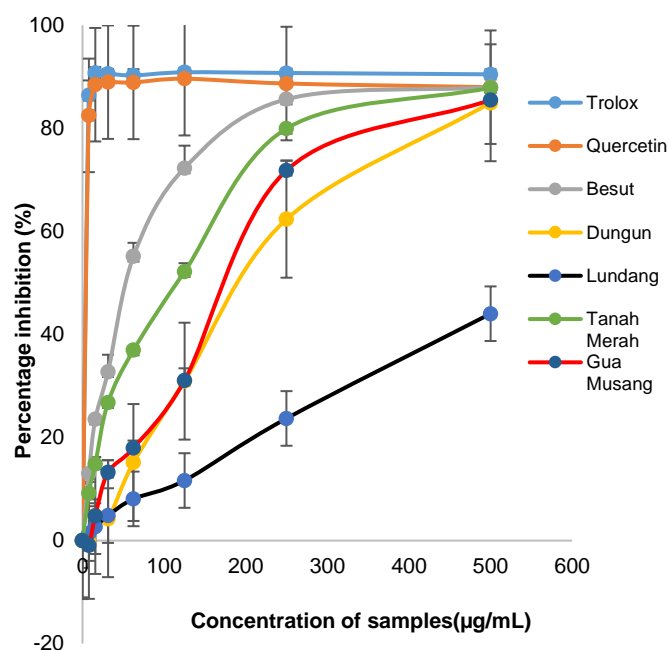


Fig. 2 Percentage of inhibition of DPPH free radical scavenging activity at different concentrations of *G. thoracica* propolis from Besut (BST), Dungun (DGN), Lundang (LDG), Tanah Merah (TM) and Gua Musang (GM).

Besides percentage of inhibition, IC_{50} values could also be determined from this assay. IC_{50} is defined as the concentration of extract required to scavenge the DPPH free radical by 50%. Propolis from BST showed lowest IC_{50} with 53 μ g/mL while the highest IC_{50} was showed by propolis from DGN with 190 μ g/mL and propolis from LDG was inactive. The lower the IC_{50} , the greater the radical scavenging activity. Thus, propolis from BST had the highest radical scavenging and antioxidant activities. These variations might due to the differences of chemical composition in the propolis extracts (Socha *et al.*, 2015). Study by Ibrahim *et al.* (2016) on the effect of propolis produced from stingless bee *H. itama* and *G. thoracica* towards the free radical scavenging activity showed that propolis from *H. itama* had stronger antiradical activity compared to propolis from *G. thoracica*. Ethanolic extracts of propolis from Italy and Russian possessed similar antioxidant activity due to the similar composition of phenolic compounds while Brazilian propolis had weak antioxidant activity due to low phenolic composition (Fabris *et al.*, 2013). The free radical scavenging activity was appeared to correlate with the presence of phenolic compounds and in particular to flavonoids which were the most abundant and effective antioxidant compounds (Kumazawa *et al.*, 2004; Moreno *et al.*, 2000; Isla *et al.*, 2001). Previous study by Yang *et al.* (2011) found that antiradical activity was depended on the compound presented in the propolis for example *p*-coumaric acid and isoferulic acids exerted higher antiradical activity towards DPPH. Propolis that collected from Anhui, China inhibited strong free radical scavenging activity and ferric reducing activity due to the presence of caffeic acid, phenethyl caffeate, cinnamyl caffeate

and benzyl caffeate (Yang *et al.*, 2011). Another study reported that methanolic extracts of Algerian propolis had strong scavenging activity and ferric reducing activity, those activities were influenced by the presence of high amounts of caffeic acid esters and flavanones, kaempferol and galanggin (Piccinelli *et al.*, 2013). Ethyl acetate extract of Kangaroo Island propolis which enriched with stilbenes also possessed a stronger scavenging activities (Abu-Mellal *et al.*, 2012). The chemical structures of compound were also contributed to the difference in antioxidant and antiradical activities of propolis extracts. The most active flavonoids, which were quercetin and kaempferol contained five or four hydroxyl groups which contributed to the antioxidant properties while galangin was less active with only three hydroxyl groups (Kumazawa *et al.*, 2004; Ahn *et al.*, 2009).

CONCLUSION

In this study, the results of phytochemical screening and determination of total phenolic content, total flavonoids content and their antioxidant activity in methanolic extracts of *G. thoracica* propolis from different locations were presented. *G. thoracica* from Besut (BST) showed various compounds in phytochemical screening and exerted highest total flavonoids content, as well as exhibited lowest IC₅₀ for DPPH free radical scavenging activity which indicated for a good source of antioxidant due to its antioxidant properties. Further investigation on identification of the compounds in propolis with their biological activity should be carried out for propolis standardization.

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