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Isolation and Identification of Potential Antineoplastic Bioactive Phenolic Compounds in Malaysian Honeys

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ABSTRACT

The role of honeys as chemopreventive agents was inferred from their phenolic composition. The present study aims to isolate and identify such phenolic compounds with anticarcinogenic potential from the sugar matrix of Malaysian honeys. Phenolic compounds in Malaysian Acacia, Gelam and Tualang honey samples were isolated using octadecyl (C18) silica solid phase extraction (SPE) technique and identification was performed using high-performance liquid chromatography (HPLC) with diode array detector (DAD). Identification of phenolic compounds was achieved by comparing chromatographic retention times of honey samples with those of authentic standard compounds. HPLC analysis confirmed the presence of anticancer phenolic compounds in all honey samples with considerable variation observed among both different and the same types of honey. Six flavonoids (quercetin, naringenin, kaempferol, rutin, hesperetin, and apigenin), two phenolic acids (*p*-coumaric acid, and ferulic acid) and two tannins (ellagic acid, and penta-*O*-galloyl-β-D-glucose [PGG]) were the bioactive anticancer compounds identified. The presence of PGG in Malaysian honey was described for the first time. This study concludes that these three types of Malaysian honey possessed anticancer properties at varying degrees. Their potential usage as natural anticancer therapeutic agents with numerous health benefits could be further explored and considered as an alternative for current anticancer drugs.

INTRODUCTION

Honey is a natural product rich in phytochemicals. A phytochemical is a substance derived from a plant source which could be any of various vitamins, minerals or bioactive compounds (Simon, 2002). Previously, honey has been consumed as a dietary supplement or used as a topical aid to cure some ailments. These days, more of its functional properties have been discovered. Evidences from *in vitro*, *in vivo* as well as clinical trials have proven that honeys harvested from places all over the world exhibit various therapeutic properties, such as anti-inflammatory, antioxidant, antimicrobial, and antidiabetic effects, with recent highlight placed on its antineoplastic (antiproliferative and anticarcinogenic) activity (Jaganathan and Mandal, 2009; Abubakar *et al*., 2012; Othman, 2012; Erejuwa *et al*., 2014). Reported studies have revealed that crude honeys are promising chemopreventive agents (Swellam *et al*., 2003; Fauzi *et al*., 2011; Kadir *et al*., 2013). These findings have attracted much interest among health practitioners, survival and in-treatment cancer patients, considering the side effects of current cancer drugs and emergence of drug resistance (chemoresistance) as well as radioresistance in cancer treatment that have triggered massive continuous searches for alternative ways in treating cancer patients.

Cancer chemoprevention is defined as the use of natural, synthetic, or biologic chemical agents to reverse, suppress, or prevent carcinogenic progression (Sporn, 1976; Surh, 2003). Cancer is a multistep process that comprises of three key stages: initiation, promotion and progression (Surh, 2003; Fresco *et al*., 2006; Othman, 2012; Erejuwa *et al*., 2014). Initiation is the first stage of carcinogenesis, a rapid and irreversible process that is characterized by the accumulation of mutated deoxyribonucleic acid (DNA) upon exposure to carcinogens (cancer-causing agents). Promotion stage, in contrast to initiation stage, is a lengthy process in which mutated (preneoplastic) cells actively proliferate and accumulate. The last stage, progression, is when the cancer cells

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metastasize (disseminate) to distant sites such as tissues and organs through the lymphatic system and bloodstreams (Surh, 2003; Erejuwa *et al*., 2014). Therefore, cancer chemopreventive agents are substances that could prevent, arrest or reverse the cancer development at various stages. Basically, there are two main categories of chemopreventive agents based on their mechanisms of action-blocking agents and suppressing agents. Blocking agents can impede initiation by either preventing carcinogens from reaching the target sites, from undergoing metabolic activation or from interacting with crucial cellular target molecules such as DNA, ribonucleic acid (RNA) or proteins. Suppressing agents, in turn, inhibit the expression of initiated cells in either the stages of promotion or progression (Surh, 2003; Fresco *et al*., 2006).

It is postulated that the role of honey as a chemopreventive agent was attributed from its bioactive composition, particularly the phenolic compounds (polyphenols) (Jaganathan and Mandal, 2009; Abubakar *et al*., 2012; Erejuwa *et al*., 2014). As a phytochemical-rich product, honey possesses a range of phenolic compounds, whose source originates from where bees forage for nectars and pollens. This natural phenomenon is one of the key factors that differentiate one type of honey from another, and even from one hive to another in the same location. It has been reported that honey comprises of phenolic acids and flavonoids as the major phenolic compounds (Jaganathan and Mandal, 2009; Abubakar *et al*., 2012). Phenolic acids namely ferulic, caffeic, *p*-coumaric acids and flavonoids including naringenin, hesperetin, apigenin, kaempferol, rutin, chrysin and quercetin are among the polyphenols that are present in honey (Jaganathan and Mandal, 2009; Abubakar *et al*., 2012; Erejuwa *et al*., 2014), and have been identified as the promising chemopreventive agents (Fresco *et al*., 2006; Gao *et al*., 2006; Alshatwi *et al*., 2013; Chen *et al*., 2013; Jaganathan *et al*., 2013). Tannins such as ellagic acid and penta-*O*-galloyl-β-D-glucose (PGG) are another class of polyphenols that have been reported to exhibit anticarcinogenic properties as well (Abubakar *et al*., 2012; Zhang *et al*., 2009). All these polyphenolic compounds were observed to act through one or more combinations of several mechanisms; either by inducing apoptosis (cell death), arresting cell cycle, inhibiting cell growth and growth factor (GF) expression or through promoting DNA repair mechanism (Surh, 2003; Fresco *et al*., 2006; Gao *et al*., 2006; Jaganathan and Mandal, 2009; Zhang *et al*., 2009; Abubakar *et al*., 2012; Othman, 2012; Alshatwi *et al*., 2013; Chen *et al*., 2013; Jaganathan *et al*., 2013; Erejuwa *et al*., 2014). Previously, column chromatography was the most popular method available for compounds separation. In recent years, SPE has been used in many experimental designs and is considered as an acceptable alternative to the conventional column chromatography. Both methods perform the separation (purification) of individual chemical compounds from mixtures of compounds. Both methods have been reportedly utilized in the isolation of honey phenolic compounds. For this purpose, C18 silica membrane disks and non-ionic polymeric Amberlite XAD resins were commonly employed in SPE (Aljadi and Yusoff, 2003; Michalkiewicz *et al*., 2008; Hussein *et al*., 2011; Khalil *et al*., 2011) and column chromatography (Ferreres *et al*., 1994; Yao *et al*., 2003; Kassim *et al*., 2010), respectively. Similarly, HPLC has been used widely for the separation and characterization of phenolic compounds in the last two decades, where both isocratic and gradient elution have been applied for analyses of phenolic compounds (Stalikas, 2007). Malaysian Tualang and Gelam honeys have been reported to exhibit anticarcinogenic properties against different types of cancer cell lines and in *in vivo* studies (Fauzi *et al*., 2011; Abubakar *et al*., 2012; Wen *et al*., 2012; Kadir *et al*., 2013), but limited published data was available for Malaysian Acacia honey. Thus, this work aimed to isolate and identify phenolic compounds with anticarcinogenic potential from the sugar matrix of these three types of Malaysian honeys in order to verify their roles as prospective natural chemopreventive agents.

MATERIALS AND METHODS

Collection of honey samples

Three types of Malaysian honeys (Gelam [*Melaleuca cajuputi*], Acacia [*Acacia mangium*] and Tualang [multifloral] honeys) were collected starting in December 2012 until February 2013 (Table 1). The locations of honey collection are shown in Figure 1. All seven samples were stored in the dark at room temperature before analysis.

Table 1: Honey samples involved in this study.

Honey samples[a]	Type	Botanical origin	Local name
G1	Monofloral	*Melalueca cajuputi*	Gelam
G2	Monofloral	*Melalueca cajuputi*	Gelam
T1	Multiforal	Mixed source	Tualang
T2	Multiforal	Mixed source	Tualang
A1	Monofloral	*Acacia mangium*	Acacia
A2	Monofloral	*Acacia mangium*	Acacia
A3	Monofloral	*Acacia mangium*	Acacia

[a] G = Gelam honey, T = Tualang honey, A = Acacia honey.

Reagents and chemicals

Analytical grade hydrochloric acid (HCl) (J.T.Baker, PA, USA), analytical grade methanol, HPLC-grade methanol and formic acid (Merck KgaA, Darmstadt, Germany), and ultrapure water (Easypure Barnstead, Thermo Scientific Inc., USA) were used for sample pre-treatment and mobile phase preparation. Seventeen reference standard compounds were purchased for identification of polyphenols in studied honey samples. Naringenin was purchased from Sigma-Aldrich Co. (Steinheim, Germany). Caffeic acid, syringic acid, ellagic acid, *p*-coumaric acid, rutin hydrate, kaempferol, apigenin, quercetin, (-)-catechin, chrysin, *trans*-cinnamic acid, luteolin, and PGG were purchased from Sigma Chemicals Co. (St. Louis, MO, USA) whereas hesperetin, benzoic acid, and *trans*-ferulic acid were supplied by Fluka (Buchs, Switzerland).

Isolation of phenolic compounds using solid phase extraction (SPE)

The extraction was carried out as described by Hussein *et al*. (2011) with modifications. Five grams of honey sample were

dissolved in 25 mL of acidified deionised water (adjusted to pH 2 with diluted HCl), stirred for 30 minutes, and filtered using Whatman No. 1 filter paper to remove solid particles. Eight milliliters of methanol and 4 mL of 0.01 M HCl were applied sequentially to precondition C18 SPE cartridges (Agilent Bond Elut C18, 500 mg, 6 mL). The filtrate was passed through the preconditioned C18 column and washed with 125 mL of 0.01 M HCl to remove sugars. The adsorbed compounds were eluted with methanol (150 mL) followed by evaporation until a dry state is reached under reduced pressure at 40 ˚C using a rotary evaporator with a water bath.

Sample extract and standard compound preparation

Prior to injection into an HPLC system, the residues were dissolved in HPLC grade methanol (1mL), filtered through 0.45 µm syringe filters and concentrated to 0.5 mL. The mixtures of seventeen reference standard compounds (100 µg/mL each), also prepared in HPLC grade methanol were filtered through 0.2 µm syringe filters prior HPLC analysis.

Identification of phenolic compounds using high-performance liquid chromatography (HPLC)

The chromatographic identification of phenolic compounds of interest was performed on Agilent 1100 series HPLC system (Agilent Technologies, USA) equipped with multichannel diode array detector (G1315B), on-line degasser (G1379A), quatpump (G1311A), autosampler (G1313A), and column heater (G1316A). Instrument control and data analysis were performed using Agilent HPLC Chemstation. The analytical column was a reversed-phase column ZORBAX Eclipse XDB-C18 (4.6 mm x 150 mm, 5 µm, Agilent Technologies, USA). The binary mobile phase consisted of 0.25% formic acid and 2% methanol in ultrapure water (solvent A) and 100% methanol (solvent B), as described by Hussein *et al*. (2011). Elution from the column was achieved with the following gradient: time 15 min: 10% B; time 18 min: 15% B; time 20 min: 20% B; time 23 min: 30% B; time 25 min: 35% B; time 28 min: 40% B; time 30 min: 45% B; time 40 min: 60% B. The final composition was kept constant up to 50 min. Prior to subsequent injection, the system was equilibrated for 10 minutes. The system was operated at temperature, flow rate and an injection volume of 28 ˚C, 1 mL/min and 20 µL, respectively. Detection of phenolic compounds was performed using UV absorption spectra monitoring at 290 nm and 340 nm. The flavonoids, phenolic acids and tannins were identified by comparing the chromatograms and retention times (RTs) of the analytes with reference standards.

RESULTS

The phenolic compounds with anticarcinogenic properties identified in seven Malaysian honey samples are presented in Table 2. In this study, two phenolic acids (*trans*-ferulic and *p*-coumaric acids), six flavonoids (rutin, hesperetin, quercetin, naringenin, kaempferol and apigenin) and two tannins (PGG and ellagic acid) were detected either at 290 nm and/or 340 nm wavelengths. Figure 2 depicts the HPLC chromatograms of the analysed Malaysian Gelam, Tualang and Acacia honey samples. *Trans*-ferulic acid, found in all analysed honey samples, is seen as the most common phenolic compound regardless of honeys' geographical location, botanical origin, and time of collection. Other compounds common to Gelam honey samples (G1 and G2) were hesperetin, kaempferol, and rutin, whereas *p*-coumaric acid was observed in both Tualang honey samples (T1 and T2). Acacia honey samples (A1, A2 and A3) contained naringenin, quercetin, and *p*-coumaric acid as another common polyphenols (Table 2).

From the HPLC chromatograms of three Acacia and two Gelam honey samples, it was shown that monofloral Acacia and Gelam honeys are rich in polyphenols as compared to multifloral Tualang honeys (Figure 2), in which an increasing number of peaks and relatively high peak intensities were observed for these two types of monofloral honeys. The polyphenols detected in this study were mostly in the form of aglycones instead of glycoside conjugates. Interestingly, two out of three Malaysian Acacia honey samples studied (A2 and A3) exhibited the presence of PGG at relatively low concentrations (Figure 2c). The presence of more unidentified peaks, especially in Acacia and Gelam honeys, indicates that more sensitive instruments such as liquid chromatography (LC) coupled with mass spectrometry (MS) is needed to further identify those compounds. Figure 3 depicts chromatograms of authentic standard compounds at both 290 nm and 340 nm wavelengths, where some compounds were better detected at 290 nm and vice versa.

Table 2: Phenolic acids, flavonoids and tannins detected in different types of Malaysian honey samples using HPLC analysis.

Compound	RT (min) at 290nm[a]	SD[b]	Sample[c]	RT (min) at 340nm[a]	SD[b]	Sample[c]
p-Coumaric acid	26.887	0.059	G1, T1, T2, A2, A3	26.887	0.059	G1, A1, A2, A3
Ferulic acid	28.244	0.056	G1, G2, T1, T2, A1, A3	28.244	0.056	T1, T2, A1, A2, A3
Penta-*O*-galloyl-β-D-glucose (PGG)	28.606	0.034	A2, A3	-	-	
Rutin	31.830	0.062	G2, A2	31.830	0.062	G1, G2, A1, A2
Ellagic acid	32.703	0.078	T1, T2, A1	32.704	0.077	G1, A1, A3
Quercetin	37.144	0.100	A1, A2	37.143	0.098	A1, A2, A3
Naringenin	37.474	0.095	G1, T1, A1, A2, A3	37.471	0.094	G1, T1, A1, A2, A3
Hesperetin	38.575	0.091	G1, G2	38.412	0.111	G1, G2, T1
Kaempferol	40.588	0.109	G1, T2	40.588	0.109	G2, A1, A2
Apigenin	41.177	0.115	G1, T1	41.177	0.115	G1

[a] RT = retention time, based on mixtures of standard compounds (100µg/mL each). Values are mean of duplicate analysis.
[b] SD = standard deviation, (n = 2). [c] G = Gelam honey, T = Tualang honey, A = Acacia honey.

Table 3: Anticarcinogenic mechanisms of action of polyphenols on cancer cells.

Cancer research	Phenolic compound[a]	Cell line (cancer)	Action[b]
Jaganathan *et al.* (2013)	*p*-Coumaric acid	HCT-15 (colon)	Induce apoptosis
Chen *et al.* (2013)	Rutin	LAN-5 (neuroblastoma)	G2/M arrest, induce apoptosis
Alshatwi *et al.* (2013)	Hesperetin	SiHa (cervical)	Induce apoptosis
Gao *et al.* (2006)	Naringenin	LNCaP (prostate)	DNA repair
Hagiwara *et al.* (2010)	Ellagic acid	HL-60 (leukemia)	Induce apoptosis
Hu *et al.* (2008)	PGG	LNCaP (prostate)	Induce apoptosis
Chai *et al.* (2010)	PGG	MCF-7 (breast)	G1 and S arrest
Gonzalez-Mejia *et al.* (2010)	Apigenin	THP (leukemia)	Induce apoptosis
Bestwick *et al.* (2007)	Kaempferol	HL-60 (leukemia)	Cell cycle arrest, inhibit growth
Lee *et al.* (2006)	Quercetin	U937 (leukemia)	Induce apoptosis, cell cycle arrest
Luo *et al.* (2008)	Quercetin	OVCAR-3 (ovarian)	Inhibit VEGF expression
Kampa *et al.* (2004)	Ferulic acid	T47D (breast)	Inhibit growth

[a] PGG = Penta-*O*-galloyl-β-D-glucose.
[b] VEGF = Vascular endothelial growth factor.

Fig. 1: Location of honey collection.

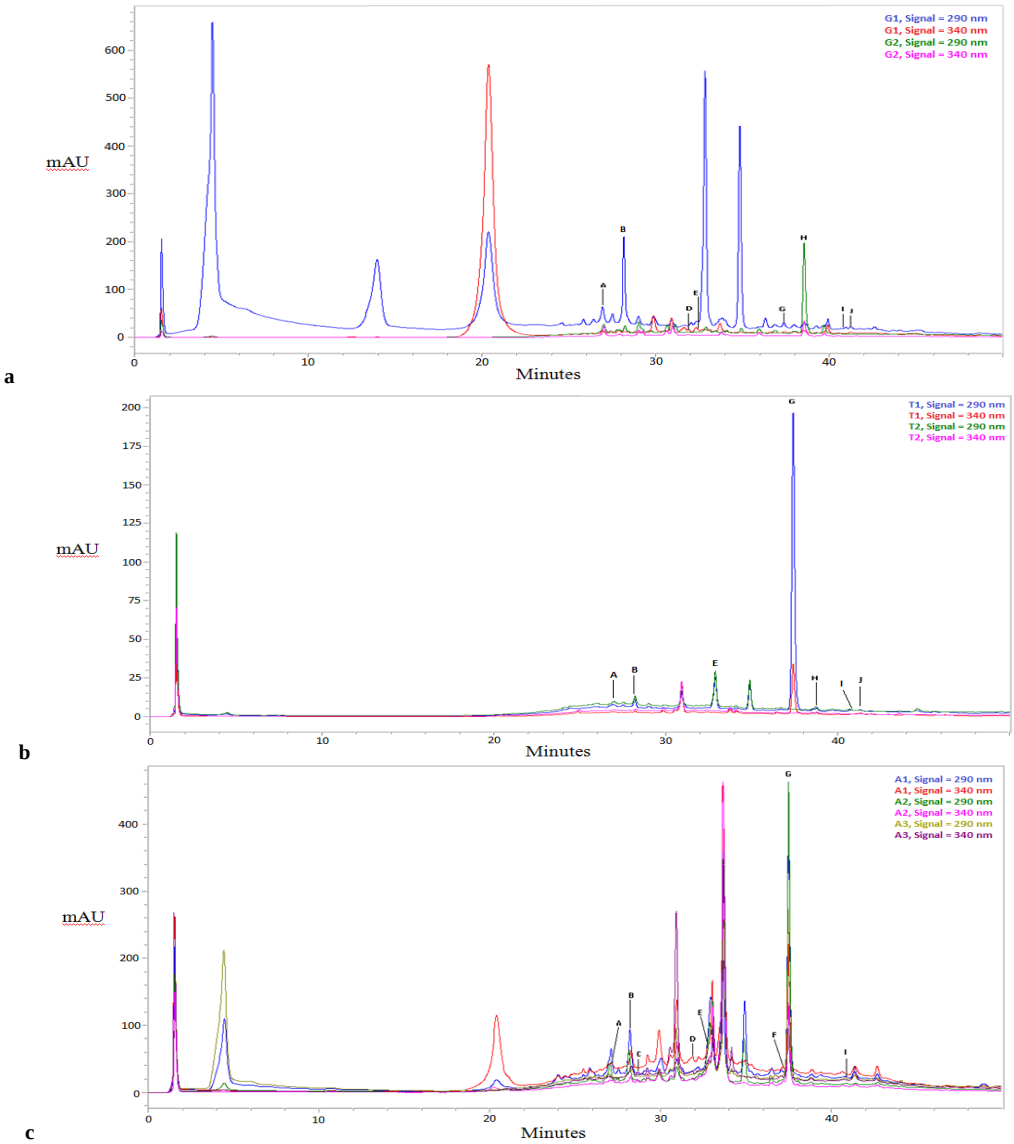

Fig. 2: HPLC chromatograms of Malaysian honeys: (a) Gelam, (b) Tualang and (c) Acacia at both 290 nm and 340 nm. Phenolic compounds identified include: A (*p*-coumaric acid), B (*trans*-ferulic acid), C (PGG), D (rutin), E (ellagic acid), F (quercetin), G (naringenin), H (hesperetin), I (kaempferol), and J (apigenin).

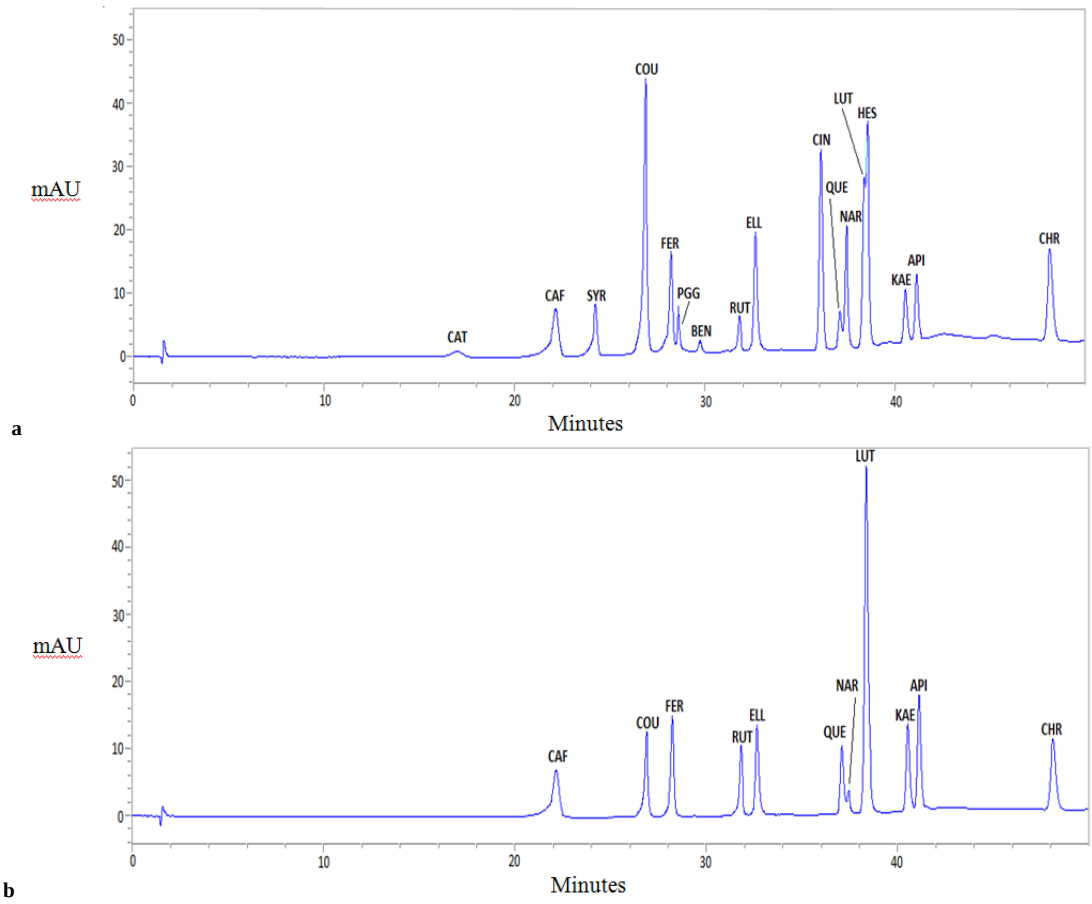

Fig. 3: HPLC chromatograms of polyphenolic standard compounds (100µg/mL) at two wavelengths: (a) 290 nm and (b) 340 nm. Phenolic compounds are: CAT (catechin), CAF (caffeic acid), SYR (syringic acid), COU (*p*-coumaric acid), FER (*trans*-ferulic acid), PGG (penta-*O*-galloyl-β-D-glucose), BEN (benzoic acid), RUT (rutin hydrate), ELL (ellagic acid), CIN (*trans*-cinnamic acid), QUE (quercetin), NAR (naringenin), LUT (luteolin), HES (hesperetin), KAE (kaempferol), API (apigenin), and CHR (chrysin).

DISCUSSION

Table 3 shows the summary of polyphenolic compounds detected in current work with their anticarcinogenic mechanisms of action on various types of cancer cells. It was demonstrated earlier that quercetin, kaempferol, hesperetin, ellagic, ferulic and *p*-coumaric acids were present in Malaysian Gelam honey samples isolated using C18 silica SPE (Aljadi and Yusoff, 2003; Hussein *et al*., 2011; Wen *et al*., 2012), and Amberlite XAD-2 resin column chromatography (Kassim *et al*., 2010). All of these compounds were seen in Gelam honeys analysed in this study, except for quercetin (Table 2). In addition, naringenin, and apigenin have been detected in the Gelam honeys in this study, as well as in an earlier study performing C18 silica SPE (Khalil *et al*., 2011). Interestingly, rutin was not detected previously (Hussein *et al*., 2011; Wen *et al*., 2012) but was present in Gelam honey samples from this study. Gelam honeys exhibited several other polyphenols which are either not analysed or absent in current work, namely phenolic acids (gallic, benzoic, cinnamic, caffeic, chlorogenic) and flavonoids (myricetin, luteolin, chrysin and catechin) (Aljadi and Yusoff, 2003; Kassim *et al*., 2010; Hussein *et al*., 2011; Khalil *et al*., 2011; Wen *et al*., 2012).

In view of the presence of these polyphenols in Gelam honeys, it could be inferred that Malaysian Gelam honey is a promising chemopreventive agent. This claim is strongly supported by the study done by Jubri *et al*. (2012) whom demonstrated that Gelam honey reduced the proliferation rate of HepG2 liver cancer cells by inducing apoptosis at a low dose without affecting normal liver cells (WRL-68), which was observable through cell shrinkage. It was also found that Gelam honey suppressed growth of HT29 colon cancer cells by inducing DNA damage which leads to subsequent apoptosis, and suppressing inflammation (Wen *et al*., 2012). In fact, oxidative DNA damage with subsequent apoptosis has been associated with the possible mechanisms of action of chemotherapy drugs and chemopreventive herbs in killing cancer cells (Wen *et al*., 2012).

Tualang honey, a mixed floral deep jungle honey, has been extensively studied so far and proven to possess anticancer activities and a potential role as an adjuvant with current cancer drugs and chemotherapy. Malaysian Tualang honey has been shown to exhibit antiproliferative effect on oral squamous cell carcinomas (OSCC) and human osteosarcoma (HOS) cell lines by inducing early apoptosis in dose- and time-dependent manners (Ghashm *et al*., 2010). In addition, Tualang honey was found to

induce apoptosis of human breast (MCF-7 and MDA-MB-231) and cervical (HeLa) cancer cell lines via depolarization of mitochondrial membrane (Fauzi *et al*., 2011). The study observed that Tualang honey displayed non-cytotoxic effect on normal breast epithelial cell lines (MCF-10A), suggesting that Tualang honey has an advantage over current anticancer agents. On top of that, an *in vivo* study revealed that Tualang honey exerted positive modulation effects on carcinogen 7,12-dimethylbenz(α)anthracene (DMBA)-induced breast cancers in rats in which; late development, smaller size and lesser number of tumor nodules (benign lesions) were the characteristics of Tualang honey-treated groups in comparison to control groups (Kadir *et al*., 2013). Thus, the study concluded that Tualang honey was a possibility when considering its use as a prophylactic or therapeutic agent to combat breast cancer growth or as an adjuvant to conventional chemotherapy (Kadir *et al*., 2013). A more recent study demonstrated that a combination of Tualang honey with Tamoxifen (TAM, a drug for breast cancer treatment) was more potent towards both MCF-7 and MDA-MB-231 breast cancer cell lines, suggesting that Tualang honey is a potential adjuvant to be used with TAM, provided that it reduces effective dose of TAM and simultaneously reduced TAM-induced adverse effects (Yaacob *et al*., 2013).

On the other hand, the contributory bioactive components that play vital roles in Tualang honey anticarcinogenic activities are yet not so clear. For that reason, the present and previous works have determined several polyphenols that might be responsible for Tualang honey anticarcinogenic properties. In this study, *trans*-ferulic acid, *p*-coumaric acid, ellagic acid, naringenin, hesperetin, kaempferol and apigenin were detected in Malaysian Tualang honey samples (Table 2). Previous studies have reported the presence of gallic, syringic, benzoic, *p*-coumaric, chlorogenic, caffeic and *trans*-cinnamic acids, naringenin, kaempferol, quercetin as well as catechin in Tualang honey samples (Khalil *et al*., 2011; Chua *et al*., 2013). Hesperetin in Tualang honey was first described in this study as it was not detected previously (Khalil *et al*., 2011). Besides, it was observed that Tualang honey exhibited less number of peaks of compounds as compared to Gelam and Acacia honeys (Figure 2). This finding suggests that the *in vitro* and *in vivo* anticancer effects of Tualang honeys could be partly attributed from their polyphenolic composition with contribution of other compounds as well. Several other compounds have been associated with honey's antioxidant properties including enzymes (glucose oxidase, catalase), ascorbic acid, organic acids, amino acids, carotenoid-like substances, and proteins (Jaganathan and Mandal, 2009).

In contrast, Malaysian Acacia honey (also known as Borneo tropical honey) has not yet gained much attention. A previous study reported that caffeic acid and catechin were the compounds detected in Acacia honey (Khalil *et al*., 2011). However, in comparison to the only data available (Khalil *et al*., 2011), findings of the current work exhibited that Malaysian Acacia honeys were found to be rich in polyphenols. To describe, eight out of ten analysed phenolic compounds were detected in Acacia honey samples examined in this work (Table 2). PGG, which is a naturally occurring gallotannin commonly found in medicinal herbs such as *Paeonia lactiflora* (Lee *et al*., 2004), and has been identified as a potential anticancer agent candidate (Lee *et al*., 2004; Zhang *et al*., 2009), was detected in Acacia honey samples in present work. This is the first time PGG is reported to be found in Malaysian honey. Therefore, presence of PGG together with other polyphenols in Malaysian Acacia honey supported its potential role in cancer chemoprevention. Previously, Italian Acacia honey has been demonstrated to induce cytotoxicity in human (A375) and murine (B16-F1) melanoma cell lines. The study affirmed that the observed effects of studied Acacia honey was mainly due to the presence of chrysin (5,7-dihydroxyflavone) (Pichichero *et al*., 2010). However, present study did not detect chrysin in analysed Acacia honey samples.

Detection of phenolic compounds was done using UV absorption spectra monitoring at 290 nm and 340 nm, taking into account that the majority of honey phenolics and flavonoids exhibit maximum UV absorption around these two wavelengths (Martos *et al*., 1997). In comparison to the findings obtained by Hussein *et al*. (2011), it was observed that modification made for HPLC analysis in current work has managed to obtain well-separated peaks of compounds of interest within a shorter run time (Figure 3). The reported study performed an analysis in 73 minutes while current work completed one analysis in 60 minutes. Total of seventeen authentic standard compounds have been separated using the HPLC-DAD system in the present study (Figure 3). However, only ten of these compounds were present in both the analysed honey samples and exhibited anticancer activity (Table 2). Therefore, this study is focusing on only these ten compounds. Additionally, the current work also successfully detects compounds with high peak intensity which is observable from the chromatograms. Previous study by Kassim *et al*. (2010) also showed peaks of compounds at higher intensities, but the amount of honey sample used for analysis was 100 g, which is 20 times higher than in the current work. Conversely, lesser peak intensities were demonstrated in the study by Khalil *et al*. (2011) with the same amount of honey sample used as in present study (5 g).

Still, direct comparisons between current findings and reported studies on phenolic composition of Malaysian honeys were limited due to different samples used, as well as extraction, isolation and HPLC procedures. Unavailability of reference standard compounds for comparison also limits the identification of all peaks of compounds that appear in the chromatograms. However, this study suggests that the presence of similar bioactive phenolic compounds in the same type of honey, despite the different extraction, isolation and HPLC procedures applied, could be regarded as a way of confirming the polyphenolic profile of that type of honey. Furthermore, it should be emphasized that similar types of honey in terms of floral source may vary greatly in phenolic compositions and proportions depending on geographical location, climatic conditions and bee species.

CONCLUSION

The presence of identified phenolic compounds in Malaysian Gelam, Tualang and Acacia honeys at varying degrees is therefore supporting their prospective roles as natural cancer chemopreventive agents. From the present findings, it could be deduced that most of the polyphenols found in analysed Malaysian honeys have the ability to induce apoptosis of cancer cells. This is coherent with the reported observations where induction of apoptosis was observed when Malaysian honeys were introduced to cancer cells. It is suggested that combination of honey with cancer drugs during chemotherapy could help to reduce the effective dose and side effects of drugs, as well as inhibit progression of cancer cells. Hence, the results of present study encourage more *in vivo* studies and clinical trials to be conducted to further evaluate the efficacy of Malaysian honeys in cancer chemoprevention.

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