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STUDIES ON THE ANTIOXIDANT PROPERTIES OF TUALANG HONEY OF MALAYSIA

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Abstract

Honey has been used since ancient times for its nutritional as well as curative properties. Tualang honey is collected from wild honey bees' hives on Tualang trees found in the Malaysian rain forest. It has been used traditionally for the treatment of various diseases, where its therapeutic value has partly been related to its antioxidant properties. This study therefore assessed the colour intensity, total phenolic content, antioxidant activity and antiradical activity of gamma irradiated Tualang Honey. The colour intensity at ABS_{450} was 489.5 ± 1.7 mAU, total phenolic content was 251.7±7.9 mg $_{gallic\ acid}$ /Kg honey, total antioxidant activity by FRAP assay was 322.1±9.7 (µM Fe(II)) and the antiradical activity by DPPH assay was 41.30 ± 0.78 (% inhibition). The data confirms that the antioxidant properties of gamma irradiated Tualang honey are similar to other types of honeys reported in the literature.

Key words: Tualang honey, Malaysia, Total antioxidant activity, Gamma radiation, Phenolic content, DPPH assay.

Introduction

Oxidative stress has been implicated in the development of many chronic diseases (Halliwell et al., 1992). The therapeutic role of honey in the treatment of various ailments has been receiving considerable attention recently, and its therapeutic value has been partly attributed to its antioxidant properties (Aljadi and Kamaruddin 2004; The National Honey Board, 2003; Gheldof and Engeseth, 2002). In Malaysia, Tualang honey has been used in the local community for the treatment of various diseases. The content and composition of the different types of honey vary with different floral sources as well as climatic and environmental conditions (Gheldof et al., 2002; Aljadi and Kamaruddin 2004; Küçük et al., 2007). Studies on the antioxidant activity of different types of honey from different countries and different botanical origins have been carried out (Aljadi and Kamaruddin 2004; Al-Mamary et al., 2002; Beretta et al.,2005; Estevinho et al., 2008; Socha et al., 2009), but the antioxidant properties of the local Tualang honey, have not been well documented in terms of above parameters.

Honey is frequently contaminated with various microorganisms during harvesting and packaging. In order to use honey for research in medicine, a suitable method of sterilization like gamma irradiation is highly recommended. The aim of this present study was to assess the antioxidant properties of gamma-irradiated Tualang honey of Malaysia (irradiated with 25 kGy) by using a combination of tests including colour intensity, phenolic contents, anti radical activity and total antioxidant activity (Beretta et al., 2005).

Materials and Methods

The Tualang Honey used in this study was supplied by Federal Agricultural Marketing Authority (FAMA), Malaysia. It was harvested from *Apis dorsata* bees' nectar on the Tualang tree in the Rain Forest of Kedah in Peninsular Malaysia in March 2008. The honey had been previously filtered to remove solid particles, concentrated (20% w/v water) by oven drying at 40^0C by FAMA, Malaysia and subjected to gamma irradiation at 25 kGy at Sterilgamma (M) Sdn. Bhd. (Selangor, Malaysia) prior to submitting to us for analysis. All the chemicals and solvents used were of analytical grade.

Assays for in vitro antioxidant properties of Tualang Honey
Color intensity: Abs 450 (Beretta et al., 2005)

Tualang honey was diluted to 50% (w/v) with warm water (45–50 ◦C), vortex-mixed for 5 mins and then filtered (0.45µm pore size, AGILENT TECHNOLOGIES, MILAN, ITALY) to eliminate large particles. The net absorbance was defined as the difference between spectrophotometric absorbance at 450 and 720 nm.

Phenol content (PC)

The total phenol content was determined with Folin's reagent and the result was expressed as mg gallic acid /Kg honey (Beretta et al., 2005). Tualang honey was first mixed with warm distilled water (500 mg/5mL water), and vortex-mixed for 5 mins. Then 100 microlitre of the solution, corresponding to 10 mg of honey was added to 1mL of Folin-Phenol reagent (SIGMA, USA) [pre diluted with distilled water (1:10)]. The mixture was vortex-mixed for 2 mins, and was then transferred into a 1.5mL cuvette (1 cm path). The absorbance was determined against a blank on a spectrophotometer. The blank consisted of honey solution with distilled water to eliminate honey colour interference. The solutions with gallic acid (SIGMA, USA; dissolved in methanol/water: 1:1) concentrations in the range of 10-250µg/ml were used for calibration.

Antiradical activity: DPPH assay

The scavenging activity against 1,1-diphenyl-2-picrylhydrazil (DPPH; SIGMA, USA) radical was used in this study (Chen et al., 2000; Aljadi and Kamaruddin, 2004). Briefly, 0.75ml of the honey solution (0.1g/ml) in warm water was mixed with 1.5ml of 0.09mg/ml DPPH in methanol. The mixture was then incubated at 25ºC in a water bath for 5 mins after which the absorbance was measured at 517nm against a blank sample consisting of honey solution with distilled water. The absorbance of a radical blank was also measured using 0.75ml of distilled water. The radical scavenging activity (RSA) of honey was expressed in terms of percentage inhibition of DPPH radical by honey and was calculated (Batrušaitytė et al., 2007) as follows:

RSA ($DPPH^{\cdot}$ Inhibition, %) = $[(A_B-A_T)/A_B] \times 100$

Where, A_B = Absorbance of radical blank ($DPPH^{\cdot}$ without honey)

A_T = Absorbance of test sample ($DPPH^{\cdot}$ with honey)

Total antioxidant activity: FRAP assay

The reducing ability of honey was determined by FRAP assay (Benzie and Strain, 1999; Beretta et al., 2005) with some modifications. Briefly, working FRAP reagent was prepared by mixing 10 volumes of 300 mmol/L acetate buffer, pH 3.6 with 1 volume of 10mmol 2,4,6-tripyridyl-s-triazine (TPTZ; SIGMA, USA) in 40mmol/L hydrochloric acid and with 1 volume of 20mmol/L ferric chloride. Two hundred µl of honey solution (0.1g/ml) was added to a test tube containing 1.5ml of freshly prepared FRAP reagent. The mixture was subsequently incubated at 37ºC for 4 mins after which the absorbance value were measured at 593nm against a reagent blank (200 µl of distilled water). The difference between this absorbance and the sample blank (honey solution with distilled water), was calculated to get the final absorbance. Aqueous solutions of known Fe^{II} concentration, in the range of 100-1000 µmol/L (FeSO4.7H2O) were used for calibration. The reducing ability of honey was expressed as µM of Fe^{II} equivalent/L.

Data presentation

All the determinations were conducted in quadruplicate from a single honey sample. Values are expressed as mean ± standard deviation.

Results and Discussion

Honey contains many compounds that can act as antioxidants such as polyphenolics, organic acids, vitamins, catalase and glutathione peroxidase (Aljadi and Kamaruddin, 2004; Batrušaitytė et al., 2007). Beretta et al. (2005) standardized the protocols to study the antioxidant properties of honey by a combination of spectrophotometric assays such as color intensity [Abs_{450}], total phenolic content, FRAP assay and DPPH assay. The results of the colour intensity of Tualang honey as well as its phenolic content and antioxidant activities were given in Table 1.

Table 1: In vitro antioxidant properties of Tualang honey

Parameters	(Mean ± SD)*
Colour intensity, ABS_{450} (mAU, 50w/v)	489.5 ± 1.7
Total phenolic content (mg $_{gallic\ acid}$ /Kg honey)	251. 7 ±7.9
Radical scavenging activity by DPPH assay (% inhibition)	41.3 ± 0.78
Anti-oxidant activity by FRAP assay (µM Fe(II))	322.1 ± 9.7

*All determinations were carried out in a single honey sample in quadruplicate

When compared to the other types of honey, the net absorbance of Tualang honey was in the range of the different types of Slovenian honey such as Chest nut, Fir, Spruce, Multifloral and Forest (Bertoncelj et al., 2007; Table 2) as well as Multiflora and honeydew honey (Beretta et al., 2005; Table 2). The colour of the honey is usually related to the contents of the mineral, pollen and phenolic compounds (Batrušaitytė et al., 2007). Honey has also been shown to have a wide range of antioxidant activities depending on the botanical source, and high correlations have been reported between the antioxidant activity and colour, and total phenolic content of the honey (Al-Mamary et al., 2002; Berrata et al., 2005; Vela et al., 2007; Al et al., 2009). The total phenolic content of Tualang honey is also within the reported range of Slovenian honey (Chestnut, Fir, Spruce, Multifloral and Forest honey), Romanian honeys like Acacia, Lime, Sunflower, Chestnut and Honeydew honey (Bertoncelj et al., 2007; Al et al., 2009; Berrata et al., 2005;Table 2). DPPH assay reflects the activity of water soluble antioxidant (Frankel et al., 1998). The radical scavenging activity (RSA) of Tualang Honey, in terms of percentage inhibition of DPPH (~40%), is once again comparable with that reported for other types of honey such as Herb honey (pine and marigold) and Romanian honeys (Socha et al., 2009;.Al et al., 2009; Table 2) . Unlike DPPH assay, FRAP assay directly measures the total antioxidant activity in the honey (Aljadi and Kamaruddin 2004; Beretta et al., 2005). In our study, we used this assay to measure the total antioxidant activity of gamma radiated Tualang honey. The total antioxidant activity for Tualang honey also appears to be within the range reported for some Slovenian honeys (Bertoncelj et al., 2007; Table 2).

Many studies indicated that the colour intensity of the honey at ABS_{450} reflects its total phenolic content and could be correlated to its antioxidant activity (Al-Mamary et al., 2002; Berrata et al., 2005; M.L. Al et al., 2009). Thus, in the present study, it was concluded that Tualang honey has good colour intensity and contains phenolic compounds that possess relatively good antioxidant activity, which is comparable with that reported for other types of honey. However further studies are required to identify and quantify the biologically active components present in the Tualang honey, which could serve as a source of nutraceuticals.

Table 2: Comparison of antioxidant properties between Tualang honey and other types of honey

	Honey types	Colour intensity ABS_{450} (mAU, 50w/v)	Total Phenolic content (mg $_{gallic\ acid}$ /Kg honey)	Anti-oxidant activity by FRAP assay (μM Fe(II))	Radical scavenging activity by DPPH assay (~% inhibition)
Malaysian honey* (One honey sample)	Tualang honey	489.5 ± 1.7	251.7 ±7.9	322.1 ± 9.7	41.3 ± 0.78
Slovenian honey# reported by *J. Bertoncelj et al. / Food Chemistry 105 (2007) 822–828*	Acacia	70±15	44.8 ±14.8	71.0 ± 10.2	-
	Lime	123±25	83.7±14.3	118.8± 20.3	-
	Chestnut	495±73	199.9±34.1	360.1± 66.5	-
	Fir	405±60	241.4 ±39.5	478.5± 95.5	-
	Spruce	417±35	217.5± 20.6	395.3± 69.6	-
	Multifloral	344 ±57	157.3± 20.9	224.8±24.7	-
	Forest	467±74	233.9±21.7	426.4±41.5	-
Herbhoneys reported by *R. Socha et al. / Food Chemistry 113 (2009) 568–574*	Aloe	-	-	-	31
	Blackchokeberry	-	-	-	67
	Chamomile	-	-	-	25
	Hawthorn	-	-	-	85
	Marigold	-	-	-	42
	Mint	-	-	-	34
	Nettle	-	-	-	36
	Pine	-	-	-	41
	Raspberry	-	-	-	81
	Thym	-	-	-	80
Honeys* reported *by G. Beretta et al. / Analytica Chimica Acta 533 (2005) 185–191*	Strawberry tree	3413	789.6±13.8	1501.4±60.2	-
	Buckwheat	2245	482.2 ±2.4	800.7±23.8	-
	Honeydew	466	255.6 ±7.5	772.0±21.5	-
	Chestnut	610	211.2 ±5.5	388.6±8.2	-
	Multi-flora	415	170.4 ±1.7	361.9±10.8	-
	Acacia	25	55.2 ±2.8	79.5±3.7	-
Romanian honeys## reported by *M.L. Al et al. / Food Chemistry 112 (2009) 863–867*	Acacia	-	20–390	-	35.80–45.27
	Lime	-	160–380	-	36.60–40.91
	Sunflower/	-	200–450	-	40.65–49.19
	Honeydew	-	230–1250	-	40.67–64.83

*All determinations were carried out in quadruplicate and the values were expressed as mean ± SD
All determinations were carried out in triplicate and the data were expressed as means ± SD
##Data presented as the minimum and maximum values obtained for every honey type

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