DETERMINATION OF ANTIOXIDANT ACTIVITY AND TOTAL PHENOLICS OF SELECTED THAI HONEYS

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Abstract: The aim of this research was to determine the bioactive properties of seven floral honeys originated in Thailand including sesame *(Sesamum indicum* L.), lychee *(Litchi chinensis* Sonn.*)*, longan *(Dimocarpus longan* Lour.), sunflower *(Helianthus annuus*), korlan *(Nephelium hypuleucum* Korz.*)*, sabsue *(Eupatorium odoratum* L.*)* and plaunoi *(Croton stellatopilosus* Ohba). Results indicated that reducing sugar content ranged from 66.5 to 73.0%. Total phenolic content ranged from 58.7 to 165.2 mg GAE/100 g, flavonoid content varied between 4.8 to 27.5 mg QE/100 g. Three different methods were used to evaluate the antioxidant activity; the 2,2-diphenyl-1-picrylhydrazyl radical scavenging capacity (DPPH) assay, the 2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonic acid (ABTS) assay and the ferric reducing/antioxidant power (FRAP) assay. Inhibition concentration at 50% (IC_{50}) by DPPH assay and ABTS assay were found in the range from 0.02 to 0.12 g/ml and from 0.25 to 1.03 g/ml, respectively and the ferric reducing antioxidant power (FRAP) assay was found in the range from 147.5 – 550.0 µmol Fe (II)/100 g of honey. Antioxidant capacity of honey was positively correlated between the total phenolics and flavonoid contents.

Introduction: Botanical diversity and availability throughout the year has made Thailand a growing potential for honey production and export. The phytochemical constituents of a honey are known to be associated with their origin and in turn the quality of the honey.[1] Honey is a supersaturated solution of sugars which has been consumed for its high nutritive value and its association in human health. The composition of honey depends on the plant species that honeybees visited as well as the environmental, processing and storage conditions.[2] The use of honey in therapeutical treatment and prevention of numerous diseases resulted from oxidative damages is due to its antioxidant capacity. Phenolic compounds acting as minor constituents demonstrate antioxidant properties including phenolic acids and flavonoids of which its antioxidant activity varies greatly depending on the honey floral source.[3] However, there is a lack of information on the comparative biochemical properties of Thai honeys from various floral sources. Therefore, the objective of this study was to investigate the antioxidant properties of Thai honeys as to establish values for consumers.

Methodology:

Materials: Seven honey samples of known floral origin; lychee (*Litchi chinensis* Sonn.) (A), longan (*Dimocarpus longan* Lour.) (B), korlan (*Nephelium hypoleucum* Kurz.) (C), sunflower (*Helianthus annuus*) (D), plaunoi (*Corton stellatopilosus* Ohba.) (E), sesame (*Sesamum indicum* L.) (F), and sabsue (*Eupatorium odoratum* L.) (G) were obtained directly from beekeepers farms of two provinces in the Northern part of Thailand and maintained at cool temperature (20°C± 5) in their closed bottles prior to analysis. All the samples were informed of being freshly harvested and manufactured with less than 3 months olds by the manufacturers. The chemicals used in determination of antioxidant capacity were all analytical grade.

Determination of reducing sugar: All honeys were analyzed in triplicates according to the method proposed by the International Honey Commission[4] and in agreement with the Codex Alimentarius.[5]

Determination of total phenolic content: The total phenolic content was determined by the Folin-Ciocalteu Method [6] with some modification and the results were expressed as mg gallic acid equivalent (GAE)/100g of honey, as the average of 3 replications.

Estimation of flavonoid contents: A method described by Meda et al.[7] for honey samples was used and the results were expressed in mg quercetin equivalent (QE)/100 g of honey, as the average of 3 replications.

DPPH radical scavenging activity: The DPPH (2,2-diphenyl-1-picryl-hydrazyl) radical scavenging effect of honey samples was measured as per the described method.[8] The DPPH scavenging activity was calculated using the following formula:

DPPH scavenging activity (%) = $100(A_{control}-A_{sample})/A_{control}$

Where A= Absorbance at 515 nm

Results were expressed in terms of percentage of 50% inhibition of radical (IC_{50}).

Ferric reducing/antioxidant power (FRAP) assay: The reducing power of the ethanolic extracts of honey was determined according to the method of Alvarez-Suarez et al.[9] The absorbance was measured at 593 nm using a spectrophotometer. The results were expressed as µmoles of ammonium ferrous sulfate per 100 g of honey (µmol Fe(II)/100g of honey).

ABTS free radical scavenging activity: The antioxidant capacity assay of the honey samples was carried out by ABTS [2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonic acid] cation radical decolorization assay.[10] Results were expressed in terms of percentage of 50% inhibition of radical at 734 nm (IC_{50}). All spectrophotometric measurements were done with a UV-VIS Shimadzu 1601, Japan.

Statistical analysis: Each antioxidant activity assay was conducted in triplicate and data received were subject to analysis of variance for mean comparison followed by Duncan's multiple range tests. In all assays the results were expressed as mean ± standard deviation (SD). Differences at $p<0.05$ were considered to be statistically significant.

Results, Discussion and Conclusion:

The total reducing sugar content in the honey samples ranged from 66.5% to 73.0% (Table 1). Reducing sugars, which include mainly glucose and fructose, has been reported to be the major constituents of honey.[4] The total content of glucose and fructose for all honey samples is over 60 g/100g of honey in accordance with the international regulations.

The concentration and type of phenolic substances are primarily responsible for biological activities of honey.[11] The results are presented in Table 1 and the values represent the mean ± SD of three determinations. The total phenolic content (mg GAE/ 100 g of honey) of Thai honeys was found in the range of 58.7 to 165.2, which was determined using gallic acid as standard (R^2= 0.9988). The total phenolic substances were higher in korlan, sabsae and plaunoi. The values obtained for Thai honeys were similar to those obtained from Indian and Algerian honeys for which the phenolic content varied from 47 to 98 and 64 to 1304 mg GAE/100g, respectively.[2,12]

Using the standard curve generated by quercetin (R^2=0.9992), the total flavonoid content of Thai honey samples (mg of QE/100 g of honey) varied from 4.8 to 27.5 with the highest and the lowest levels observed in plaunoi and lychee, respectively. These values are similar to the average values found for some previous reports.[3,10] Phenolic compound occurring in honeys have been classified into three groups: flavonoids, cinnamic acids and benzoic acids.[13] The concentration of phenolics also varies among honey of different

geographical origins.[11] Due to the beneficial effects of polyphenols in human health that have been reported elsewhere and based on our data, Thai honey is therefore considered the natural polyphenol food sources.

Table 1: Reducing sugar content, total phenolic compound, total flavonoid content of Thai honeys

Content	Honey samples*						
	A	B	C	D	E	F	G
Reducing sugar (g/100g)	70.0±0.2^{b}	66.5±1.1^{d}	69.6±0.2^{b}	73.0±0.2^{a}	66.5±0.4^{d}	68.1±0.2^{c}	70.4±0.4^{b}
Total phenolic compound (mg GAE/ 100g honey)	58.7±0.2^{e}	81.4±0.6^{d}	104.0±0.7^{c}	82.7±0.9^{d}	165.2±1.0^{a}	83.6±0.5^{d}	124.7±2.0^{b}
Total flavonoid content (mg QE/100 g honey)	4.8±0.2^{f}	5.8±0.4^{f}	11.6±0.8^{c}	9.2±0.1^{d}	27.5±0.6^{a}	7.9±0.7^{e}	13.3±0.3^{b}

*A=lychee, B=longan, C=korlan, D=sunflower, E=plaunoi, F=sesame, G=sabsue

In our study, DPPH was used to test the free radical scavenging ability of honeys. The higher is the DPPH scavenging activity, the higher is the antioxidant activity of the sample. The strong absorption maxima at 517 nm as obviously seen in purple are resulted from the presence of an odd electron. When the unpaired electron of DPPH forms a pair with hydrogen donated by a free radical scavenging antioxidant, the decolorization of DPPH occurs from purple to yellow. The IC_{50} values calculated as the concentration of sample required for 50% inhibition of radical (DPPH and ABTS) of Thai honey samples ranged from 0.02-0.12 g/ml and 0.25-1.03 g/ml, respectively as shown in Table 2. The lower IC50 values the higher the antioxidant capacity of the sample. The results indicated that plaunoi honey has the highest antioxidant capacity followed by sabsue and korlan honeys, respectively. It should be noted that honeys derived from wild species of plants seem to pose higher antioxidant power than that from fruit or flower plants. Our results were in agreement with those of Sangsrichan and Wansorn[8] in that the radical scavenging assay of honey samples in $ABTS^{+}$ reaction system was slightly higher than that of DPPH reaction.

The FRAP assay of all the Thai honeys are shown in Table 2. The FRAP values were found the highest in plaunoi, whereas lychee showed the lowest values. A relatively higher FRAP value indicated more reduction of ferric ions to ferrous ions. Similar observations have also been observed for Indian and Turkish honeys.[11,12]

In conclusion, all the seven Thai honeys demonstrated antioxidant activities which are characterized by content of phenolic compounds they contain and their content presents variable values for the different plant origin. The richest honey in total phenols and total flavonoids is plaunoi which presents the highest percent of inhibition. With respect to the antioxidant properties, supplementation of Thai honeys in food may be recommended for polyphenol complement.

Table 2: Antioxidant capacity of Thai honeys

Determination	Honey samples*						
	A	B	C	D	E	F	G
DPPH assay (IC_{50} g/mL)	0.12±0.01^{a}	0.06±0.01^{b}	0.03±0.03^{cd}	0.06±0.06^{b}	0.02±0.09^{d}	0.05±0.08^{bc}	0.04±0.04^{c}
ABTS assay (IC_{50} g/mL)	1.03±0.03^{a}	0.44±0.01^{c}	0.37±0.01^{d}	0.59±0.02^{b}	0.25±0.01^{e}	0.41±0.02^{c}	0.35±0.01^{d}
FRAP µmol Fe(II)/ 100 g honey	147.5±3.16^{e}	252.5±5.5^{d}	437.5±13.7^{bc}	272.5±12.7^{d}	550.0±25.2^{a}	337.5±13.7^{c}	425.0±6.5^{b}

* A=lychee, B=longan, C=korlan, D=sunflower, E=plaunoi, F=sesame, G=sabsue

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