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Physicochemical parameters, multi-elemental composition and antiradical activity of multifloral honeys from *Apis cerana cerana* in Hainan province, China

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Abstract

A summarized physicochemical profile of nine multifloral honeys produced by *Apis cerana cerana* Fabricius in Qiongzhong region located in Hainan province was exhibited, regarding pH (3.72-4.02), moisture (19.20-22.53%), fructose (34.40-38.70 g 100 g^{-1}), glucose (31.60-35.05 g 100 g^{-1}), sucrose (less than 3%), color (31.00-80.00 mm Pfund), ash content (0.17-0.45 g 100 g^{-1}), soluble solid (75.87-79.10 °Brix), and electrical conductivity (343.67-678.33 μS cm^{-1}). Potassium showed the highest concentration, followed by calcium. Manganese, boron, iron, aluminium, and zinc were the main micro-elements while cadmium and hydrargyrum were not detected in all honey samples. The antiradical activity was shown to be significantly negative correlated with total phenolic content (TPC) (r^2 = −0.756) and total flavonoid content (TFC) (r^2 = −0.477). Among all tested honey samples, sample A exhibited the highest levels of TPC (45.46 mg GAE 100 g^{-1}), TFC (10.02 mg RE 100 g^{-1}), and antiradical activity (DPPH IC_{50} = 2.63 mg mL^{-1}). Our results will be useful in determining to set the standard for *A. cerana cerana* honey.

Keywords: *Apis cerana cerana*; multifloral honey; physicochemical parameters; element analysis; antiradical activity.

Practical Application: The multifloral honeys from *A. cerana cerana* in Hainan province can be used as potential functional food.

1 Introduction

Honey is widely employed in the food industry, particularly in the cosmetic and health care. In fact, honey quality varies substantially depending on botanical and geographical origins (Gregório et al., 2021), bee species, and climatic conditions of the production site (Kavanagh et al., 2019). Further processing and improper storage condition also influence the quality of honeys indirectly (Chua et al., 2012).

Honey is composed of over 200 substances, the most of which are sugar and water, as well as minerals essential for human health (Kędzierska-Matysek et al., 2018). The physicochemical parameters of honey can be used for the assessment of quality and floral origins, such as color, sugar content, pH, moisture content, and so on (Conti, 2000). The elemental profile of honeys is important not only for determining the environmental condition, but also for ensuring honey quality (Chua et al., 2012). The content of minerals in honey ranges from 0.04 to 0.2% in floral honey (Vanhanen et al., 2011), while that in honeydew honey varies between 0.40 and 0.63% (Oroian et al., 2017). Moreover, honey possesses strong antioxidant activity which varies greatly and depends on the floral and geographical origin (El-Haskoury et al., 2019), and it has been demonstrated that phenolic compounds in honeys can act as antioxidants and scavenging free radicals (Can et al., 2015).

Increasing consumer preferences for honey products has led to increased demand for particular characteristics in honey which are associated with honey's health benefits (Nascimento et al., 2022). Honey from *Apis cerana* has been used in Chinese medicine over thousands of years for its medical properties, and one of the most significant properties that has led to its widespread usage is its antimicrobial effect (Wang et al., 2019). Therefore, the price of *A. cerana* honey is commonly three to ten times more than that of *A. mellifera* honey because of its low yield, consumer impression of higher antioxidant and antibacterial activities (Park et al., 2018), and more bioactive substances (Habib et al., 2014; Zhao et al., 2017). Despite the fact that *A. cerana* hive products have been disregarded economically for decades, the native Asian honeybee is now regarded as an essential and valuable genetic resource to preserve, and its honey is becoming increasingly appreciated (Soares et al., 2018). Due to a variety of ecological and climatic circumstances, as well as a rich flora that provides blossoming plants all year, Hainan province is a key producer of *A. cerana cerana* honey. Qiongzhong region is located in the centre of Hainan province, being the largest honey-producing region in Hainan province, where multifloral honeys produced by *A. cerana cerana* are obtained.

The aim of this study was to (1) evaluate and compare the physicochemical parameters, elements, and antiradical activity of nine multifloral honeys from different sites in the same production region, and (2) identify potential relationships between physicochemical parameters, elements and antiradical

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activity, in order to assist in the promotion of *A. cerana cerana* honey as potential functional food.

2 Materials and methods

2.1 Sample collection

A total of nine multifloral honey samples from *A. cerana cerana* were collected from the selected beekeeping apiaries in Qiongzhong region as indicated in Figure 1, and the floral origins of surrounding the hives mostly included *Alpinia oxyphylla* Miq., *Areca catechu*, *Bidens pilosa* L., and other wild flowers. The honey samples were harvested from March to April 2020 without any processing and treatment and stored at room temperature until analysis.

2.2 Chemicals and reagents

Sodium carbonate anhydrous, nitric acid, and hydrogen peroxide were purchased from Xilong Scientific Co., Ltd, China. Methanol was purchased from Guangzhou Chemical Reagent Factory of China. Rutin was purchased from Shanghai yuanye Bio-Technology Co., Ltd, China. Folin-Ciocalteu reagent and gallic acid were obtained from Sigma-Aldrich Corporation of the USA. 1,1-diphenyl-2-picrylhydrazyl (DPPH) was obtained from Aladdin (Shanghai). Aluminum chloride was purchased from Shanghai Macklin Biochemical Co., Ltd, China.

2.3 Physicochemical analysis

The parameters, including ash content, moisture, pH, soluble solid, fructose, glucose, and sucrose were carried out according to the methods described by Wu et al. (2020). Briefly, the ash content was determined via incinerated samples in Muffle furnace at 640 °C for 6 h. The moisture level was analyzed using a portable refractometer (model ATC-005, China) at 25 °C. The pH was measured using a pH-meter (Leici PHSJ-4A, China) at 25 °C in 20% (w/v) honey solution. The fructose, glucose, and sucrose concentration determined via a HPLC system (Waters ALLIANCE e2695) with a refractive index detector; and the sugars were separated on a Xbridge Amide column (4.6 mm × 150 mm, 3.5 μm, Waters) with a mobile phase of acetonitrile/water/triethylamine (80 : 20 : 0.2, v/v). Electrical conductivity was measured using a conductivity meter (Leici DDS-307A). Color measurement was performed using a honey color analyzer (Hanna Instruments, HI96785).

2.4 Multi-elemental analysis

The five macro-elements including magnesium (Mg), sodium (Na), potassium (K), phosphorus (P), and calcium (Ca),

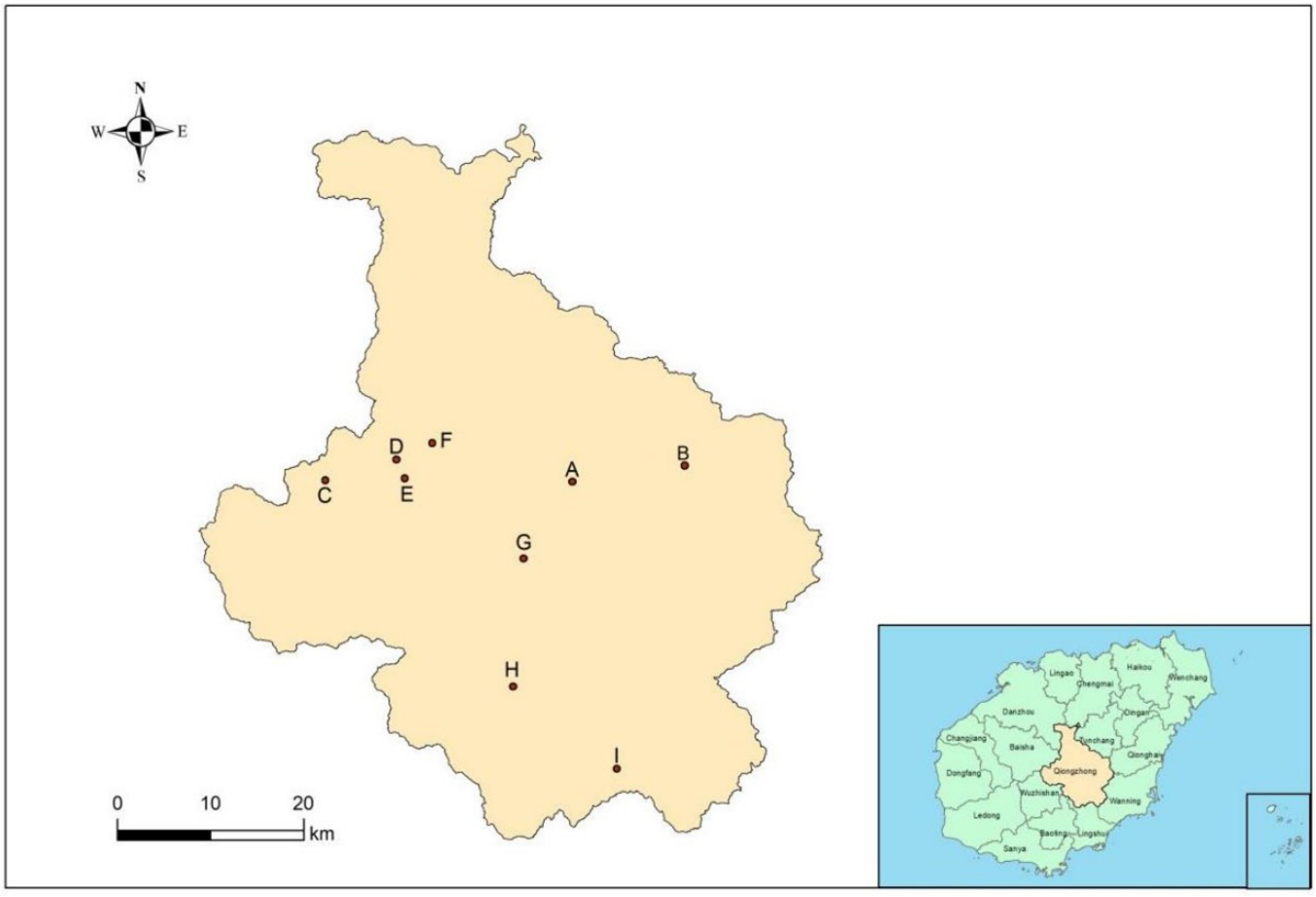

Figure 1. Geographical origin of honey samples from Hainan province.

and 16 micro-elements including chromium (Cr), vanadium (V), aluminium (Al), boron (B), zinc (Zn), copper (Cu), nickel (Ni), cobalt (Co), iron (Fe), manganese (Mn), lead (Pb), barium (Ba), cadmium (Cd), molybdenum (Mo), arsenic (As), and hydrargyrum (Hg), were determined by ICP-MS (NeXION 300X, Perkin Elmer, USA) after microwave digestion, according to the methods reported by Wu et al. (2020). Briefly, 0.5 g honey was mixed with 5 mL high grade nitric acid and 1 mL hydrogen peroxide in a digestion tube. The solution was predigested for 40 min at 110 °C on an electric platen, and then digested in a 190 °C microwave for 40 min at 1600 W. After cooling, the digested sample was diluted to 50 mL with high purity water for analysis.

2.5 Total Phenolic Content (TPC)

The TPC was constructed by the method described by Singleton & Rossi (1965) with slight modification. Briefly, 1.0 mL honey solution (0.1 g mL^{-1}, w/v in ultrapure water) and 1.0 mL Folin-Ciocalteu reagent were mixed. The total volume was adjusted to 10 mL with ultrapure water after adding 5.0 mL 1 M sodium carbonate. The sample was shaken at room temperature for 1 h in the dark, and then the absorbance was measured at 760 nm by using Tecan's Infinite® 200 PRO (Switzerland). A standard curve was plotted for the standard solution of gallic acid in a range of concentrations from 0 to 10 μg mL^{-1} ($y = 62.45x + 0.0872$, $R^2 = 0.9998$), and TPC was expressed as gallic acid equivalent (mg GAE 100 g^{-1}).

2.6 Total Flavonoid Content (TFC)

The TFC assay was performed as described in Aumeeruddy et al. (2019) with slight modification. Briefly, 3 mL honey solution (0.1 g mL^{-1}, w/v in ultrapure water) was mixed with 1 mL of aluminum chloride (1%), and the total volume was adjusted to 10 mL with ethanol (95%). After ten minutes, the absorbance was measured at 405 nm by using Tecan's Infinite® 200 PRO (Switzerland). The TFC was calculated from a standard curve of rutin in a range of concentrations from 0 to 40 μg mL^{-1} ($y = 12.382x + 0.0538$, $R^2 = 0.9999$), and expressed as rutin equivalent (mg RE 100 g^{-1}).

2.7 Determination of free radical scavenging activity

The DPPH assay was performed as described in Cheng et al. (2014) with slight modification. Briefly, 1 mL of honey solution (40 mg mL^{-1}, 20 mg mL^{-1}, 10 mg mL^{-1}, 5 mg mL^{-1}, 2.5 mg mL^{-1}) were mixed with a methanolic solution of DPPH (0.025 mg mL^{-1}, 4.0 mL), and then they were left in the dark for 1 h. Absorbance reading was performed at 517 nm on a Tecan's Infinite® 200 PRO (Switzerland) and the results were expressed as percentage inhibition of DPPH radicals by honey samples based on (Equation 1):

$$\text{Inhibition (\%)} = \frac{A_0 - A_t}{A_0} \times 100 \quad (1)$$

Where A_0 is the control (blank, without sample) and A_t is the absorbance of honey samples. The IC_{50} values (the concentration of the test honeys required to reach the inhibition of DPPH radicals to 50%) were calculated.

2.8 Statistical analysis

All experiments were performed with three replicates, and the results were expressed as means ± standard deviation. Prior to One-way analysis of variance (ANOVA) followed by Tukey's test, the normality of the distribution (original data or transferred data) was checked using the test of Homogeneity of Variances. As the data differed from a normal distribution, the statistical significance and level were checked with Nonparametric test followed by Kruskal-Wallis one-way ANOVA. The correlation analysis was carried out using the Pearson mode. Differences between means at confidence levels of 95% and 99% ($p < 0.05$ and $p < 0.01$, respectively) were considered statistically significant. Statistical analyses were performed using SPSS 24 software (IBM, New York, USA).

3 Results and discussion

3.1 Physicochemical analysis

Table 1 showed basic descriptive statistics for pH, moisture, sugars, color, ash content, soluble solid, and electrical conductivity of multifloral honeys. Honey pH values are of great importance during extraction and storage, as they influence the texture, stability, and shelf-life of honeys (Terrab et al., 2004). Most bacteria grow in a neutral and mildly alkaline environment, while yeasts and moulds are capable of growth in an acidic environment (pH = 4.0-4.5) (Conti, 2000). Honey produced by *A. cerana cerana* had a pH within the range of 3.72-4.02 (mean 3.87 ± 0.10), in which sample F and sample G might be more susceptible to bacterial contamination.

The moisture content of honey depends on various factors, like the harvesting season, the degree of maturity reached in the hive and climatic factors (Lazarević et al., 2012), therefore the parameter can be used to detect honey maturity (Kanbur et al., 2021). The moisture in the analyzed honeys ranged from 19.20 to 22.53% (mean 20.77 ± 1.23%), which is similar to the moisture content (20.12%) in *A. cerana* honeys (Joshi et al., 2000). Except sample A, C, E, and F, the moisture contained in the other honeys exceeded the maximum acceptable content of 20% (Codex Alimentarius Commission, 2001), indicating that sample A was the most mature.

The major constituents in honey are sugars, such as fructose and glucose (Solayman et al., 2016). The fructose and glucose ranged from 34.40 to 38.70 g 100 g^{-1} (mean 36.54 ± 1.30 g 100 g^{-1}) and 31.60-35.05 g 100 g^{-1} (mean 33.94 ± 1.00 g 100 g^{-1}), respectively. Additionally, all analyzed samples had a sucrose content of less than 3%, the maximum limit regulated by Codex Alimentarius Commission (2001), which could indicate that the honey was not adulterated with sugar syrup and was properly matured before harvesting. It has been stated that the amount of sucrose varies according to the degree of ripening of the honey and the content of the nectar, and the value of sucrose in immature honey harvested very early was higher (Kolayli et al., 2016). Although sugar contents in all honey samples did not differ significantly (Table 1), sugars make up the largest proportion

of dry matter in honey, and their qualitative and quantitative composition is an important criterion in the quality assessment of honey.

The color of honey samples in this study ranged from 31.00 to 80.00 mm Pfund (mean 46.63 ± 16.53 mm Pfund), in which sample A was significantly darker than sample I ($p < 0.05$) (Table 1). These values were lower compared to the results as reported in Malaysian (76-113 mm Pfund) and Algerian honey (111-150 mm Pfund) (Khalil et al., 2012). According to the standard scale of honey color from water white (< 8 mm Pfund) to dark amber (> 140 mm Pfund), the colors of the analyzed honeys were divided into three groups: white (sample I, E, and H), extra light amber (sample B, C, and G), and light amber (sample D, F, and A). The color of honey is also largely determined by its degree of crystallization and the conditions in which physicochemical changes take place during storage (Kędzierska-Matysek et al., 2021).

Ash content is a parameter used for the determination of the botanical origin (floral, mix or honeydew) (Saxena et al., 2010). The range of ash content in *A. cerana cerana* honeys was from 0.17 to 0.45 g 100 g^{-1} (mean 0.30 ± 0.09 g 100 g^{-1}), which falls within the limit allowed for floral honeys (0.6%).

The soluble solid was not only directly related to sugars and the water levels but also served as an indicator parameter of the rate in organic acids, minerals, sugars and so on (Biluca et al., 2016). The analyzed samples presented Brix degrees ranging from 75.87 to 79.10 (mean 77.61 ± 1.16 °Brix).

The EC of honey reflects its botanical origin, the content of mineral salts, proteins, and organic acids (Rodríguez et al., 2019), being used as a honey quality indicator, assisting in the identification and distinction of floral honey and honeydew (Bettar et al., 2019). In this study, the results obtained for the honey samples varied between 343.67 and 678.33 µS cm^{-1} (mean 549.56 µS cm^{-1}), which complied with the Codex Standard for honey to be < 0.8 mS cm^{-1} (European Union, 2001), suggesting that these samples were blossom honey or a blend of blossom with honeydew honey. Our study results were similar to the honey from the Black Sea region of Turkey (0.54 ± 0.38 mS cm^{-1}) (Bîdecî & Karasalihoğlu, 2022). Specifically, the average EC value in our study was lower than that of multifloral honey from Italy (0.63 ± 0.37 mS cm^{-1}) (Conti et al., 2018) and *A. cerana* honey (0.65 mS cm^{-1}) in Nepal (Joshi et al., 2000).

3.2 Multi-elemental analysis

The composition of mineral and trace elements has been suggested as a useful parameter for the identification of both botanical (Chen et al., 2014) and geographical origins of honey (Baroni et al., 2015). However, the variation in micro-element content in different honey types is primarily due to botanical origin rather than geographical and environmental exposition of nectar sources (Bogdanov et al., 2007). For the macro-elements, it was found that sample C had the highest total element content (1,605.70 ± 10.90 mg kg^{-1}), followed by sample G (1,587.67 ± 41.15 mg kg^{-1}) and sample F (1,582.50 ± 28.69 mg kg^{-1}). It was clear that the highest content of the total element in all honey samples was mainly due to the presence of K in high concentration (Table 2), which was in agreement with that K is the abundant element (Conti, 2000; Pisani et al., 2008; Terrab et al., 2004). Ca ranged from 103.07 to 228.00 mg kg^{-1} (mean 181.45 ± 39.64 mg kg^{-1}), and Na and Mg were present in moderate amounts in the honey samples, with average contents of 22.88 ± 9.52 and 61.97 ± 19.46 mg kg^{-1}, respectively.

Besides the macro-elements, Mn, B, Fe, Al, and Zn were the main micro-elements, 4.81 ± 1.33, 4.30 ± 1.19, 2.45 ± 0.82, 1.92 ± 0.70, and 1.00 ± 0.38 mg kg^{-1}, respectively, while the contents of Cr, V, Cu, Ni, Co, Pb, Ba, Cd, Mo, As, and Hg were detected at the concentration less than 1 mg kg^{-1}, in which Cd and Hg were not detected in all honey samples at the level of mg kg^{-1} (Table 3). It is also believed that some portion of Al content might be attributed to secondary sources such as metallic containers used for storage during harvesting and handling processes (Pisani et al., 2008). The trace contents of Co, Cr, Cu, Fe, Mn, and Zn are currently considered essential for human nutrition (European Food Safety Authority, 2017). The average concentration of Mn (4.81 ± 1.33 mg kg^{-1}) was less than the highest Mn content (5.5 mg kg^{-1}) set by World Health Organization (2011). Apart from the view of elemental nutrients, honey is also sensitive to environmental or anthropogenic contaminants (Fechner et al., 2020). The origin of contamination can be the environment (air, water, plants, and soil) and beekeeping practices (Costa-Silva et al., 2011). As and Pb are currently the most concern environment contaminants.

Table 1. The physicochemical parameters of multifloral honeys from *A. cerana cerana*.

Sample	pH	Moisture (%)	Fructose (g 100 g^{-1})	Glucose (g 100 g^{-1})	Sucrose (g 100 g^{-1})	Color (mm Pfund)	Ash content (g 100 g^{-1})	Soluble solid (°Brix)	Electrical conductivity (µS cm^{-1})
A	3.81 ± 0.05^{ab}	19.20 ± 0.00^{a}	36.20 ± 0.14^{a}	34.10 ± 0.00^{a}	1.75 ± 0.07^{a}	80.00 ± 0.00^{a}	0.24 ± 0.00^{A}	79.10 ± 0.00^{a}	487.33 ± 4.51^{A}
B	3.79 ± 0.04^{ab}	22.10 ± 0.00^{ab}	35.20 ± 0.28^{a}	33.60 ± 0.28^{a}	2.40 ± 0.14^{a}	36.67 ± 0.58^{ab}	0.21 ± 0.00^{A}	76.40 ± 0.00^{ab}	467.00 ± 2.65^{B}
C	3.83 ± 0.01^{ab}	19.80 ± 0.00^{ab}	34.40 ± 0.14^{a}	33.90 ± 0.14^{a}	1.25 ± 0.07^{a}	45.00 ± 0.00^{ab}	0.45 ± 0.02^{B}	78.50 ± 0.00^{ab}	678.33 ± 5.69^{C}
D	3.98 ± 0.02^{ab}	20.80 ± 0.00^{ab}	36.65 ± 0.21^{a}	34.70 ± 0.42^{a}	-	52.00 ± 0.00^{ab}	0.22 ± 0.01^{A}	77.60 ± 0.00^{ab}	445.00 ± 3.00^{D}
E	3.85 ± 0.01^{ab}	20.00 ± 0.00^{ab}	36.60 ± 0.28^{a}	34.45 ± 0.64^{a}	1.70 ± 0.00^{a}	33.33 ± 0.58^{ab}	0.34 ± 0.00^{C}	78.30 ± 0.00^{ab}	632.00 ± 4.36^{E}
F	4.00 ± 0.02^{b}	19.40 ± 0.00^{ab}	38.70 ± 0.28^{a}	31.60 ± 0.57^{a}	-	68.67 ± 0.58^{a}	0.38 ± 0.02^{C}	78.90 ± 0.00^{ab}	591.33 ± 0.58^{F}
G	4.02 ± 0.01^{b}	22.53 ± 0.12^{b}	37.70 ± 0.00^{a}	35.05 ± 0.64^{a}	-	39.00 ± 0.00^{ab}	0.35 ± 0.03^{C}	75.87 ± 0.06^{b}	630.00 ± 3.00^{E}
H	3.85 ± 0.01^{ab}	22.30 ± 0.00^{b}	37.55 ± 0.07^{a}	34.05 ± 0.35^{a}	-	34.00 ± 0.00^{ab}	0.37 ± 0.01^{C}	76.20 ± 0.00^{b}	671.33 ± 11.24^{C}
I	3.72 ± 0.03^{a}	20.80 ± 0.00^{ab}	35.90 ± 0.57^{a}	34.05 ± 0.35^{a}	2.70 ± 0.00^{a}	31.00 ± 0.00^{b}	0.17 ± 0.01^{D}	77.60 ± 0.00^{ab}	343.67 ± 1.15^{G}
Mean	3.87 ± 0.10	20.77 ± 1.23	36.54 ± 1.30	33.94 ± 1.00	1.96 ± 0.55	46.63 ± 16.53	0.30 ± 0.09	77.61 ± 1.16	549.56 ± 112.96

"-" for not detected. For each column, values followed by different lower case letters indicate significantly different values ($p < 0.05$) and different capital letters indicate significantly different values ($p < 0.01$).

Table 2. Content of macro-elements (mg kg^{-1}) in *A. cerana cerana* honeys.

Sample	K	Ca	Na	Mg	P	Total
A	791.00 ± 6.24^{A}	178.67 ± 3.79^{A}	25.53 ± 1.04^{A}	65.00 ± 0.40^{Aab}	47.47 ± 1.07^{ABa}	1,107.67 ± 6.26^{Aa}
B	738.67 ± 1.15^{B}	132.67 ± 1.15^{B}	13.30 ± 0.20^{B}	38.33 ± 1.89^{Bc}	44.33 ± 1.46^{ACb}	967.30 ± 1.81^{Bb}
C	1,315.67 ± 10.07^{C}	170.33 ± 5.13^{A}	16.03 ± 0.84^{CD}	62.60 ± 2.25^{Aa}	41.07 ± 0.42^{CDc}	1,605.70 ± 10.90^{Cc}
D	658.67 ± 9.07^{D}	228.00 ± 2.00^{C}	18.73 ± 0.60^{E}	83.60 ± 2.62^{Cd}	39.70 ± 1.67^{Dcd}	1,028.70 ± 12.09^{Bd}
E	1,140.67 ± 20.03^{E}	223.67 ± 4.62^{C}	17.73 ± 0.31^{DE}	67.50 ± 2.07^{Aab}	37.30 ± 0.79^{DEde}	1,486.87 ± 16.75^{De}
F	1,228.00 ± 24.58^{F}	194.33 ± 1.15^{D}	41.00 ± 1.18^{F}	62.83 ± 1.53^{Aab}	56.33 ± 0.87^{Ff}	1,582.50 ± 28.69^{Cc}
G	1,188.33 ± 37.54^{EF}	202.67 ± 2.08^{D}	37.10 ± 0.80^{G}	86.63 ± 2.76^{Cd}	72.93 ± 1.01^{Gg}	1,587.67 ± 41.15^{Cc}
H	1,215.00 ± 23.64^{F}	199.67 ± 2.52^{D}	21.37 ± 0.29^{H}	68.50 ± 2.51^{Ab}	50.83 ± 0.67^{Bh}	1,555.37 ± 26.47^{CDc}
I	565.00 ± 10.82^{G}	103.07 ± 4.24^{E}	15.10 ± 0.17^{BC}	22.73 ± 1.02^{De}	35.53 ± 1.15^{Ee}	741.43 ± 14.78^{Ef}
Mean	982.33 ± 278.09	181.45 ± 39.64	22.88 ± 9.52	61.97 ± 19.46	47.28 ± 11.28	

For each column, values followed by different lower case letters indicate significantly different values ($p < 0.05$) and different capital letters indicate significantly different values ($p < 0.01$).

Table 3. Content of micro-elements (mg kg^{-1}) in *A. cerana cerana* honeys.

Element	Honey sample									
	A	B	C	D	E	F	G	H	I	Mean
Cr	0.13 ± 0.01^{ab}	0.05 ± 0.00^{a}	0.07 ± 0.00^{ab}	0.09 ± 0.00^{ab}	0.06 ± 0.00^{ab}	0.24 ± 0.01^{b}	0.07 ± 0.00^{ab}	0.05 ± 0.00^{a}	0.06 ± 0.00^{ab}	0.09 ± 0.06
V	0.01 ± 0.00^{Aa}	0.01 ± 0.00^{Bb}	0.01 ± 0.00^{BCbc}	0.01 ± 0.00^{Ccd}	0.01 ± 0.00^{Bb}	0.01 ± 0.00^{BCcd}	0.01 ± 0.00^{Cd}	0.01 ± 0.00^{Bb}	0.02 ± 0.00^{Aa}	0.01 ± 0.00
Al	2.44 ± 0.10^{Aa}	1.37 ± 0.05^{Bb}	1.68 ± 0.02^{DEc}	1.54 ± 0.03^{CDd}	1.47 ± 0.03^{BCbd}	2.36 ± 0.08^{Aa}	1.80 ± 0.02^{Ec}	1.15 ± 0.04^{Fe}	3.50 ± 0.14^{Gf}	1.92 ± 0.70
B	4.20 ± 0.16^{A}	4.22 ± 0.10^{A}	3.74 ± 0.14^{B}	7.22 ± 0.04^{C}	3.17 ± 0.19^{D}	4.99 ± 0.10^{E}	4.02 ± 0.08^{AB}	4.00 ± 0.04^{AB}	3.12 ± 0.14^{D}	4.30 ± 1.19
Zn	0.84 ± 0.01^{A}	0.64 ± 0.00^{B}	1.64 ± 0.02^{C}	1.30 ± 0.03^{D}	0.89 ± 0.02^{A}	1.01 ± 0.02^{E}	1.06 ± 0.06^{E}	1.32 ± 0.03^{D}	0.31 ± 0.01^{F}	1.00 ± 0.38
Cu	0.25 ± 0.01^{Aa}	0.13 ± 0.00^{Bb}	0.19 ± 0.00^{Cd}	0.23 ± 0.01^{ADa}	0.06 ± 0.00^{Ec}	0.20 ± 0.01^{DCd}	0.41 ± 0.01^{Fe}	0.15 ± 0.00^{Bf}	0.10 ± 0.01^{Gg}	0.19 ± 0.10
Ni	0.06 ± 0.00^{ab}	0.02 ± 0.00^{ab}	0.03 ± 0.00^{ab}	0.02 ± 0.00^{ab}	0.02 ± 0.00^{b}	0.04 ± 0.00^{b}	0.07 ± 0.00^{a}	0.02 ± 0.00^{ab}	0.02 ± 0.00^{b}	0.33 ± 0.02
Co	0.01 ± 0.00^{Aa}	0.03 ± 0.00^{Bb}	0.02 ± 0.00^{Cc}	0.01 ± 0.00^{Ad}	0.01 ± 0.00^{De}	0.03 ± 0.00^{Ef}	0.02 ± 0.00^{Fg}	0.04 ± 0.00^{Gh}	0.04 ± 0.00^{Hi}	0.02 ± 0.01
Fe	2.67 ± 0.09^{Aa}	1.17 ± 0.03^{Bb}	2.70 ± 0.03^{Aa}	2.96 ± 0.05^{Aa}	2.26 ± 0.18^{Cc}	4.10 ± 0.10^{Dd}	2.66 ± 0.19^{Aa}	1.92 ± 0.15^{DEe}	1.64 ± 0.03^{Ee}	2.45 ± 0.82
Mn	4.52 ± 0.06^{A}	2.50 ± 0.05^{B}	5.40 ± 0.10^{C}	6.98 ± 0.18^{D}	5.35 ± 0.10^{C}	5.93 ± 0.06^{E}	3.83 ± 0.12^{F}	5.36 ± 0.12^{C}	3.38 ± 0.06^{G}	4.81 ± 1.33
Pb	0.05 ± 0.00^{Aa}	0.02 ± 0.00^{Bbc}	0.02 ± 0.00^{Bb}	0.09 ± 0.00^{Cd}	0.02 ± 0.00^{Bbc}	0.02 ± 0.00^{Bc}	0.02 ± 0.00^{Bbc}	0.02 ± 0.00^{Bbc}	0.02 ± 0.00^{Bbc}	0.03 ± 0.02
Ba	0.50 ± 0.01^{A}	0.40 ± 0.02^{B}	0.80 ± 0.00^{C}	0.29 ± 0.02^{D}	0.28 ± 0.01^{D}	0.26 ± 0.02^{DE}	0.25 ± 0.01^{DE}	0.57 ± 0.00^{F}	0.23 ± 0.01^{E}	0.40 ± 0.18
Cd	-	-	-	-	-	-	-	-	-	-
Mo	0.01 ± 0.00^{ABab}	0.01 ± 0.00^{ABac}	0.01 ± 0.00^{ABac}	0.01 ± 0.00^{Ab}	0.01 ± 0.00^{ABac}	0.01 ± 0.00^{Bc}	0.02 ± 0.00^{Cd}	0.00 ± 0.00^{De}	0.00 ± 0.00^{De}	0.01 ± 0.00
As	0.01 ± 0.00^{ab}	0.01 ± 0.00^{ab}	0.01 ± 0.00^{a}	0.01 ± 0.00^{ab}	0.01 ± 0.00^{ab}	0.01 ± 0.00^{ab}	0.01 ± 0.00^{ab}	0.00 ± 0.00^{b}	0.01 ± 0.00^{ab}	0.01 ± 0.00
Hg	-	-	-	-	-	-	-	-	-	-
Total	15.73 ± 0.42^{A}	10.57 ± 0.20^{B}	16.32 ± 0.18^{A}	20.77 ± 0.17^{C}	13.61 ± 0.22^{D}	19.21 ± 0.24^{E}	14.26 ± 0.41^{DF}	14.62 ± 0.21^{F}	12.46 ± 0.22^{G}	

"-" for not detected. For each row, values followed by different lower case letters indicate significantly different values ($p < 0.05$) and different capital letters indicate significantly different values ($p < 0.01$).

A PTMI of 1.75 mg (0.25 mg d^{-1}) for a 70-kg person (0.025 mg kg^{-1} body weight/wk) was designated for Pb (World Health Organization, 2011). For the tested sample D with the highest Pb (0.09 ppm), a 20-g daily honey consumption translates into a weekly intake of 0.0126 mg. This intake only represents about 0.72% of the PTWI for Pb, indicating that heavy metal intake from honey is well below the recommended dose, and consumption of these honeys is not considered dangerous for human health.

3.3 Total phenolic content, total flavonoid content and antiradical activity

The flavonoids and phenolic acids are deemed as of the significant group of components specified in honey having an antiradical activity (Alotibi et al., 2018). In the current study, the TPC of *A. cerana cerana* honeys ranged from 18.85 to 45.46 mg GAE 100 g^{-1} (mean 27.64 ± 8.16 mg GAE 100 g^{-1}) (Table 4), which was lower than a mean TPC of 408.9 mg GA kg^{-1} reported by Zhao et al. (2017), and our result was similar to Yemen honey (30.81 ± 1.94 mg GA kg^{-1}) (Habib et al., 2014). The TFC varied from 3.38 to 10.02 mg RE 100 g^{-1} (mean 6.02 ± 2.18 mg RE 100 g^{-1}), which was similar to that in Yemeni multifloral honey (mean 5.29 ± 0.44 mg QE kg^{-1}) (Wabaidur et al., 2020) and Brazilian honey (21.6-109.1 mg QE kg^{-1}) (Sant'Ana et al., 2012). Table 4 showed the comparative data of DPPH radical scavenging activity was determined by the IC_{50} values of the different honey samples. Sample A had the highest TPC and TFC and, in consequence, the highest antiradical activity with IC_{50} value of 2.63 ± 0.17 mg mL^{-1}.

Figure 2 showed that the TPC, TFC, and color were negatively correlated with the IC_{50} value of honey samples ($p < 0.01$). The higher TPC and TFC or the darker color would result in the lower IC_{50} value, indicating the honey has stronger antiradical activity. In addition, the higher correlation between IC_{50} value and TPC than between IC_{50} value and TFC suggested that the phenolic acids are one of the most important compounds contributing to the antiradical activity of honey (Cheung et al., 2019). The color of *A. cerana cerana* honeys was positively correlated with TPC and TFC (Figure 2), which means that the dark-colored

Table 4. Total phenolic contents, total flavonoid contents and antioxidant activity of multifloral honeys from *A. cerana cerana*.

Parameter	Honey samples									
	A	B	C	D	E	F	G	H	I	Mean
TPC (mg GAE 100 g^{-1})	45.46 ± 0.37^{a}	31.35 ± 0.17^{ab}	25.80 ± 0.50^{ab}	30.53 ± 0.32^{ab}	19.21 ± 1.05^{b}	32.92 ± 0.35^{ab}	22.74 ± 0.39^{ab}	21.94 ± 0.15^{ab}	18.85 ± 0.05^{b}	27.64 ± 8.16
TFC (mg RE 100 g^{-1})	10.02 ± 0.25^{ab}	3.38 ± 0.02^{c}	6.23 ± 0.47^{abcd}	6.87 ± 0.10^{ac}	4.01 ± 0.10^{abcd}	8.66 ± 0.53d	6.11 ± 0.42^{abcd}	3.84 ± 0.13^{abcd}	5.06 ± 0.20^{abcd}	6.02 ± 2.18
DPPH (IC_{50} mg mL^{-1})	2.63 ± 0.17^{Aa}	4.06 ± 0.78^{ABab}	4.50 ± 0.50^{BCbc}	4.53 ± 0.40^{BCbc}	7.27 ± 0.75^{CDde}	4.87 ± 1.05^{BCbcd}	6.02 ± 1.46^{BCbcd}	6.76 ± 0.22^{BCDcd}	11.10 ± 2.12^{De}	5.75 ± 2.51

For each row, values followed by different lower case letters indicate significantly different values ($p < 0.05$) and different capital letters indicate significantly different values ($p < 0.01$).

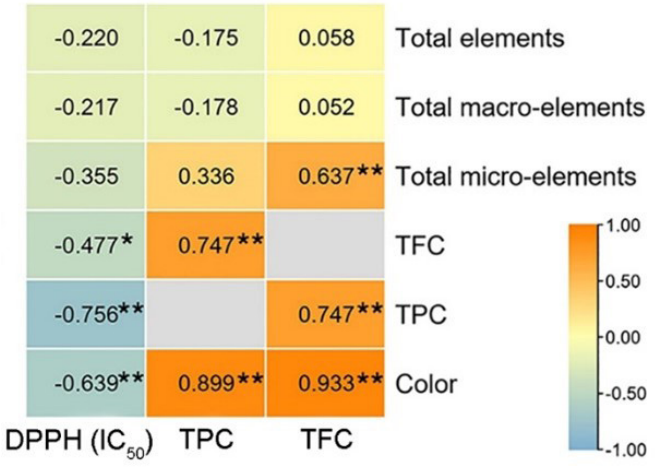

Figure 2. Heatmap of the correlation coefficients for DPPH antiradical capacity (IC_{50}) versus elements, TPC, TFC, and color by Pearson mode. Orange indicates positive correlation and blue indicates negative correlation.

honeys possessed higher antiradical activity as compared to honey with a light color (Bertoncelj et al., 2007).

4 Conclusions

In the present study, we analyzed and evaluated the major differences among the nine multifloral honeys produced by *A. cerana cerana* from Qiongzhong region in Hainan province in terms of physicochemical parameters, elements, and antiradical activity. The levels of heavy metals and toxic elements in the analyzed honeys were low or not detected. Moreover, close relationships were shown between antiradical activity and TPC as well as color. In general, the honeys produced in Qiongzhong region of Hainan province were high in phytochemicals (phenols and flavonoids) and displayed strong antiradical activities. We also discovered that moisture, electrical conductivity, and sugars in the certain *A. cerana cerana* honeys did not meet *A. mellifera* honey standards. Our findings clearly illustrated the functional food potential of multifloral honeys from *A. cerana cerana* in Hainan province, as well as provided important baseline data for setting *A. cerana cerana* honey standards.

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