Research Article



Pollen Analysis and Evaluation of the Antioxidant Activity of Honeys from Nine Localities in Côte d'Ivoire

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ABSTRACT

Similar to certain food products, honey from developing countries remains undervalued in international markets due to a lack of information about their floral origin and chemical composition. This study aims to assess ten (10) honey samples from nine (9) locations in Côte d'Ivoire. To achieve this, a pollen analysis followed by an evaluation of the antioxidant activity of these honeys was conducted. Melissopalynology identified thirty pollen taxa grouped into eighteen families. By analyzing the obtained pollen spectrum, the studied honeys were classified into two categories: monofloral and multifloral honeys. Furthermore, through hierarchical cluster analysis (HCA) and principal component analysis (PCA), four groups were identified, with quantitative and geographical pollen variability linked to phenology. Regarding the evaluation of antioxidant activity, the honey samples showed lower activity in the FRAP test compared to reference antioxidants. However, for the DPPH method, ethyl acetate selective extracts exhibited significant activity compared to chloroform extracts. The antioxidant capacity of the analyzed honeys may result from the presence of phytoconstituents in foraged plants such as *Mangifera indica, Parkia biglobosa*, and *Vitellaria paradoxa*, which are rich in phenolic compounds. In conclusion, this study highlights that Ivorian honey has the ability to neutralize free radicals, making it a beneficial food for health. Additionally, its plant composition rich in phytochemicals also contributes to this ability to neutralize free radicals.

Keywords: Honey, pollen composition, antioxidant activity, melissopalynology.

INTRODUCTION

any conditions, such as cancer, cardiovascular diseases, and diabetes, could result from oxidative damage caused by free radicals and reactive oxygen species. Indeed, these unstable and highly reactive chemical particles due to their unpaired electron can have significant adverse effects on the essential components (proteins, lipids, RNA, DNA) of living matter. ^{1,} ² Fortunately, they can be captured or neutralized by natural antioxidant substances present in medicinal plants, fruits, vegetables, and honey. ^{1, 3} The latter, naturally produced by bees from flower nectar and plant secretions, is recognized for its antioxidant and enzymatic properties. Indeed, honey is a complex mixture of sugars, also containing other constituents such as minerals, proteins, vitamins, organic acids, phenolic compounds (flavonoids), and enzymes. ^{4, 5} Furthermore, these properties could also result from the combined action of these components through synergistic effects. ^{6, 7} However, data related to the mechanism and antioxidant capacity, both the reducing power and the radical scavenging activity of honey from tropical countries, remain little known.^{8,9}

The annual global honey production in African countries is estimated at 198,668 tons, representing less than 1.5% of the world's production. Among these countries, those in West Africa have the lowest rate, with 21,484 tons. ¹⁰ The beekeeping sector there is mainly traditional. ^{11;12} Fortunately, in recent years, some countries, notably Côte d'Ivoire, have adopted modern practices to revalue this sector, thus increasing productivity and preserving biodiversity. ^{13;14} However, these nectars are often considered of poor quality on international markets due to the lack of reliable data on their botanical origin and quality. One of the essential quality indicators is the type of plant pollen, as it allows understanding the true nature of honey and its composition, which varies depending on floral sources. In this context, our work aims to study honey samples produced in nine (09) regions of Côte d'Ivoire by analyzing their pollen content and evaluating their antioxidant potential.

MATERIALS AND METHODS

1. Materials

The material consists of ten honey samples collected from private beekeepers located in nine cities in Côte d'Ivoire (Table I). These samples were carefully stored in glass bottles, in a clean place, protected from light, and at room temperature. The selection was made taking into account the geographical distribution of beekeepers across the Ivorian territory. Additionally, some samples were chosen based on the location of the apiaries, particularly in Biankouma (M4 and M5) (Table 1).



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Samples	Collection Sites	Geographic location	GPS Coordinates
Mı	Bouna (Bounkani Region)	North-East	9°16′ N 3°00′W
M2	M₂ Ferkessédougou No (Tchologo Region)		9°35′37″ N 5°11′50″ W
M3	Séguéla (Worodougou Region)	North-West	7°57′36″ N 6°40′22″ W
M₄(town)	Biankouma	West-Central	7°44'00'' N
M₅ (forest edge)	(Tonkpi Region)		7°37′00′′ W
M6	Dimbokro (N'zi Region)	Central	6°39′ N 4°42′ W
M7	Molonoublé (Lacs Region)	Central	7°24'00'' N 4°59'00'' W
M8	Prikro (Iffou Region)	East-Central	7°38′ N 3°59′ W
M9	Guezon (Moyen Cavally Region)	West	6°44'00'' N 7°07'00'' W
M 10	Dianra (Béré Region)	Central	8°57′11″ N 6°15′17″ W

Table 1: Presentation of the honey sam	ples
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2. Methods

2.1 Pollen analysis

The pollen analysis was conducted following the methodology described in the works of Louveaux. ¹⁵ For this purpose, 10 g of honey were dissolved in 20 ml of distilled water and shaken for 10 to 15 minutes. The mixture was then centrifuged at 1500 rpm for 15 minutes using the SIGMA 2-7 centrifuge. After centrifugation and recovery of the pellet, 10 ml of distilled water were added to the pellet, which was then subjected to another centrifugation at the same speed and for the same duration. The pellet was recovered again and mixed with 5 ml of glycerol-water solution in a 1:1 ratio (water/glycerol; v/v). After 30 minutes, this mixture was centrifuged at 1500 rpm for 15 minutes. The resulting pellet was placed in the center of a microscope slide, likely coated with 5 mm² of glycerol jelly. The slide was gently heated on a hot plate to dissolve the glycerol jelly, then covered with a coverslip and sealed with paraffin wax. Finally, the slide was observed under a microscope from Optikos microscopes Italy to visualize the pollen grains. The identification of these grains was carried out using a library of reference pollen slides, ¹⁶ and their classification followed the approaches of Feller-Demalsy & Parent (1989). 17

2.2. Antioxidant Activity

2.2.1. Free radical scavenging activity

The evaluation of the antiradical potential of selective honey extracts was carried out following the methods described in the literature. ^{2,18} The DPPH radical was dissolved in absolute ethanol, resulting in a solution with a concentration of 0.3 mg/ml. Different concentration ranges

(4 mg/ml, 2 mg/ml, 1 mg/ml, 0.5 mg/ml, 0.25 mg/ml, and 0.125 mg/ml) for each honey extract were prepared in absolute ethanol. In dry and sterile tubes, 1 ml of extract and 2 ml of ethanolic DPPH solution were introduced, respectively. After agitation, the tubes were placed in the dark for 30 minutes. The absorbance of the mixture was then measured at 517 nm against a control consisting of 1 ml of pure ethanol and 2 ml of DPPH solution. Quercetin was used as the positive reference control. The percentage reduction of DPPH (PR) was calculated according to formula (I):

PR = (Ac - As) x 100 / Ab (I)

PR: percentage reduction; **Ab:** absorbance of control; **Ae:** absorbance of sample

The effectiveness of the extracts was evaluated using their median concentration (CR_{50}), which represents the quantity required to reduce the initial concentration of the DPPH free radical by 50%. This value was calculated using Microsoft Excel 2010 software.

2.2.2. Ferric Reducing (FRAP) Method

The method described by Benzie & Strain (1996) ¹⁹ was used to conduct the FRAP test. In this method, 500 μ l of each honey sample (at a concentration of 0.25 g/ml) was mixed with 2.5 ml of the FRAP solution, which consisted of a mixture of 25 ml of CH₃COONa buffer (300 mM, pH 3.6); 2.5 ml of TPTZ (10 mM) dissolved in HCl (40 mM), and 2.5 ml of FeCl3 solution (20 mM). After 5 minutes of incubation at 37°C, the absorbance was measured at 593 nm. The concentrations of Fe³⁺ reduction to Fe²⁺ were determined from a calibration curve established with a Trolox standard extract at different concentrations (1.5; 0.75; 0.375; 0.187;



0.0935; and 0.04675 mM). This standard was prepared under the same conditions as the honey samples. Quercetin was used as the reference antioxidant. The concentrations of reducing compounds were expressed in milligrams of Trolox equivalents per gram of honey (mg TE/g of honey).²⁰

2.2.3. Statistical Analysis

The statistical analysis of the pollen data from the honey samples was conducted using IBM SPSS Statistics 25 software. This analysis included Principal Component Analysis (PCA) as well as Hierarchical Cluster Analysis (HCA). The correlation was verified using the Kaiser-Meyer-Olkin criterion. Additionally, the HCA and dendrogram were constructed based on matrices calculated using the Euclidean distance between pairs of honey samples.

RESULTS AND DISCUSSION

1. Pollen Spectrum

The pollen analysis indicated the presence of various pollens in the honey samples from Côte d'Ivoire (Table 2).

Honey	Botanical origin	Dominant pollens (≥ 45 %)	Accompanying pollens (15- 45%)	Minor pollens (3-15%)	Very minor pollens (≤ 3%)
Mı	Monofloral	V. paradoxa (46.30%)	P. Biglobosa (18.52%)	M. indica (6.48%) A.occidentale(9.26%)	A.digitata (0.92%),A.africana (0.92%),T. guineensis (0.92%),Ixora sp (0.92%),D. guineense (0.92%), C. pulcherrima (0.92%), H. squamosus(0.92%), B. buonopozense (1.85%),C. pentandra (1.85%),C. procera (1.85%),T. neriifolia (1.85%),A. cordifolia (2.78%), T. grandis (2.78%)
M ₂	Multifloral	-	V. paradoxa (32.26%) M. indica (23.39%)	A. cordifolia (3.23%), T. grandis (3.23%), A.occidentale (7.26%), A. digitata (8.06%), P. biglobosa(13.71%)	C. roseus (0.81%),C. javanica (0.81%), T. neriifolia(0.81%), C. sanderianum (0.81%), C. pulcherrima (0.81%), H. squamosus (0.81%), C. pentandra (2.42%), A.africana (1.61%)
M₃	Multifloral	-	V. paradoxa (30%) M. indica (19.28%)	C. pentandra (3.57%), A. cordifolia (3.57%), A. digitata (5.71%), P. biglobosa (11.43%), A. Occidentale (12.14%)	H. sublobata (0.71%), D. guineense (0.71%), C. laurifolia (0.71%), P. santalinoides (0.71%), C. sanderianum (0.71%), T. guineensis (1.43%), C. procera (1.43%), Ixora sp (1.43%), B. buonopozense (2.14%), A.africana (2.14%), T. neriifolia (2.14%)
M4	Multifloral	-	B. buonopozense (31.54%)	C. pentandra (3.85%), A. cordifolia (3.85%), T. neriifolia (3.85%), A. occidentale (4.61%), E. guineensis (8.46%), A. africana (8.46%), M. indica (10.77%), V. paradoxa (13.08%),	P. biglobosa (0.77%),M. oppositifolius (0.77%),M. arboreus (0.77%),P. reclinata (0.77%), P. santalinoides (0.77%), H. squamosus (0.77%), C. Arabica (2.31%), Ixora sp (2.31%), C. pulcherrima (2.31%)
M5	Multifloral	-	V. paradoxa (20.35%),	A. cordifolia (4.07%), M. arboreus (4.07%), T. guineensis (4.07%), M. indica (4.65%), E. guineensis (6.39%), A. africana (7.56%), C. Arabica (8.72%), C. pentandra (11,63%)	T. grandis (0.58%), C. javanica (0.58%), H. sublobata (0.58%), Ixora sp (0.58%), T. neriifolia (0.58%), P. angolensis (0.58%),D. gilgiana (0.58%), C. laurifolia (0.58%), P. santalinoides (0.58%), P. biglobosa (1.16%), C. roseus (1.16%), A. occidentale (1.74%) M

Table 2: Distribution of Pollens in Honey Samples from Côte d'Ivoire



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				B. buonopozense (14.53%)	oppositifolius (1.74%), C. pulcherrima (2.91%)
M ₆	Monofloral	M. indica (49.14%)		B. buonopozense (4.31%), V. paradoxa (8.62%), A. occidentale (8.62%), E. guineensis (11.21%)	P. biglobosa (0.86%), C. Arabica (0.86%), M. oppositifolius (0.86%), C. procera (0.86%), P. reclinata (0.86%), P. santalinoides (0.86%), C. sanderianum (0.86%), C. pulcherrima (0.86%), T. grandis (1.72%), H. squamosus (1.72%), A. digitata (2.59%), C. pentandra (2.59%), T. neriifolia (2.59%)
M7	Monofloral	C. pentandra (45.87%)	B. buonopozense (15.60%)	V. paradoxa (3.67%), E. guineensis (7.34%) A.digitata (7.34%), A. Africana (11.93%)	C. Arabica (0.92%), C. roseus (0.92%), H. sublobata (0.92%), P. santalinoides (0.92%), C. pulcherrima (0.92%), D. guineensis (1.43%), P. biglobosa (2.75%)
M8	Multifloral	_	V. paradoxa (22.09%)	B. buonopozense (4.07), H. squamosus (4.07%), M. indica (5.23%), A. cordifolia(6.39%), C. pulcherrima (6.39%), A. occidentale (8.14%), C. arabica (8.14%), C. pentandra (9.88%), E. guineensis (9.88%), A. Africana (10.46%),	A.digitata (0.58%), H. sublobata (0.58%), P. santalinoides (0.58%), P. biglobosa (1.16%), M. oppositifolius (1.16%), Ixora sp (1.16%)
M9	Multifloral	-	M. indica (20.93%) E. guineensis (20.93%)	A. cordifolia (4.07%), C. arabica (5.23%), M. arboreus (5.23%), T. guineensis (5.23%), B. buonopozense (6.40%), C. pentandra (6.40%), V. paradoxa (6.40%) A. africana (6.40%)	P. reclinata (0.58%), C. laurifolia (0.58%), H. sublobata (1.16%), D. gilgiana (1.16%), P. santalinoides (1.16%), M. oppositifolius (1.74%), P. angolensis (1.74%), H. squamosus (1.74%), Ixora sp (2.91%)
M ₁₀	Monofloral	P. biglobosa (52.46%)		A.digitata (4.10%), C. pentandra (5.74%), A. africana (5.74%), M. indica (11.47%), A. occidentale (11.47%)	E. guineensis (0.82%), T. grandis (0.82%), C. roseus (0.82%),C. javanica (0.82%), P. reclinata (0.82%), P. angolensis (0.82%), C. laurifolia (0.82%), C. pulcherrima (0.82%), H. squamosus (2.46%)

According to the research conducted by Feller-Demalsy & Parent (1989) ¹⁷, these pollens can be classified into four (4) categories: highly frequent taxa with pollen counts exceeding 45%; frequent taxa, also known as "accompanying pollens," representing between 15 to 45% of the total pollen count; then less frequent taxa characterized by pollen counts between 3 and 15%; and finally rare taxa present in quantities less than 3%. The taxa identified in our study were grouped into eighteen (18) plant families as follows: Fabaceae (4 species), Malvaceae and Apocynaceae (3 species each), Bombacaceae, Anacardiaceae, Arecaceae, Rubiaceae, and Euphorbiaceae

(2 species each), Sapotaceae, Mimosaceae, Asteraceae, Verbenaceae, Urticaceae, Ulmaceae, Convolvulaceae, Myristicaceae, Putranjivaceae, and Amaryllidaceae (1 species each) (Table 1). The honeys from Bouna (M1), Dimbokro (M6), Molonoublé (M7), and Dianra (M10) contain dominant pollens (Table I), with proportions exceeding 45% (\geq 45%). Consequently, these honeys can be considered monofloral honeys. According to the research of Von Der Ohe *et al.*, (2004) ²¹, honey is classified as monofloral when the number of pollens from this taxon exceeds 45%. Thus, the honey from Bouna (M1) in the Bounkani region (Northeast) can be identified as *Vitellaria*

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paradoxa honey; that from Dimbokro (M6) in the N'zi region (Central) is Manaifera indica honey; finally, the honeys from Molonoublé (M7) in the Lac region (Central) and Dianra (M10) in the Béré region (Central) correspond respectively to Ceiba pentandra and Parkia biglobosa honey. Furthermore, the honey samples from Ferkéssédougou (M2), Séguéla (M3), Biankouma (M4 and M5), Prikro (M8), and Guezon (M9), located respectively in the North, North-west, West-Central, East-Central, and West, can be considered multifloral honeys, due to the number of identified taxa. Indeed, these honeys contain several types of pollen, the quantity of which is less than 45%. The pollen composition of the honey collected in Biankouma also reveals differences, indicating the locations of the apiaries. For example, the apiaries from which the honey sample M4 originates are located in small plots within the city, while those of M5 are on the edge of the forest. This environmental diversity provides bees with a wide range of floral species (such as (the silk-cotton tree, the coffee tree, etc.) to forage. Moreover, although the apiaries from which the honey samples of Bouna (M1), Séguéla (M3), Dimbokro (M6), Prikro (M8), Guezon (M9), and Dianra (M10) originate are located in cashew plantations (Anacardium occidentale), we observed a low density of this plant in the pollen spectra of these honeys. Several factors may explain this situation, including the flowering period of cashew trees, bee behavior, as well as the selectivity and availability of floral species. ^{22, 23} These results are consistent with those of Massé et al., (2018)²³, who mentioned the low presence of Anacardium occidentale in honeys from three locations in Séguéla (Bobi, Forona, and Wongué), despite the fact that the apiaries from which these honeys originate are located in cashew fields. However, the information gathered has allowed us to determine the floral origin of the honeys produced in Côte d'Ivoire.

2. Pollen variability

The pollen composition of the various collected honey samples was analyzed using Hierarchical Cluster Analysis (HCA) and Principal Component Analysis (PCA). The aim was to highlight potential pollen variability (Figure 1 and 2).

The dendrogram obtained from the HAC revealed the presence of four (04) distinct groups among the ten (10) honey samples studied: Group I (M1, M2, M3, and M6, 4 samples), Group II (M10, 1 sample), Group III (M4, M5, M8, and M9, 4 samples), and Group IV (M7, 1 sample). These results are consistent with the clustering observed in the PCA (Figure 2). The first two principal components of the PCA, representing 48.339% and 18.168% of the total variability, allowed to characterize the pollen composition of the different honey samples.

The geographical distribution of the honey samples in Group I (M1, M2, M3, and M6) is marked by predominance in the northern part of Côte d'Ivoire. Indeed, the honeys M1 from Bouna, M2 from Ferkéssédougou, and M3 from Séguéla were collected respectively in the North-East, North, and North-West, except for M6 which comes from the Central region of Côte d'Ivoire. The honey from *Ceiba pentandra* (M7) belonging to Group IV, as well as that from *Parkia biglobosa* (M10), from Group II, are from the Central region of the country. The honey samples from Group III were collected in the West-Central region for samples M4 and M5 (Biankouma), and in the East-Central region for sample M8 (Prikro). Finally, honey M9 (Guezon) collected in the West also belongs to Group III.



Figure 1: Hierarchical Ascendant Classification (HAC) Dendrogram of the Pollen Composition of 10 Honey Samples



Figure 2: Principal Component Analysis (PCA) of the pollen composition of 10 honey samples

Statistical analysis revealed that the pollen composition varies depending on the geographical location of the honey samples within the identified groups. This pollen variability could be influenced by phenological factors, although genetic factors cannot be completely ruled out. Phenology seems to play an essential role in explaining the variability of botanical species, allowing bees to select and forage different floral species depending on the seasons. ^{22, 23}

3. Antioxidant Activity

3.1. Free radical scavenging activity (DPPH)

The histograms in Figures 3 and 4 illustrate the antioxidant effects of the different honey samples (chloroformic and ethyl acetate) against DPPH.





Figure 3: Percentages of DPPH reduction by chloroformic honey extracts



Figure 4: Percentages of DPPH reduction by ethyl acetate honey extracts



Figure 5: Concentrations of Fe³⁺ reduction to Fe²⁺ by honey samples

Chloroformic extracts showed relatively insignificant antiradical activity, with percentages reduction (PR) lower than 50% compared to quercetin (standard) (Figure 3). However, the most active extract comes from Dimbokro (M6), with the highest percentages reduction at 4 mg/ml (PR=41.31%) and 2 mg/ml (PR=34.42%), followed by samples from Biankouma (M5) and Molonoublé (M7). Regarding ethyl acetate extracts, relatively moderate antiradical activity was observed overall, except for samples from Biankouma (M5), Dimbokro (M6), Molonoublé (M7), Prikro (M8), and Dianra (M10), which exhibited PR \ge 30% at certain concentrations (Figure 4). Ethyl acetate extracts of honey with PR \geq 50% are those from Biankouma (M5) (61.24% at 4 mg/ml and 51.01% at 2 mg/ml) and Molonoublé (M7) (53.49% at 4 mg/ml; 51.68% at 2 mg/ml and 50.84% at 1 mg/ml). These two extracts appear to be the most active. The median concentration (CR₅₀) of these honey samples is respectively 1.93 mg/ml and 0.8 mg/ml for M5 and M7. Comparing these values to that of quercetin (CR₅₀ < 0.125 mg/ml), we can affirm that the Biankouma and Molonoublé samples are less active than quercetin. According to the literature, the lower the CR₅₀, the more effective the extract. ²⁴ Furthermore, the Biankouma (M5) and Molonoublé (M7) samples exhibit notable antioxidant

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potential compared to *Vitellaria* honey from Burkina Faso, which showed IC_{50} values between 1.37 \pm 0.03 and 2.43 \pm 0.08. 25

3.2. Ferric reducing Method

The honey samples reduce Fe³⁺ to Fe²⁺ at concentrations ranging from 0.065 \pm 0.004 to 0.429 \pm 0.001 mg ET/g of honey (Figure 5). These values were obtained by projecting the data on the Trolox calibration curve (y = 0.9864x + 0.0353 with R² = 0.9884).

The honey from Dianra (M10) showed the highest reduction concentration (0.429 \pm 0.001 mg ET/g of honey), while the one from Bouna (M1) had the lowest (0.065 ± 0.004 mg ET/g of honey). Some samples, such as those from Biankouma (M5) (0.371 ± 0.005 mg ET/g of honey) and Dimbokro (M6) $(0.339 \pm 0.001 \text{ mg ET/g of honey})$, exhibited noteworthy reduction concentrations. However, these values are much lower than those of catechin (2339.96 mg ET/g), quercetin (359.69 mg ET/g), and tannic acid (293.46 mg ET/g), which are standard antioxidants in pure molecule form. Compared to honey samples from Argentina (Province of Buenos Aires and Andean-Patagonian Region) (0.19 \pm 0.06 to 0.43 \pm 0.16 μg ET/mg) 20 and Mexico (Tabasco) (48 to 152 mg ET/mg) ²⁶, we can conclude that Ivorian honeys possess a significant antioxidant character. Thus, this activity detected in all Ivorian honey samples is attributed to their richness in phenolic compounds, especially flavonoids. ²⁷ Literature reports that leaves of Vitellaria paradoxa and roots of Mangifera indica are rich in anthracenosides, anthocyanins, and terpenes, respectively. ²⁸ Moreover, leaves of Vitellaria paradoxa contain antioxidant compounds with a strong presence of flavonoids. ²⁸It is noteworthy that Ivorian honey, due to the plant species it contains, has the ability to neutralize free radicals, which has a beneficial effect on health.

CONCLUSION

The objective of this study was to valorize Ivorian honey by analyzing its pollen composition and evaluating its antioxidant activity. The results revealed the presence of thirty (30) pollen taxa, grouped into eighteen (18) families, in honey samples from ten localities in Côte d'Ivoire. Based on the pollen spectrum, the studied honeys were classified into two categories: monofloral and multifloral. Hierarchical Cluster Analysis (HCA) and Principal Component Analysis (PCA) highlighted quantitative and geographical variability of pollens within the four identified groups.

The antioxidant activity of the tested honeys in the Ferric Reducing Antioxidant Power (FRAP) assay is lower than that of standard antioxidants such as quercetin, catechin, and tannic acid. Among the extracts, those from the Béré, Tonkpi, and N'zi regions exhibited the highest antioxidant activity according to the FRAP test. Regarding the antioxidant activity measured by the 2,2-diphenyl-1picrylhydrazyl (DPPH) method, selective ethyl acetate extracts showed significant activity compared to chloroform extracts. The extracts from Biankouma (M5) and Molonoublé (M7) also showed notable activity, with estimated CR_{50} values of 1.93 mg/ml and 0.8 mg/ml, respectively. In conclusion, the antioxidant activity of these honeys is partly attributable to phytochemicals present in the plant species. We therefore recommend these honeys to consumers, as they may help to address nutritional deficiencies and possess therapeutic properties.

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