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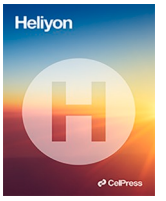

Research article

Physicochemical properties and antioxidant potential of honey from Cameroon agroecological zones

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ABSTRACT

Cellular respiration produces reactive oxygen species (ROS), which can lead to oxidative stress and significant health issues, including chronic diseases and cancer. Antioxidants play a critical role in neutralizing ROS. This study investigates the physicochemical properties and antioxidant activities of honey sourced from five distinct agroecological zones in Cameroon. Multifloral honey samples (n = 9) were collected from local beekeepers and analyzed for parameters including density, pH, total sugar content, total phenolic content (TPC), flavonoid content (FC), and antioxidant potential (DPPH, FRAP, TAC). The samples ranged in color from light amber to dark amber, with densities between 1.43 and 1.51 g/mL and sugar contents of 70.33 %–83.16 %. pH levels varied from 3.30 to 4.10. Antioxidant analysis revealed phenolic contents ranging from 26.75 to 85.06 mg GAE/100 g and flavonoid contents between 5.22 and 14.47 mg QE/100 g. Significant differences in antioxidant activity were noted, particularly in correlation with color intensity and pH. Honeys with more reddish and greenish hues exhibited better FRAP values, while those with a pH around 4 showed improved DPPH activity. This preliminary study underscores the importance of regional differences in honey quality and its potential health benefits, advocating for further research on the diverse honey types in Cameroon.

1. Introduction

Cellular respiration in living organisms leads to the production of reactive oxygen species (ROS), which can pose significant health risks. The natural metabolic processes that produce these oxygen molecules also generate free radicals as byproducts. Extended exposure to free radicals results in oxidative stress, which damages proteins, lipids, and nucleic acids. This oxidative damage tends to increase with age, contributing to chronic diseases, weakened immune responses, and serious health conditions, including cancer, diabetes, and neurodegenerative disorders [1,2]. The role of oxidative stress in various chronic illnesses is well-documented, making it crucial to explore strategies for mitigation. Antioxidants—both endogenous and exogenous—play a vital role in neutralizing ROS and

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reactive nitrogen species (RNS), thereby protecting cellular integrity [3,4]. The food industry has increasingly favored natural antioxidants from plant sources over synthetic alternatives due to concerns over toxicity at high concentrations [5,6].

Among these natural antioxidants, honey stands out due to its recognized medicinal properties [7]. Produced by bees (*Apis*

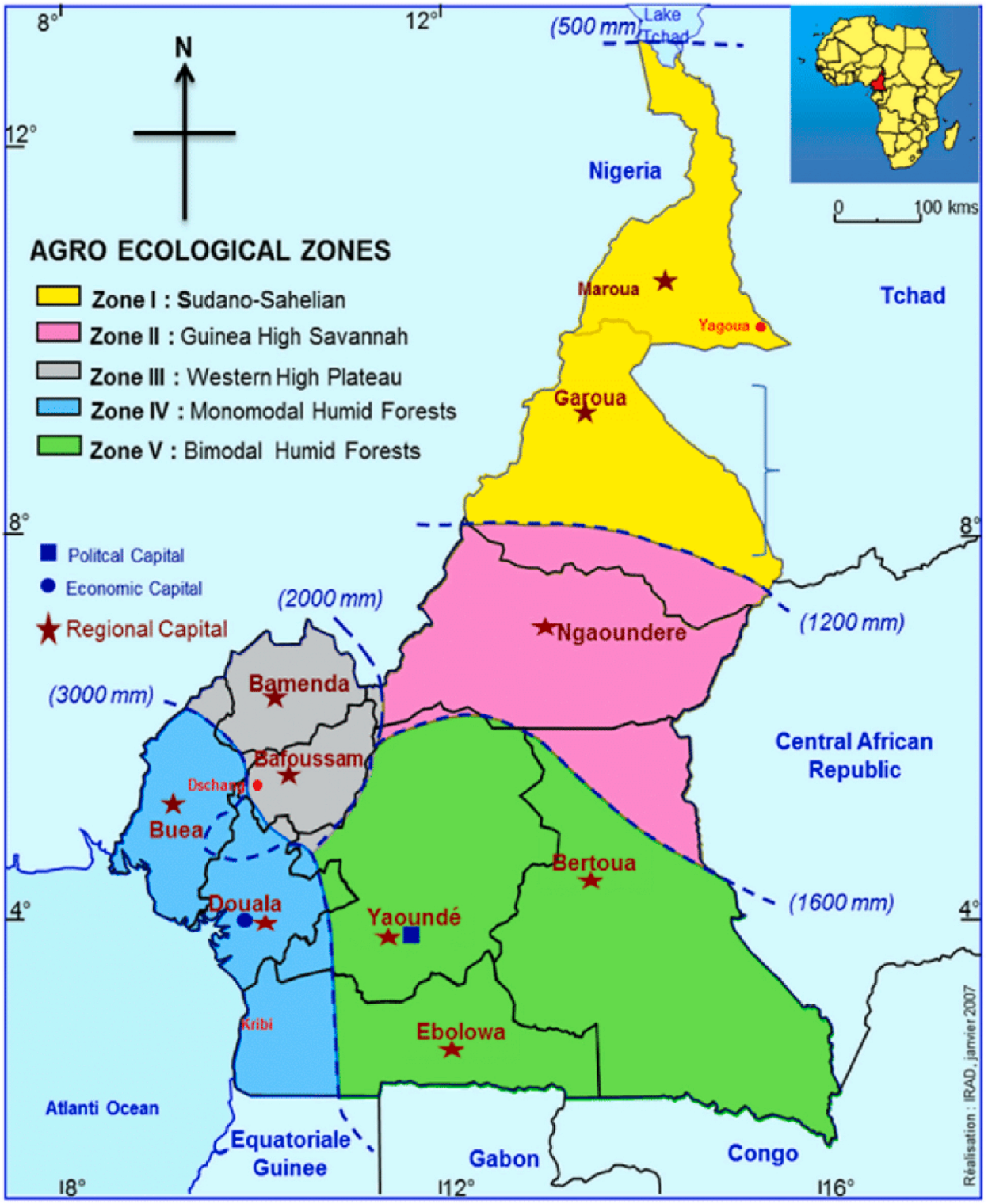

Fig. 1. Map of Cameroon and its five agroecological zones.

mellifera) from nectar and plant secretions, honey consists of approximately 80 % sugars, primarily glucose and fructose, along with water, protein, and trace minerals [8,9]. Honey is rich in phytochemicals, organic acids, vitamins, and enzymes, making it a valuable dietary source of antioxidants [10]. Historically, honey has been used for medicinal purposes, including wound treatment [11,12]. Its antioxidant properties play a crucial role in preventing both acute and chronic conditions, such as inflammatory and cardiovascular diseases, as well as cancer [7]. This antioxidant activity can be attributed to various compounds, including polyphenols, ascorbic acid, and enzymes, which help scavenge free radicals and reduce oxidative stress [13].

The physicochemical properties of honey can vary significantly based on factors such as bee species, geographical origin, and floral sources. For instance, the quality and antioxidant capacity of honey are influenced by environmental conditions, seasonal changes, and processing methods [14,15]. In Cameroon, the presence of five distinct agroecological zones—Sudano-Sahelian, Guinea High Savannah, Western High Plateau, Monomodal Humid Forest, and Bimodal Humid Forest (Fig. 1) indicates a rich diversity of flora, which may affect the characteristics of honey produced in different regions. There is scarce data about the honey types from this country.

This preliminary study aims to investigate the physicochemical properties and antioxidant activities of honey from various agroecological areas in Cameroon, highlighting the significance of regional differences in honey quality and its potential health benefits.

2. Material and methods

2.1. Honey samples collection

Nine honey samples coded M1 to M9 were collected during the year 2021 from the five distinct agroecological zones of Cameroon previously mentioned. They were procured from beekeepers and apicultural associations and transported to the laboratory at ambient temperature for subsequent analysis. These samples were multifloral nectar.

2.2. Physicochemical analysis

The collected honey samples underwent the measurement of various physicochemical parameters in triplicate, including density, pH, total sugar content, and color intensity.

2.2.1. Density

First, the weight equivalent to 10 mL of the sample was measured. The density (D) was estimated using the following formula:

$$D = \frac{\mathrm{m}}{V}$$

Where m is the weight and V is the volume.

2.2.2. Determination of sugar content

The sugar concentration in the honey samples was assessed using a spectrophotometric method adapted from the AOAC (2000) protocol, with slight modifications following the procedure outlined by Akharaiyi & Lawal [16]. In summary, 0.5 mL of honey sample was mixed with 3 mL of a hydroalcoholic solution (1 mL of ethanol and 2 mL of sterile distilled water). Subsequently, 10 mL of boiling ethanol was added to the mixture, which was then vigorously shaken and centrifuged at 4000 rpm for 10 min to yield a clear supernatant containing free sugars for analysis. This supernatant was further diluted tenfold using sterile distilled water. Colorimetric analysis was conducted by combining 1 mL of the diluted supernatant with 0.5 mL of 5 % phenol and 2.5 mL of concentrated H_2SO_4. Sterile distilled water served as a blank control. The test tubes were incubated at 25 °C for 10 min, after which the optical density was measured at 490 nm. Glucose was used as the standard reference (0–50 mg/mL).

2.2.3. pH

Ten grams (10 g) of honey were carefully measured and dissolved in 75 mL of distilled water. The pH level of the resulting solution was determined using a pH meter, specifically the Hanna HI 98127 pHEP 4 model [17].

2.2.4. Colour analysis

Photographs of the honey samples were captured using a LUMIX 005A-02 camera. The RGB (Red, Green, and Blue) values of the images were assessed using the ImageJ software application [18].

2.3. Phytochemical analysis

2.3.1. Total polyphenol content (TPC) assessment

The total polyphenol content was assessed using the Folin-Ciocalteu method, as outlined by Singleton and Rossi [19], with gallic acid as the standard reference. In this procedure, 50 μL of a diluted honey solution (tenfold) was dispensed into tubes, followed by the addition of 2.5 mL of 10 % Folin-Ciocalteu reagent and 2 mL of 7.5 % Na_2CO_3. After thorough mixing, the tubes were incubated in the dark for 30 min. Absorbance was read at 765 nm, and results were reported as milligrams of gallic acid equivalent (GAE) per gram of

dry matter (mg GAE/g DM).

2.3.2. Total flavonoid content (TPC) assessment

The colorimetric method utilizing aluminum chloride was employed in this study. Initially, a 0.2 mL aliquot of honey extract was combined with 0.2 mL of 10 % aluminum chloride ($AlCl_3$), followed by the addition of 0.2 mL of 1 M potassium acetate (CH_3COOK) and 1.12 mL of distilled water. The mixture was thoroughly mixed and incubated at room temperature for 30 min, after which the absorbance was measured at 415 nm against the reagent blank. Quercetin (ranging from 0 to 1000 μg/mL) was used as the standard reference. The findings were expressed in milligrams of quercetin equivalent per gram of dry matter (mg QE/g DM) [20].

2.4. Evaluation of antioxidant potential

The antioxidant capacity of the honey samples was assessed through three distinct methods: DPPH free radical scavenging, iron reduction (FRAP), and total antioxidant capacity (TAC) analysis.

2.4.1. Trapping of the radical DPPH (2,2-diphenyl-1-picrylhydrazyl)

The capacity of honey samples to scavenge free radicals against DPPH (1,1-diphenyl-2-picrylhydrazyl) was assessed following a

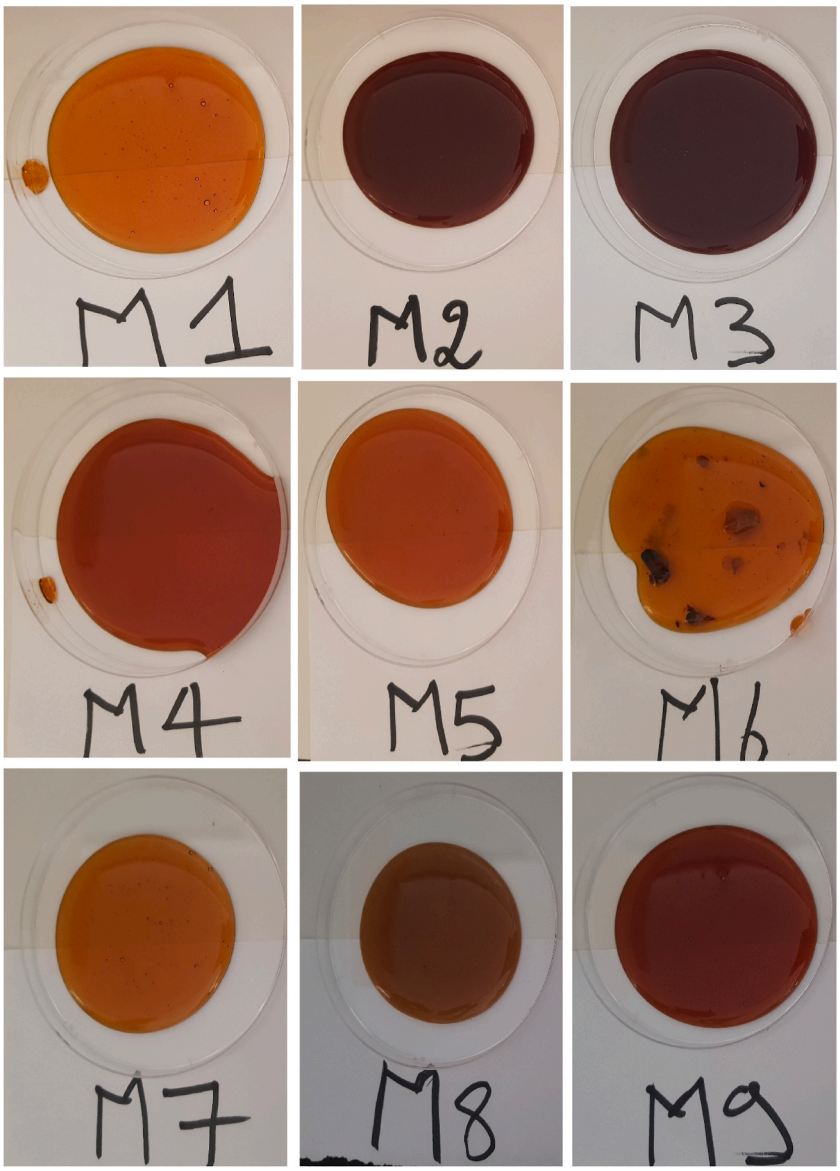

Fig. 2. Overview of the different honey samples collected (M1-M9).

slightly adapted protocol from Katalinić et al. [21]. In this procedure, 50 μL of the honey solution was combined with 2.5 mL of freshly prepared DPPH solution (55 μM) in ethanol. The mixture was thoroughly mixed and incubated in the dark for 30 min at room temperature, followed by measurement of absorbance at 517 nm. A control sample containing only DPPH without extracts was also prepared. Ascorbic acid served as the standard antioxidant. The inhibition percentage (%I) was calculated using the following equation:

$$I\% = [(Abs0 - Abs1)/Abs0] \times 100$$

Where Abs0 is the absorbance of the control and Abs1 is the absorbance in the presence of the test compound.

The IC_{50} (half maximal inhibitory concentration) was determined graphically using the calibration curve generated by plotting the sample concentration against the corresponding scavenging effect (I%, inhibition percentage).

2.4.2. Reducing ferric capacity (FRAP: ferric reducing antioxidant power)

The reducing power of the honey extracts was evaluated using the method outlined by Oyaizu [22]. Initially, 1 mL of honey extract was combined with 2.5 mL of a 0.2 M sodium phosphate buffer (pH 6.6) and 2.5 mL of 1 % potassium ferrocyanide. This mixture was then incubated in a water bath at 50 °C for 20 min. Next, 2.5 mL of 10 % trichloroacetic acid was added, followed by centrifugation at 650*g* for 10 min. The resulting supernatant (2.5 mL) was mixed with 2.5 mL of distilled water and 0.5 mL of 0.1 % ferric chloride solution. The intensity of the resultant blue-green color was measured spectrophotometrically at 700 nm. Ascorbic acid was employed as the positive control.

2.4.3. Determination of total antioxidant capacity

The total antioxidant capacity of the extract was determined through the formation of a phosphomolybdenum complex, following the method described by Prieto et al. [23]. Specifically, 0.2 mL of the honey extract was combined with 2 mL of a reagent solution containing 0.6 M H_2SO_4, 28 mM sodium phosphate, and 4 mM ammonium molybdate. The mixture was then boiled for 60 min, and absorbance was measured at 695 nm. Ascorbic acid served as the standard reference, and total antioxidant capacity was expressed as milligrams of ascorbic acid equivalent per gram of dry matter (mg AAE/g DM).

2.5. Statistical analyses

Data were analyzed using the Statistical Package for Social Sciences (SPSS) Version 27.0 (SPSS, Inc., IBM Corporation, Chicago, USA). An analysis of variance (ANOVA)-Tukey HSD test was conducted to evaluate the significance of the differences observed between the mean values ($P < 0.05$). All experiments were conducted in triplicate. Furthermore, the Pearson correlation test was utilized to determine the strength of the association between the tested parameters.

3. Results

Fig. 2 depicts the various honey samples examined, showing differences in appearance primarily related to color, ranging from Light Amber to Dark Amber. Notably, honey samples collected from the same agroecological zone also exhibited variations. For instance, samples from the Guinea High Savannah zone (M2, M6, and M8) and the Western High Plateau (M1, M3, and M7) displayed

Table 1
Physicochemical characteristics of the honey samples.

Honey samples	Sampling region	Agroecological zone	Density (g/L)	Sugar content (%)	pH	Color parameters		
						Red	Green	Blue
M1	West	Western High Plateau	1.51 ± 0.02^{a}	77.47 ± 1.1^{a}	4.10 ± 0.3^{a}	184.59 ± 1.80^{a}	84.27 ± 0.41^{a}	24.86 ± 0.91^{c}
M2	Adamaoua	Guinea High Savannah	1.46 ± 0.01^{a}	77.98 ± 1.15^{a}	3.90 ± 0.1^{a}	69.84 ± 0.59^{f}	34.18 ± 0.41^{f}	31.97 ± 0.63^{b}
M3	West	Western High Plateau	1.47 ± 0.01^{a}	70.33 ± 1.3^{a}	3.80 ± 0.15^{a}	65.52 ± 0.23^{g}	40.68 ± 0.19^{e}	43.48 ± 0.12^{a}
M4	Littoral	Monodal Humid Forests	1.43 ± 0.03^{a}	75.79 ± 1.25^{a}	3.50 ± 0.2^{a}	134.32 ± 0.36^{d}	44.91 ± 0.28^{d}	31.90 ± 0.90^{b}
M5	Centre	Humid Forests	1.41 ± 0.01^{a}	80.10 ± 1.11^{a}	3.60 ± 0.17^{a}	153.90 ± 1.54^{b}	57.80 ± 0.64^{c}	31.20 ± 1.11^{b}
M6	Adamaoua	Guinea High Savannah	1.43 ± 0.03^{a}	78.55 ± 1.31^{a}	3.70 ± 0.25^{a}	144.67 ± 1.03^{c}	62.80 ± 0.79^{b}	14.48 ± 1.07^{d}
M7	Nord West	Western High Plateau	1.47 ± 0.01^{a}	83.16 ± 1.4^{a}	3.40 ± 0.3^{a}	138.95 ± 1.18^{d}	63.64 ± 0.62^{b}	22.09 ± 0.77^{c}
M8	Adamaoua	Guinea High Savannah	1.45 ± 0.02^{a}	82.83 ± 1.32^{a}	3.30 ± 0.2^{a}	81.08 ± 0.60^{e}	39.26 ± 1.63^{e}	23.54 ± 2.78^{c}
M9	Littoral	Monodal Humid Forests	1.48 ± 0.01^{a}	81.86 ± 1.12^{a}	3.90 ± 0.1^{a}	82.38 ± 0.55^{e}	28.84 ± 0.30^{g}	26.14 ± 0.58^{c}

*Within the same column, values followed by the same letter are significantly similar ($p < 0.05$).

hues from light amber to dark amber.

3.1. Physicochemical attributes of the honey samples

The physicochemical analysis results for the nine collected honey samples are summarized in Table 1. Across various parameters—including density, sugar content, and pH—no significant differences were observed among the honey samples from different agroecological zones. Density ranged from 1.43 to 1.51 g/mL, sugar content varied between 70.33 % and 83.16 %, and pH values ranged from 3.30 to 4.10. Color analysis indicated that all samples exhibited a predominant red hue, with minimal green or blue tones. However, there were notable variations in the intensity of the red, green, and blue components. For example, sample M1 from the Western High Plateau had the highest Red (184) and Green (84) values, while sample M3 from the same area appeared the darkest with a Blue value of 43.48.

3.2. Phytochemical contents and antioxidant activities of the samples

The phytochemical contents and antioxidant properties are presented in Table 2. Phenolic content ranged from 26.75 to 85.06 mg GAE/100 g of honey, while flavonoid content varied from 5.22 to 14.47 mg QE/100 g. IC50 values showed considerable variability, with the lowest and highest values recorded for FRAP (67.75 and 33.88 mg/mL), TAC (1.61 and 0.71 mg/mL), and DPPH (48.12–17.58 mg/mL). Significant disparities were observed among samples across all tested parameters, particularly when comparing those from the Western High Plateau (M1, M3, and M7) and the Guinea High Savannah (M2, M6, and M8).

3.3. Correlation between the tested parameters and antioxidant activities of honey samples

As shown in Table 3, no statistically significant correlations were found between the antioxidant activities of the honey samples and their polyphenol or flavonoid content ($p > 0.05$). However, 64.8 % of the polyphenol data and 45.8 % of the flavonoid data suggested a correlation with TAC.

The relationships between physicochemical parameters and phytochemical content/antioxidant activities are detailed in Table 4. A significant negative correlation was observed between the ferric ion reducing capacity (IC50 FRAP) and the intensity of red color in honey ($R^2 = 0.76$), as well as with the green aspect ($R^2 = 0.69$). Additionally, a significant negative correlation was noted between pH and IC50 DPPH. Specifically, 72 % of the data indicated that honey samples with a pH around 4 had lower IC50 DPPH values than those with a pH of 3. Furthermore, only 41 % of the data showed a correlation between sugar content and DPPH, which was not statistically significant.

4. Discussion

The honey samples analyzed in this study generally meet the standard limits set by the Codex Alimentarius and the International Honey Commission (pH 3.40–6.10 and not less than 65 % for reducing sugars). Their potential medicinal properties may be attributed to their acidic nature, possibly due to organic acids that create an environment inhibiting the growth of pathogenic microbes [24]. Density measurements ranged from 1.41 to 1.51 g/mL, with seven samples exceeding the average standard (1.39–1.44 g/mL at 20 °C), although no significant differences were noted. The color of the honey samples varied from light amber to dark amber, consistent with findings by Amanah [25]. Notably, honey color has been associated with its nutritional value, as darker honeys tend to have higher

Table 2
Phytochemical characteristics of the honey samples.

Honey samples	Sampling region	Agroecological zone	Phenolic content (mg GAE/100 g)	Flavonoid content (mg QE/100 g)	IC50 FRAP (mg/mL)	IC50 TAC (mg/mL)	IC50 DPPH (mg/mL)
M1	West	Western High Plateau	80.32 ± 1.61^{a}	7.61 ± 0.4^{a}	33.88^{b}	1.01^{a}	17.58^{a}
M2	Adamaoua	Guinea High Savannah	85.06 ± 1.10^{a}	14.47 ± 0.37^{b}	60.9^{a}	1.2^{a}	22.6^{b}
M3	West	Western High Plateau	48.21 ± 1.96^{b}	5.95 ± 0.10^{c}	46.67^{b}	0.99^{a}	22.67^{b}
M4	Littoral	Monodal Humid Forests	45.83 ± 1.84^{b}	5.46 ± 0.93^{c}	41.24^{b}	1.61^{b}	38.77^{c}
M5	Centre	Humid Forests	35.83 ± 1.35^{b}	3.20 ± 0.48^{d}	41.14^{b}	1.01^{a}	23.04^{b}
M6	Adamaoua	Guinea High Savannah	30.91 ± 1.79^{b}	$5.01 \pm 0.\ 61^{c}$	49.23^{b}	0.71^{c}	23.04^{b}
M7	Nord West	Western High Plateau	30.75 ± 1.50^{b}	3.43 ± 0.41^{d}	42.11^{b}	0.96^{a}	48.12^{d}
M8	Adamaoua	Guinea High Savannah	62.65 ± 1.62^{a}	8.06 ± 0.18^{a}	67.75^{a}	1.02^{a}	30.79^{c}
M9	Littoral	Monodal Humid Forests	26.75 ± 1.47^{b}	5.22 ± 0.3^{c}	50.88^{b}	0.93^{a}	28.29^{c}

*Within the same column, values followed by the same letter are significantly similar ($p < 0.05$).

Table 3
Correlation between total phenolic content, total flavonoids and antioxidant activities of samples.

Parameter	Antioxidant test	Correlation
Phenolic content	DPPH	$R^2 = 0.434$ $p = 0.320$
	FRAP	$R^2 = 0.501$ $p = 0.648$
	TAC	$R^2 = 0.648$ $p = 0.487$
Flavonoid content	DPPH	$R^2 = 0.378$ $p = 0.233$
	FRAP	$R^2 = 0.289$ $p = 0.589$
	TAC	$R^2 = 0.458$ $p = 0.361$

Table 4
Change of phytochemical content and antioxidant activities of honey samples with their measured physicochemical parameters.

Variables	Phenolic content	Flavonoid content	IC50 FRAP	IC50 TAC	IC50 DPPH
Density	0.33	0.23	−0.18	−0.20	−0.13
Sugar content	−0.23	−0.14	0.27	−0.27	0.41
Red	−0.09	−0.45	−0.76[a]	−0.05	0.01
Green	0.09	−0.32	−0.69[a]	−0.26	−0.10
Blue	0.22	0.17	−0.07	0.46	−0.17
pH	0.38	0.36	−0.30	−0.17	−0.72[a]

[a] Significant correlation ($p < 0.05$).

mineral content [26].

The total flavonoid content found in this study (3.20–14.47 mg/100 g) was lower than the range of 8.0–17.0 mg QE/100 g reported by Nayik et al. [27] from India. Similarly, the total phenolic content (TPC) of the tested samples (26.75–85.06 mg GAE/100 g) was lower than the values reported by Meda et al. [28], who studied 27 honey samples in Burkina Faso, which included 18 multifloral, 2 honeydew, and 7 unifloral types, with TPC ranging from 32.59 to 114.5 mg/100 g. However, the TPC values in this study were higher than those reported by Yaoa et al. [29], who found values ranging from 2.13 to 12.11 mg/100 g in their analysis of various honey types using HPLC.

The antioxidant activity of each honey sample was evaluated based on its free radical scavenging activity, measured through the IC50 parameter, which indicates the concentration needed to inhibit 50 % of free radicals. A lower IC50 value reflects a greater capacity to neutralize free radicals, a common method for evaluating honey samples [30,31]. Table 2 presents the scavenging abilities of the multifloral honey samples expressed as IC50 values. For the DPPH radical, values ranged from 17.58 to 48.12 mg/mL, which is lower than those reported by Ferreira et al. [32] for Portuguese honey samples (ranging from 106.67 to 168.94 mg/mL), highlighting the quality of honey from Cameroon.

The antioxidant potential of honey contributes to its protective and therapeutic properties for humans. Regardless of the testing method used (DPPH, TAC, or FRAP), significant differences were observed, even among samples from the same agroecological zone. Various factors influence the antioxidant activity of honey, including floral source, geographic origin, collection season, storage conditions, bee species, and interactions between chemical compounds and enzymes in the honey. Other factors such as concentration, temperature, light exposure, substrate type, and the presence of micro-components acting as pro-oxidants or synergists have also been cited [33], which may explain the observed variations.

Polyphenols, including flavonoids, acids, and other phenolic compounds, are crucial contributors to the antioxidant capacity of many foods. They are of particular interest in medical and nutritional research due to their functional properties. Besides acting as radical scavengers, polyphenols can function as effective immune modulators and inhibitors of hormone action [34]. They are believed to be potent scavengers of peroxyl radicals, primarily due to the high mobility of hydrogen in their molecular structures [35]. However, this study found no significant correlation between TPC and flavonoid content in honey samples and their antioxidant potential. Gheldof et al. [13] reported that the antioxidant capacity of honey arises from the combined effects of various compounds, including phenolics, peptides, organic acids, enzymes, Maillard reaction products, and possibly other minor components. Variations in polyphenol content among honey samples were likely due to factors such as floral source, environmental conditions (e.g., soil type and climate), genetic influences, and processing methods, as documented in the literature [35–37].

Data analysis revealed that only color parameters and pH significantly influenced the antioxidant activity of honey samples. Samples with more pronounced red and green hues exhibited lower IC50 FRAP values. Moniruzzaman et al. [38] noted that the intensity of honey color reflects the presence of pigments with antioxidant properties, such as carotenoids and flavonoids. Similarly, Montenegro et al. [39] found that darker honey typically has higher total phenolic content, leading to increased antioxidant capacity. In contrast, honey samples with a pH around 4 showed lower IC50 DPPH values. Nayik et al. [27] observed similar results when adjusting the pH of Indian honey from 3 to 6, possibly due to the various antioxidant groups present.

5. Conclusion

This study examined the physicochemical properties and antioxidant activities of honey from various agroecological zones in Cameroon. The samples adhered to pH and sugar content regulations, indicating compliance with international standards. While antioxidant capabilities showed no significant variation based on the agroecological zone, the visual color—characterized by reddish and greenish tones—and mild acidity emerged as promising indicators of their potential health benefits. Given their notable antioxidant properties, encouraging the consumption of these honeys among the populace is advisable.

CRediT authorship contribution statement

Huguette Yangoua: Writing – review & editing, Supervision, Project administration, Conceptualization. **Ruth Edwige Kemadjou Dibacto:** Writing – original draft, Methodology, Formal analysis, Data curation. **Boris Ronald Tonou Tchuente:** Writing – review & editing, Methodology, Formal analysis, Data curation. **Emilienne Carine Nyobe:** Writing – review & editing, Methodology, Formal analysis, Data curation. **Maxwell Wandji Nguedjo:** Writing – review & editing, Methodology, Formal analysis, Data curation. **Tchuenchieu Kamgain Alex Dimitri:** Writing – review & editing, Supervision, Data curation, Conceptualization. **Melanie Flore Godam Kamini:** Writing – review & editing, Project administration, Methodology, Conceptualization.

Data availability

Data will be made available on request.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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