Heliyon 10 (2024) e30839

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon

Research article

Bio-functional properties and phytochemical composition of selected *Apis mellifera* honey from Africa

Nelly N. Ndungu [a,1], Timothy M. Kegode [a,1], Justus K. Kurgat [a], Steve B.S. Baleba [a], Xavier Cheseto [a], S. Turner [b], Geraud C. Tasse Taboue [c], J.M. Kasina [d], Sevgan Subramanian [a], Beatrice T. Nganso [a,*]

[a] *International Centre of Insect Physiology and Ecology (icipe), Nairobi, Kenya*
[b] *Malaika Honey Company, Kampala, Uganda*
[c] *Institute of Agricultural Research for Development, Bangangté, Cameroon*
[d] *Apiculture and Beneficial Insects Research Institute, Kenya Agricultural and Livestock Research Organization, P.O. Box 32-30403, Marigat, Kenya*

ARTICLE INFO

Keywords:
African honeys
Phytochemicals
Antioxidant/antimicrobial activity
Volatile compounds

ABSTRACT

Globally, the demand for natural remedies such as honey to manage ailments has increased. Yet, the health benefits and chemical composition of African honeys are not well understood. Therefore, this study aimed to characterise the bio-functional properties and the phytochemical composition of 18 *Apis mellifera* honeys from Kenya, Uganda, and Cameroon in comparison to the popular and commercially available Manuka 5+ honey from New Zealand. The 2,2-diphenyl-1-picrylhydrazyl radical scavenging assay (DPPH-RSA) was used to determine the antioxidant property, whilst the agar well diffusion and broth dilution (Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC)) assays were used to determine antimicrobial property. Further, colorimetric methods were used for phytochemical analysis. Our results showed that honeys collected from Rift Valley region of Kenya (e.g. Poi, Salabani and Mbechot) and Western region of Cameron (e.g. Bangoulap) had the highest antioxidant (DPPH RSA of 41.52–43.81%) and antimicrobial (MIC (3.125–6.25% w/v) and MBC (6.25–12.5% w/v)) activities. Additionally, the total flavonoid (770–970 mg QE/100 g), phenol (944.79–1047.53 mg GAE/100 g), terpenoid (239.78–320.89 mg LE/100 g) and alkaloid (119.40–266.57 mg CE/100 g) contents reached the highest levels in these bioactive African honeys, which significantly and positively correlated with their bio-functional properties. The functional and phytochemical composition of these bioactive African honeys were similar to or higher than those of the Manuka 5+ honey. Furthermore, gas chromatography-mass spectrometry analysis of African honeys revealed 10 most prominent volatile organic compounds that contribute to their geographical distinction: triacontane, heptacosane, (Z)-9-tricosene, tetracosane, 6-propyl-2,3-dihydropyran-2,4-dione, octacosane, 1,2,4-trimethylcyclohexane, 1,3-bis(1,1-dimethylethyl) benzene, 2-methylheptane and phytol. Overall, our findings suggest that some of the tested African honeys are natural sources of antimicrobial and antioxidant therapies that can be exploited upon further research and commercialized as high value honey.

* Corresponding author.
E-mail addresses: bnganso@icipe.org, ngansobeatrice@gmail.com (B.T. Nganso).
[1] equal contribution.

https://doi.org/10.1016/j.heliyon.2024.e30839
Received 29 January 2024; Received in revised form 9 March 2024; Accepted 6 May 2024
Available online 9 May 2024

1. Introduction

Honey is a natural product that honey bees (*Apis mellifera* L.) produce using nectar collected from a single species of flowering plant (referred to as mono-floral honey) or from a number of flowering plant species (referred to as poly-floral honey). The distinctive characteristic of each type of honey in terms of aroma, flavour, colour, and chemical composition is influenced by its geographical, botanical, and seasonal origin [1,2]. Its chemical composition is further influenced by the harvest and post-harvest processing methods and storage conditions [3]. Honey is comprised of more than 200 compounds, primarily carbohydrates (constituting 80%, majorly fructose and glucose) and water (approximately 17%), accompanied by trace quantities of other constituents (accounting for 3%) [4,5]. These include amino acids (predominantly proline), proteins, minerals, vitamins, enzymes, organic acids, and an array of phytochemical compounds such as flavonoids, phenolic acids, alkaloids, terpenoids, among others. The multifaceted array of compounds within honey imparts it with a wealth of nutritional and medicinal attributes. Consequently, there has been a substantial surge in demand for honey across the food, pharmaceutical, cosmetic and beverage industries over centuries due primarily to its antimicrobial and antioxidant properties [6], and it is anticipated that by this year 2024, the consumption of honey may hit 2.8 million tons worldwide [7].

The ability of honey to inhibit microbial growth is measured quantitatively using well/disk diffusion assay, agar dilution methods, broth (micro) dilution assay, and/or time-kill assay in the laboratory [8]. It is worth noting that the antimicrobial property of honey is attributed to its high osmotic pressure, low moisture content, low pH and acidity, and the presence of compounds with antibacterial effects such as hydrogen peroxide (H_2O_2), methylglyoxal, bee defensin-1, and polyphenols (flavonoids and phenolic acids) and volatile compounds [2,5,9]. These numerous components can act upon different target sites additively or synergistically, making it difficult for any bacterium to develop resistance [5]. Whereas the presence of polyphenolic compounds (flavonoids, phenolic acids and their derivatives), enzymes (such as catalase and peroxidase), proteins, amino acids and other compounds acting through several mechanisms contribute to the antioxidant property of honey [10]. The latter is generally determined spectro-photometrically using the 2, 2-diphenyl-1-picrylhydrazyl (DPPH) scavenging method [11]. In terms of value, the New Zealand/Australian mono-floral Manuka honey is the famous and has been widely used for a variety of medical applications over conventional antibiotics because it displays increased antimicrobial, antioxidant, anticancer and antibiofilm capacity, among others [12,13].

Globally, intensive research on antimicrobial (against antibiotic susceptible and resistant bacterial strains in humans) and antioxidant properties of honey including its bioactive chemical constituents has been carried out in Asia, Europe, and America, with Africa still lagging [14]; albeit a high number of endemic and medicinal plant species reported in the African continent when compared to others [15]. It is not fully understood how these biological activities together with the phytochemical constituents vary among different African honeys, and how they compare with those of the highly valued Manuka honey. Specifically, only few studies carried out in Ethiopia [16], Kenya [17,18], South Africa [19], and Cameroon [20] have comparatively characterized these biological activities and/or phytochemical contents just between their country-level honey and the Manuka honey. This study therefore aimed for the first time to compare the *in vitro* antimicrobial and antioxidant activities as well as phytochemical constituents among honeys from distinct geographical regions in Kenya, Uganda, and Cameroon in comparison to the Manuka 5+ honey. Further, the volatile organic compounds (VOCs) of the studied African honeys were analysed and compared using gas chromatography coupled to mass spectrometry (GC-MS). The outcomes of this investigation offer valuable insights into the potential value of these honeys as natural antimicrobial and antioxidant agents thereby directly contributing to the Sustainable Development Goals (SDGs) 3, while concurrently establishing a distinctive geographical signature for each African honey variety.

2. Materials and methods

2.1. Chemicals

Analytical grade chemicals: Colchicine, ferric–III–chloride (FeCl3), 1,10-phenanthroline, hydrochloric acid (HCl), Gallic acid, Folin–Ciocalteu's reagent, sodium carbonate (Na_2CO_3), aluminum chloride ($AlCl_3$), sodium nitrite ($NaNO_2$), quercetin, sodium hydroxide (NaOH), chloroform, linalool, sulphuric acid (H_2SO_4), absolute ethanol, and 2, 2-diphenyl-1-picrylhydrazyl (DPPH) were purchased from Merck (Massachusetts, USA) through Kobian, Kenya Ltd. The Mueller-Hinton agar (MHA) was purchased from Himedia Laboratories Pvt. Ltd (Mumbai, India) through F&S Scientific, Nairobi, Kenya. Methanol and n-hexane were purchased from Merck (Darmstadt, Germany).

2.2. Honey sampling

Eighteen *Apis mellifera* honey samples were collected from apiaries located in different geographical areas in three African countries including Kenya, Uganda, and Cameroon (Fig. 1). The description of the climatic conditions within each apiary site is provided in Table S1. From each location, honeys were collected from five randomly selected hives and pooled to represent one sample per individual apiary. All these samples were sent to the African Reference Laboratory for Bee Health at *icipe* and were immediately analysed as described below. In this study, the mono-floral Manuka 5 + honey from New Zealand purchased in a supermarket in Kenya was used as a positive control.

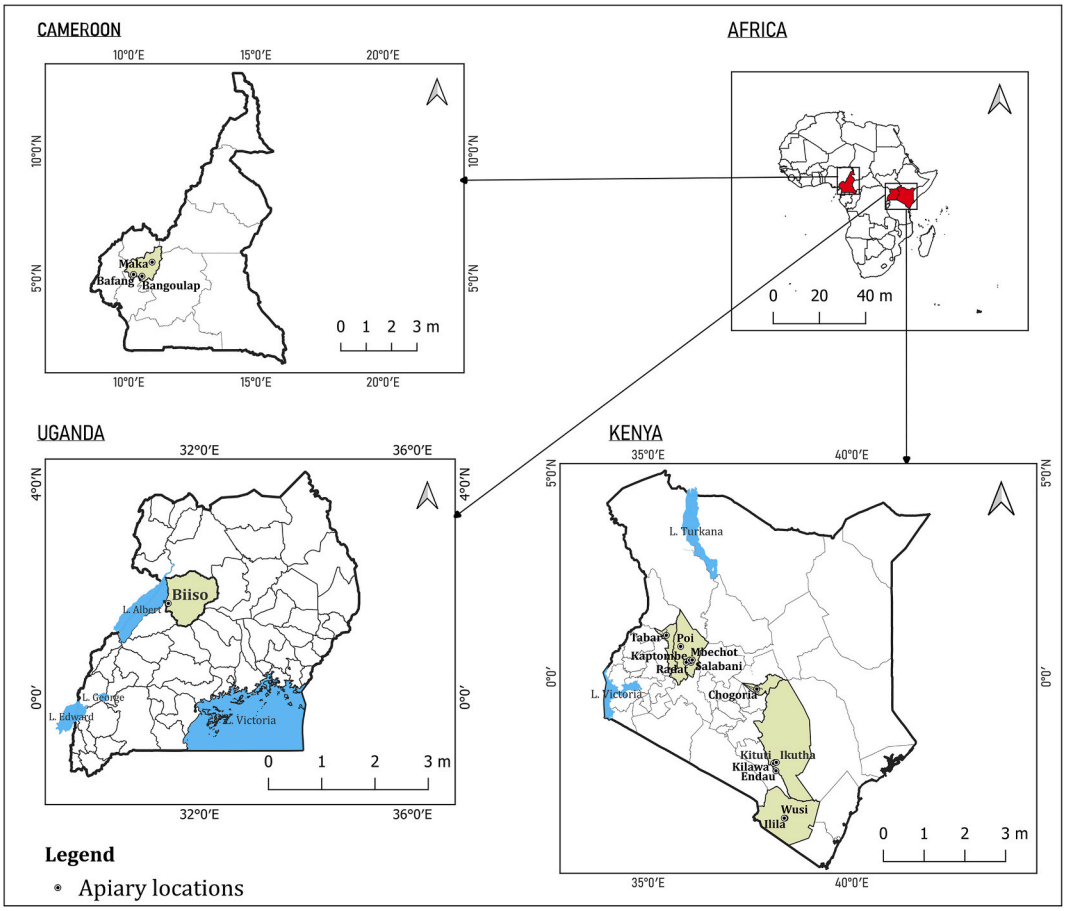

Fig. 1. Map showing the 17 apiary sites (white dots) in Cameroon, Uganda and Kenya.

2.3. Biological activities of the honey samples

2.3.1. Antioxidant activity (2,2-diphenyl-1-picrylhydrazyl radical scavenging activity (DPPH-RSA)) of the honey samples

One (1) g of each honey sample was dissolved in 10 ml of absolute methanol for *in vitro* analysis of antioxidant activity or free RSA. Thereafter, the DPPH assay was performed by spectrophotometry as previously described by Mokaya et al. [18], but with slight modifications. Briefly, to 0.75 ml of each dissolved honey sample (10% w/v), 1.5 ml of DPPH solution (2 mg/100 ml methanol) was added. This mixture was incubated for 15 min in the dark at room temperature afterwards the absorbance was measured at 517 nm. For this assay, 0.75 ml of methanol mixed with 1.5 ml of DPPH solution was used as control. Meanwhile, the blank sample consisted of 0.75 ml of each dissolved honey solution mixed with 1.5 ml of methanol. For the positive control, quercetin prepared at different concentrations (10–100 μg/ml) was used to generate a standard curve ($y = 0.591x + 38.413$, $R^2 = 0.9988$) and the results were calculated as quercetin equivalent. Each honey sample was assayed three times, the results obtained were averaged and used to determine the free RSA, expressed as the percentage of inhibition using the formula below:

$$\% \text{ Inhibition} = [(\text{Control absorbance} - \text{Sample absorbance}) / \text{Control absorbance}] \times 100$$

2.3.2. Antimicrobial activity of the honey samples

2.3.2.1. Bacterial growth and maintenance. Single colonies of each susceptible Gram-positive (*Bacillus subtilis* ATCC 6633 and *Staphylococcus aureus* ATCC 205923) and Gram-negative (*Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853) bacteria sub-cultured on Mueller-Hinton Agar (MHA) for 24 h at 37 °C were used. To achieve a turbidity of 0.5 McFarland ($\approx 1 \times 10^8$ CFU/mL), these colonies were inoculated into sterile distilled water by measuring the optical density (O.D.) = 0.132 at 600 nm. Streptomycin was used as positive control for antimicrobial activity against both the Gram-positive and Gram-negative bacteria.

2.3.2.2. Agar well diffusion assay. This assay was performed in sterile MHA prepared in different clean Petri dishes (90 mm in diameter

and 17 mm deep) and the average area of inhibition zone (mm^2) for each honey sample assayed in three biological replicates was measured as previously described by Mokaya et al. [18].

2.3.2.3. Determination of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of the honey types. The MIC was determined using broth microdilution method as previously described [21], but with slight modifications. Different concentrations were prepared (1.25, 2.5, 3.125, 6.25, 12.5, 18.75, 25% w/v) from stock solution of each honey sample (50% w/v) using Mueller Hinton broth (MHB) medium. The bacterial cultures of 0.5 McFarland standard (1×10^8 CFU/ml) prepared above were used. Two hundred (200) μl of each honey concentration was aseptically put in triplicate into 96-well micro titre plates (Agilent Biotec Epoch) before adding 10 μl of the bacteria to all columns. For positive control, the bacterial cultures were added to the MHB medium alone in one well, then for negative control the bacterial cultures, MHB medium and streptomycin were all added in one well and for sterility control only medium was put in one well. The positive, negative and sterility controls were each replicated three times. After all the plates were prepared, the O.D. was analysed using BioTek microplate reader (Agilent, California, USA) at 600 nm at time 0 h (t0h) before incubating them for 24 h in a shaker (Eppendorf, Incubator Shaker Series, Germany). The O.D. was measured again after 24 h (t24 h). The MIC was determined by checking the O.D changes between t0 and t24 h. Viability tests were done for confirmation of MIC and determination of MBC by culturing the contents of five wells (two wells each below and above the determined MIC concentration, and the well with the determined MIC concentration) in the MHA plate. The plates were incubated for 24 h at 37 °C and the bacterial growth was observed afterwards. This indicated that the wells with no change in O.D. indeed had no growth. The MBC for each honey sample was thereafter determined as the concentration at which there was no bacterial growth.

2.4. Quantification of phytochemicals in the honey samples

2.4.1. Sample preparation

One (1) gram of each honey sample was dissolved in 10 ml of distilled water for quantification of phytochemicals.

2.4.2. Total phenol content (TPC)

The TPC of each honey sample, expressed in mg of gallic acid equivalents (GAE)/100 g honey, was determined in triplicates following the Folin–Ciocalteu method as previously described by Mokaya et al. [18]. The calibration curve (0–250 μg/ml) was generated using gallic acid as the standard ($y = 0.0073x + 0.0233$, $R^2 = 0.9992$).

2.4.3. Total flavonoid content (TFC)

The TFC of each honey sample, expressed in mg of quercetin equivalents (QE)/100 g honey, was determined in triplicates following the aluminium chloride ($AlCl_3$) colorimetric assay as previously described by Mokaya et al. [18]. The calibration curve (20–200 μg/ml) was generated using quercetin as the standard ($y = 0.0006x + 0.0028$, $R^2 = 0.9981$).

2.4.4. Total alkaloid content (TAC)

The TAC of each honey sample, expressed in mg of colchicine/100 g honey, was determined in triplicates according to the 1,10-phenanthroline method as previously described by Kegode et al. [22]. The calibration curve (0.1–1.5 mg/ml) was generated using colchicine as the standard ($y = 1.866x + 0.2332$, $R^2 = 0.9844$).

2.4.5. Total terpenoid content (TTC)

The TTC of each honey sample, expressed in mg of linalool equivalents/100 g honey, was quantified in triplicates according to the colorimetric method as previously described [22]. The calibration curve (10–500 mg/ml)) was generated using colchicine as the standard ($y = 0.0009x - 0.0158$, $R^2 = 0.9914$).

2.5. Gas chromatography coupled to mass spectrometry (GC-MS) analysis of honey samples

One (1) gram of each honey sample was weighed and diluted with 1 ml of ultra-pure water. The resultant mixture was then vortexed for 2 min for complete homogeneity, then n-hexane (4 ml) added. Subsequently, the mixture was centrifuged at 1500 rpm for 2 min and left to stand for 10 min to allow formation of a biphasic system. The organic phase was transferred into a separate clean glass vial and residue was used for a second and third cycle of extraction. The first, second and third extracts were combined and centrifuged at 2500 rpm for 10 min and 1 ml of top layer was withdrawn, dried through anhydrous sodium sulphate, into 2 ml clear glass and immediately analysed by GC-MS.

Chromatographic separation of the honey extracts was carried out using GC on an Agilent Technologies series A 7890 linked to a 5975C inert XL EI/CI MS, equipped with a (5%-phenyl)-methylpolysiloxane (HP-5 MS) column (30 m in length × 250 μm internal diameter × 0.25 μm film thickness) (Agilent, Palo Alto, CA, USA). One μl of each extract was injected into the GC-MS in the splitless mode at an injector temperature of 270 °C. The oven temperature was held at 35 °C for 3 min, followed by a gradual increase rate of 10 °C/min to 280 °C and maintained at this temperature for 10 min for a total of 50 min. Helium was used as a carrier gas at a flow rate of 1.2 ml/min. The temperature of the ion source was set at 240 °C and the ionization energy at 70 eV and fragment ions analysed in scan mode over 40–450 *m/z* mass range. Experiment-specific retention indices (RIs) were calculated in reference to C_5–C_{32} n-alkanes.

The relative integration of each identified peak was determined using the ChemStation integrator and is presented as percentage

(%) relative abundance in Table S6. To eliminate potential column, or solvent contamination, blank runs were conducted and subsequently analysed. Detected peaks were initially identified through a comparative analysis of mass spectral data against reference spectra published by library–MS databases: National Institute of Standards and Technology (NIST) 05, 08, and 11 as well as by considering retention times and retention indices. When authentic standards were available, the compounds identifications were definitively confirmed by comparing their fragmentation patterns, retention times, and retention indices with those of commercially available standards.

2.6. Statistical analysis

The R-Software version 4.2.2 [23] and the PAleontological Statistics (PAST) version 3.12 [24] software were used to compute all statistical analyses. To compare the phytochemical contents, the antibacterial and antioxidant activities among the 18 honey samples, a Kruskal-Wallis test followed by post-hoc Dunn's test was ran after confirming that the data for the parameters were not normally distributed and the variances were not homogeneous using the Shapiro-Wilk test ($P < 0.05$) and Bartlett's test ($P < 0.05$), respectively. Principal components analysis (PCA) was performed for visualization and analysis of the phytochemical contents among the different honey samples. Spearman's rank order correlation was conducted to establish the existence of relationships between the studied parameters. The *heatmap* function embedded in the R software was used to generate the heatmaps illustrating the mean abundance of the different volatile organic compounds arising from each honey sample. To assess the variation in chemical profiles among different honey samples, a one-way analysis of similarities (ANOSIM) using the Bray–Curtis dissimilarity matrix was conducted. Furthermore, the similarity percentages (SIMPER) analysis was employed to determine the relative contributions of various compounds to the dissimilarity observed among the volatiles in different honey samples. These results were then visualized using the non-metric multidimensional scaling (NMDS) method.

3. Results and discussion

3.1. Biological activities of the honey samples

3.1.1. Antioxidant activity of the honey samples

The antioxidant activity of the honey samples was measured based on their ability to neutralize the DPPH free radicals leading to a colour change from purple to colourless. Our results revealed that the ability to neutralize the DPPH free radicals differed significantly between the Manuka 5+ honey (control) and the African honeys investigated herein (Kruskal-Wallis test: $H = 55.54$, $df = 18$, $p < 0.0001$) (Fig. 2). Among the studied African honeys, the Poi (DPPH RSA of 43.81%) and Salabani (DPPH RSA of 41.72%) honeys from

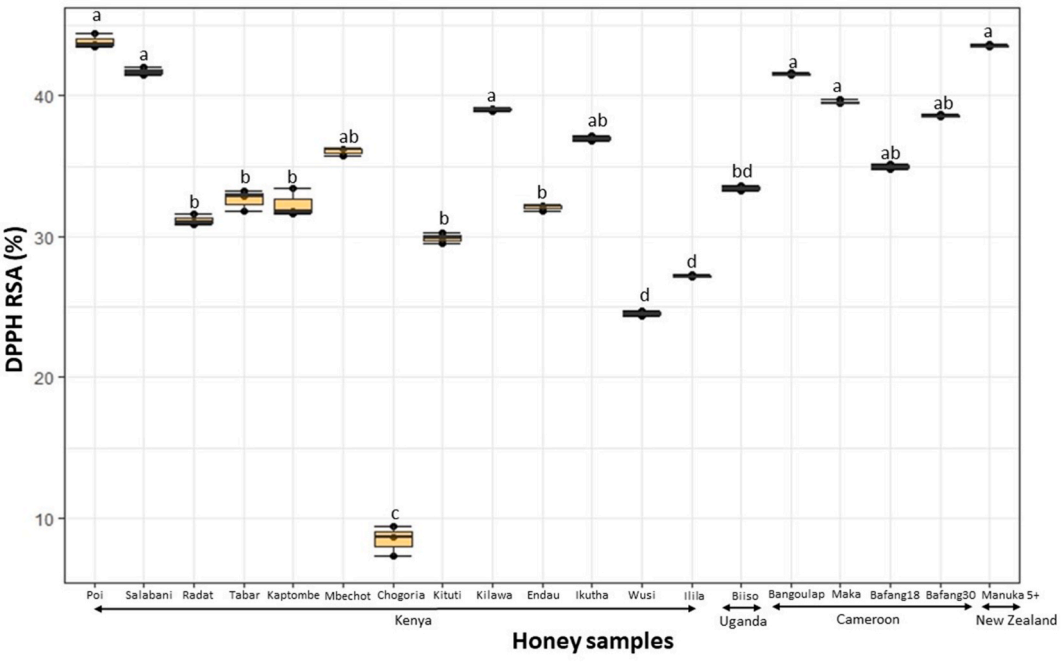

Fig. 2. Box plot diagram showing the differences existing among the honey samples from New Zealand, Kenya, Uganda, and Cameroon based on their DPPH radical scavenging activity (RSA) (%). In each boxplot, the ends of boxplot whiskers represent the minimum and maximum values of all the data and dots show individual data points (n = 3 replicates). Box plots with different letters are significantly different from each other (Kruskal-Wallis test followed by post-hoc Dunn's test, $P<0.05$).

the Rift Valley region of Kenya had the highest antioxidant capacity followed in descending order, by Bangoulap (DPPH RSA of 41.52%) and Maka (DPPH RSA of 39.55%) honeys from the Western region of Cameroon, and Kilawa honey (DPPH RSA of 39.03%) from the Eastern region of Kenya. Interestingly, the antioxidant capacity of all these poly-floral honeys was comparable with that of mono-floral Manuka 5+ honey (DPPH RSA of 43.48%), suggesting that they could be used as natural antioxidant agents to protect human cells against the harmful effects of free radicals [25]. The above DPPH RSA values were within the range reported in previous studies (31.1–86.9%) [26–30]. However, the Chogoria honey from the Eastern region of Kenya was the least potent in terms of antioxidant activity, with a DPPH RSA of 8.45%. The quercetin standard equivalent for all the studied honey samples is shown in Table S2.

3.1.2. Antimicrobial activity of the honey samples

As shown in Table 1, the area of inhibition against Gram-positive and Gram-negative bacteria differed significantly between the Manuka 5+ honey and the studied African honeys ($p < 0.0001$). Among the studied African honeys, honeys from the Rift Valley region of Kenya (specifically Poi, Salabani and Mbechot) had the highest antimicrobial activity against *B. subtilis*, *S. aureus* and *E. coli* while the Bangoulap honey from the Western Region of Cameroon, was the most bioactive against *P. aeruginosa*. Interestingly, the MIC (3.125–6.25% w/v) and MBC (6.25–12.5% w/v) values of these Rift Valley bioactive honeys against *B. subtilis*, *S. aureus*, and *P. aeruginosa* were comparable with those of the popularly studied Manuka 5+ honey (Table S3). However, their MIC (6.25% w/v) and MBC (12.5 % w/v) values against *E. coli* were higher than those of the Manuka 5+ honey (MIC (3.125% w/v) and MBC (6.25% w/v) values). Taken together, these results suggest that the bioactive Rift Valley honeys could also be used as natural therapeutic agents against infections caused by *B. subtilis*, *S. aureus*, and *P. aeruginosa*. Of note, the MIC values of these bioactive Rift Valley honeys against *S. aureus*, *E. coli*, and *P. aeruginosa* (3.125–6.25% w/v) were lower than those reported in previous studies (MIC (5–25% w/v)), but their MBC values against these pathogens (6.25–12.5% w/v) were within the range reported in previous works (6.25–50% w/v) [21, 31]. On the other hand, the MIC (6.25–12.5% w/v) and MBC (12.5–18.75% w/v) values of the Bangoulap honey against the four studied bacteria were higher than those of the Manuka 5+ honey (MIC (3.125–6.25% w/v) and MBC (6.25–12.5% w/v) values) (Table S3). The Chogoria honey from the Eastern region of Kenya displayed the least antimicrobial activity (Table S3, Table 1). The studied African honey types and the Manuka 5+ honey displayed greater effectiveness against Gram-positive than Gram-negative bacteria (Table 1), suggesting differences in bactericidal activity as reported previously [8,31].

3.2. Quantification of phytochemicals

As illustrated in Table 2, the content of phenols, flavonoids, alkaloids and terpenoids as well as the total phytochemical content (flavonoids + phenols + alkaloids + terpenoids) differed significantly between the Manuka 5+ honey and the investigated African honey types ($p < 0.0001$). In fact, the Rift Valley honeys from Kenya had the highest average values of total phenol, flavonoid, alkaloid, terpenoid and total phytochemical contents among all the studied African honey varieties, and their levels were like those of the Manuka 5+ honey. In contrast, Chogoria honey had, in general, the lowest average values of all these phytochemicals. In our findings,

Table 1
Comparison (Mean ± SEM) of the antibacterial area of inhibition (mm^2) between the Manuka 5+ honey from New Zealand and honey collected in Kenya, Uganda, and Cameroon. Different superscript letters in each column indicate significant differences among the honey samples according to Kruskal-wallis test followed by post-hoc Dunn's test, $p < 0.05$.

			Gram-positive bacteria		Gram-negative bacteria	
Country	Region	Honey samples	*Bacillus subtilis*	*Staphylococcus aureus*	*Escherichia coli*	*Pseudomonas aeruginosa*
New Zealand	ND	Manuka 5+	448.03 ± 3.33^{a}	343.75 ± 9.20^{a}	248.48 ± 0.00^{a}	241.28 ± 2.11^{a}
Kenya	Rift Valley	Poi	517.14 ± 9.85^{a}	322.95 ± 12.86^{a}	237.93 ± 7.64^{a}	191.76 ± 3.96^{a}
		Salabani	434.68 ± 17.3^{a}	307.13 ± 6.86^{a}	190.38 ± 10.84$^{a, b}$	180.67 ± 2.94^{a}
		Radat	347.13 ± 10.07$^{a, b}$	311.71 ± 8.13^{a}	206.97 ± 2.36^{a}	160.75 ± 0.73$^{a, b}$
		Tabar	297.9 ± 1.3$^{a, b}$	231.31 ± 6.93$^{a, b}$	192.22 ± 12.72$^{a, b}$	152.97 ± 1.47^{b}
		Kaptombe	301.1 ± 6.88$^{a, b}$	289.92 ± 27.3$^{a, b}$	204.66 ± 7.05^{a}	154.37 ± 0.71^{b}
		Mbechot	415.51 ± 10.03^{a}	318.98 ± 5.47^{a}	207.46 ± 4.13^{a}	178.74 ± 1.93^{a}
	Eastern	Chogoria	170.69 ± 0.68^{c}	176.99 ± 7.02$^{b, c}$	151.34 ± 2.12^{b}	124.24 ± 0.50^{c}
		Kituti	208.15 ± 23.07$^{b, c}$	258.91 ± 24.55$^{a, b}$	126.65 ± 5.18^{b}	141.04 ± 6.41^{b}
		Kilawa	183.99 ± 10.7$^{b, c}$	291.51 ± 12.3$^{a, b}$	158.79 ± 2.16$^{a, b}$	177.84 ± 7.5$^{a, b}$
		Endau	291.2 ± 16.82$^{a, b}$	231.74 ± 4.68$^{b, c}$	172.03 ± 9.46$^{a, b}$	176.67 ± 7.09$^{a, b}$
		Ikutha	253.16 ± 14.14$^{a, b}$	244.66 ± 1.83$^{a, b}$	226.48 ± 2.94^{a}	138.86 ± 2.13$^{b, c}$
	Coastal	Wusi	322.24 ± 10.84$^{a, b}$	246.17 ± 16.17$^{a, b}$	205.16 ± 12.98^{a}	139.73 ± 1.55$^{b, c}$
		Ilila	237.05 ± 14.54$^{a, b}$	196.38 ± 1.83$^{b, c}$	156.11 ± 7.25$^{b, c}$	158.80 ± 2.13$^{a, b}$
Uganda	Western	Biiso	351.55 ± 8.48$^{a, b}$	223.52 ± 0.92$^{b, c}$	170.16 ± 10.07$^{b, c}$	154.64 ± 1.61$^{b, c}$
Cameroon	Western	Bangoulap	238.22 ± 2.2$^{b, c}$	242.72 ± 1.45$^{a, b}$	197.22 ± 3.39^{a}	207.41 ± 1.23^{a}
		Maka	220.96 ± 17.85$^{b, c}$	202.41 ± 12.47$^{b, c}$	144.04 ± 3.57$^{b, c}$	175.45 ± 2.1$^{a, b}$
		Bafang18	206.37 ± 3.58$^{b, c}$	224.83 ± 15.51$^{b, c}$	137.57 ± 0.00$^{b, c}$	163.75 ± 2.21$^{a, b}$
		Bafang30	382.50 ± 38.36$^{a, b}$	219.06 ± 6.05$^{b, c}$	155.31 ± 0.00$^{b, c}$	175.5 ± 2.33$^{a, b}$
		Streptomycin	993.66 ± 0.4^{a}	714.21 ± 0.14^{a}	743.40 ± 0.17^{a}	423.60 ± 0.31^{a}
		p-value	1.425e-05	6.495e-05	2.306e-05	1.376e-05

*ND-Indicates that the information is not available on the product's label.

Table 2
Comparison (Mean ± SEM) of the content of flavonoids, phenols, alkaloids, terpenoids and total phytochemicals between the Manuka 5+ honey from New Zealand and honeys collected in Kenya, Uganda, and Cameroon. Different superscript letters in each column indicate significant differences among the honey samples according to Kruskal-Wallis test followed by post-hoc Dunn's test, $P < 0.05$.

Country	Region	Honey samples	Flavonoids (mg QE/100 g)	Phenols (mg GAE/100 g)	Alkaloids (mg CE/100 g)	Terpenoids (mg LE/100 g)	Total phytochemicals
New Zealand	ND	Manuka 5+	903.33 ± 0.00^{a}	1059.86 ± 0.00^{a}	330.55 ± 0.62^{a}	282 ± 0.64^{a}	2575.74 ± 0.63^{a}
Kenya	Rift Valley	Poi	970 ± 9.62^{a}	1047.53 ± 0.79^{a}	251 ± 0.31^{a}	285 ± 0.64^{a}	2554.10 ± 8.35^{a}
		Salabani	920 ± 0.00^{a}	1024.25 ± 0.00^{a}	226.58 ± 0.62^{a}	274.22 ± 0.00^{a}	2445.05 ± 0.62^{a}
		Radat	486.67 ± 19.25^{b}	736.58 ± 1.58^{b}	158.52 ± 0.31^{a}	176.44 ± 0.64^{b}	1558.21 ± 18.46^{b}
		Tabar	453.33 ± 9.62^{b}	$769.45 \pm 0.79^{a, b}$	136.55 ± 0.31^{b}	185.33 ± 0.64^{b}	1544.67 ± 11.21^{b}
		Kaptombe	553.33 ± 9.62^{b}	735.21 ± 0.79^{b}	118.86 ± 0.62^{b}	270.89 ± 0.00^{a}	1678.29 ± 9.16^{b}
		Mbechot	1070 ± 9.62^{a}	$998.22 \pm 0.79^{a, c}$	$266.77 \pm 0.31^{a, c}$	320.89 ± 1.28^{a}	2655.88 ± 9.96^{a}
	Eastern	Chogoria	$403.33 \pm 0.00^{b, c}$	$450.27 \pm 1.58^{b, d}$	$42.23 \pm 0.62^{b, d}$	202 ± 0.00^{b}	1097.84 ± 1.38^{c}
		Kituti	$703.33 \pm 9.62^{a, b}$	736.57 ± 2.37^{b}	$353.59 \pm 0.62^{a, c}$	$250.89 \pm 1.28^{a, b}$	$2044.39 \pm 8.24^{a, b}$
		Kilawa	$536.67 \pm 9.62^{b, c}$	644.79 ± 1.58^{b}	$186.92 \pm 0.62^{a, b, c}$	314.22 ± 0.64^{a}	1682.61 ± 9.00^{b}
		Endau	$658.89 \pm 5.56^{a, b}$	$873.56 \pm 0.79^{a, b}$	$228.72 \pm 6.19^{a, b}$	283.11 ± 0.64^{a}	$2044.29 \pm 3.19^{a, b}$
		Ikutha	$636.67 \pm 9.62^{a, b}$	$902.79 \pm 8.46^{a, b}$	145.12 ± 0.31^{b}	222 ± 0.00^{b}	1906.58 ± 17.43^{b}
	Coastal	Wusi	$570 \pm 9.62^{b, c}$	$494.11 \pm 0.79^{b, d}$	$54.02 \pm 5.09^{b, d}$	266.44 ± 1.28^{a}	1384.57 ± 10.59^{b}
		Ilila	853.33 ± 9.62^{a}	979.04 ± 1.58^{a}	153.70 ± 3.09^{a}	225.33 ± 1.28^{b}	2211.41 ± 8.79^{a}
Uganda	Western	Biiso	803.33 ± 9.62^{a}	987.72 ± 1.21^{a}	145.12 ± 0.62^{a}	$236.44 \pm 0.64^{a, b}$	2172.62 ± 8.80^{a}
Cameroon	Western	Bangoulap	770 ± 9.62^{a}	$944.79 \pm 0.79^{a, b}$	$119.40 \pm 0.62^{a, b}$	$239.78 \pm 0.00^{a, b}$	2073.97 ± 8.53^{a}
		Maka	770 ± 9.62^{a}	720.14 ± 0.79^{b}	103.86 ± 0.31^{b}	$257.23 \pm 7.60^{a, b}$	$1851.23 \pm 17.48^{a, b}$
		Bafang18	$570 \pm 9.62^{b, c}$	$462.60 \pm 0.79^{b, d}$	79.74 ± 0.62^{b}	$245.33 \pm 0.64^{a, b}$	1357.68 ± 9.48^{b}
		Bafang30	820 ± 9.62^{a}	687.26 ± 0.79^{b}	104.93 ± 0.31^{b}	260.89 ± 1.28^{a}	$1873.08 \pm 10.50^{a, b}$
		p-value	1.07e-05	9.93e-06	1e-05	1.051e-05	1.04e-05

*ND-Indicates that the information is not available on the product's label.

the average values of flavonoid and phenolic content in all the tested African honeys ranged between 403.33 and 1070 mg QE/100 g and 450.27–1059.86 mg GAE/100 g, respectively. These values were higher than those reported for mono- and poly-floral honeys in previous studies (0.86–73.02 mg QE/100 g for flavonoids and 2–142.61 mg GAE/100 g for phenols) [18,32,33].

It is worth mentioning that the high level of variability in the bio-functional properties of the studied honeys recorded herein could be partly linked to differences in the levels of several factors, especially flavonoid and phenolic compounds, as was suggested before for similar cases [18,34]. Some studies have shown that these plant-derived secondary metabolites are responsible for the scavenging activity of honey against the free radical DPPH [35–37]. Other studies have shown that they limit the development of numerous Gram-positive and Gram-negative bacteria [38]. Our results concurred with these previous findings as significant and positive correlations were observed between the phytochemicals (phenols and flavonoids) and antioxidant (DPPH RSA %)/antimicrobial activity (Fig. 3). Significant and positive correlations were also found between total terpenoid content and DPPH RSA (%) ($r = 0.48$, $p < 0.05$), the antibacterial activity against *S. aureus* ($r = 0.55$, $p < 0.05$) and *P. aeruginosa* ($r = 0.62$, $p < 0.01$) (Fig. 3). Further, levels of terpenoids significantly and positively correlated with those of flavonoids ($r = 0.51$, $p < 0.05$). These findings therefore suggest that in combination with flavonoids, terpenoids may also contribute to the biological activities of honey because the presence of terpenes from which they are derived is apparently limited in honey when compared to phenolic and flavonoid compounds [9]. Alkaloid may also contribute to the bio-functional properties of honey in combination with flavonoids due to significant and positive correlation found between this compound and flavonoids ($r = 0.51$, $p < 0.05$), and the antibacterial activity against *S. aureus* ($r = 0.68$, $p < 0.01$) and *P. aeruginosa* ($r = 0.47$, $p < 0.05$) (Fig. 3).

The quality and quantity of the phytochemicals investigated herein are partly influenced by the geographical and floral origin of the nectar [9,32,39,40]. As such, they could be used to provide a unique geographical fingerprint for different types of honey as was suggested before [41]. Clear separation of African and Manuka 5+ honey varieties based on their phytochemical contents using PCA in this study support this opinion (Fig. 4). Notably, Poi and Salabani honeys from the Rift Valley region in Kenya were close to each other and both were close to the Manuka 5+ honey. Also, they were relatively close to the Mbechot honey from the same region on the positive side of PCA 1. Poi and Salabani honeys with the Manuka 5+ honey were fairly close to those from the Eastern (e.g. Endau and Kituti) and Coastal (e.g. llila) regions of Kenya, Western regions of Uganda (e.g. Biiso) and Cameroon (Bangoulap) on the positive side of PCA 1, but were rather separated from the rest of honeys on the negative side of PCA 1. Chogoria honey from Eastern Kenya was very different from all the African honey samples investigated herein including the Manuka 5+ honey. However, since the amounts of these phytochemicals vary seasonally [42–44], elucidating the influence of seasonality on their levels and consequently on the bioactivity of these African honey types is recommended.

3.3. Volatile organic compounds in African honey samples

A total of 129 volatile organic compounds (VOCs) across all samples were detected (Tables S4 and S5). These VOCs encompassed a diverse range of chemical classes, including 9 ketones, 5 alcohols, 5 benzenoids, 25 terpenes, 4 carboxylic acids, 3 esters, and several

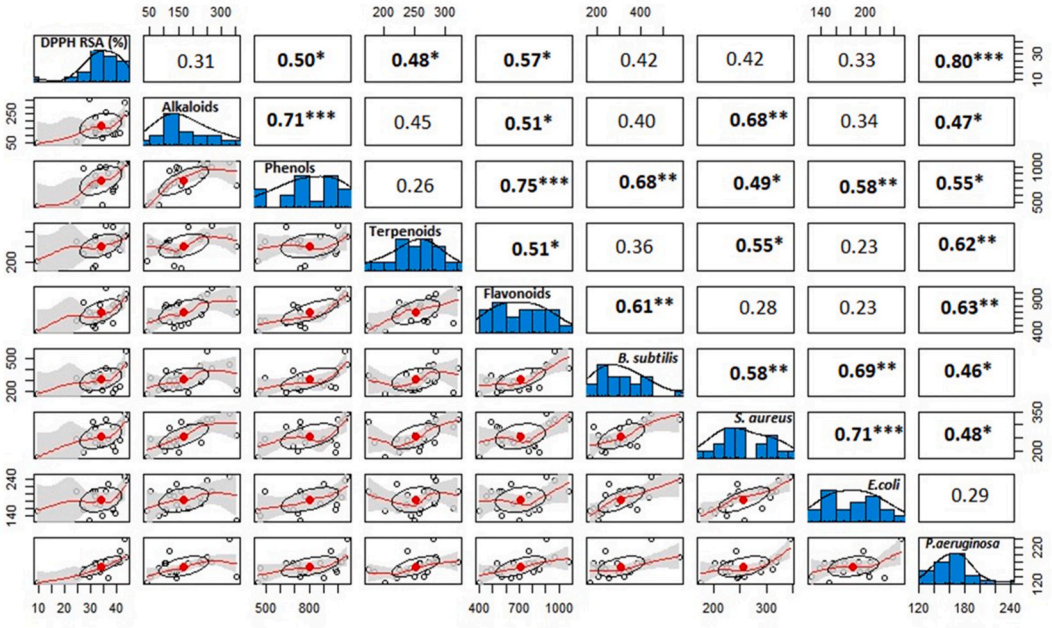

Fig. 3. The correlation between alkaloids, phenols, terpenoids or flavonoids and antioxidant activity (DPPH RSA (%))/antimicrobial activity, with corresponding correlation coefficient and significant levels. $p < 0.05$ "*", $p < 0.01$ "**", $p < 0.001$"***".

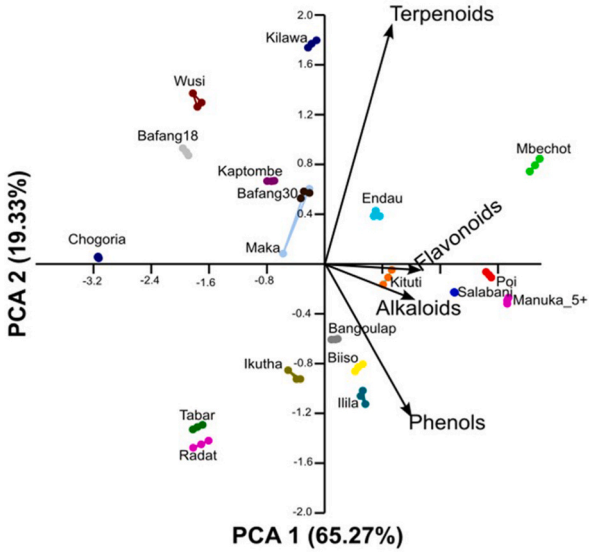

Fig. 4. Principal components analysis showing the similarities (or dissimilarities) existing among the honey samples from New Zealand, Kenya, Uganda, and Cameroon based on their phytochemical contents. Total variance explained by 83.63%.

others. Within this rich array of compounds, specific VOCs exhibited significantly higher abundances in certain honey samples compared to others (ANOSIM: R-stat = 0.089, $p < 0.001$). Noteworthy among these findings was the prevalence of identical volatile compound profiles in Endau, Tabar, and Kilawa honeys from Kenya (Fig. 5). This striking similarity is further confirmed by our non-metric multidimensional scaling (NMDS) plot (Fig. 6A), which clearly demonstrates the clustering of these three honey samples, underscoring the consistency in their volatile compositions. Conversely, certain honey samples, such as those from Chogoria and Ilila, displayed distinct profiles of volatile organic compounds when compared to samples from Poi and Maka. These differences in volatile composition were primarily attributed to the presence of the ten most abundant compounds, as illustrated in Fig. 6B, suggesting that they could also be partly used to diagnose the geographical and/or floral sources of honey. However, future studies on their antimicrobial and antioxidant properties are required. Other minor volatile compounds detected in some of tested African honeys such as α-pinene and D-limonene [45], camphor [46], eucalyptol [47,48] and camphene [49] have been demonstrated to have antioxidant and/or antimicrobial activities (Table S5). It is worth noting that there was not enough Mbechot, Kituti, and Kaptombe honeys to run

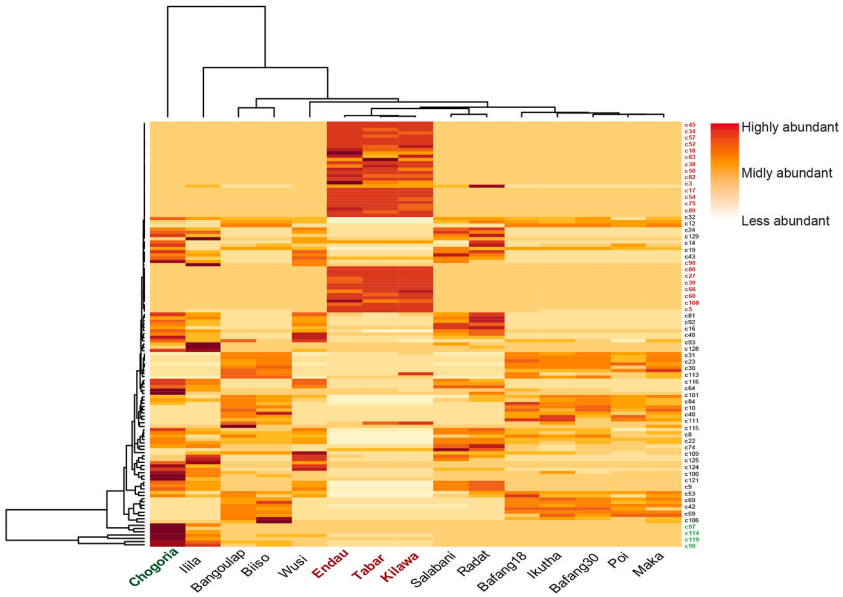

Fig. 5. The honey samples emit different volatile organic compounds (VOCs). (**A**) Heatmap showing the mean abundance of the VOCs in each honey sample. Abbreviation of the 129 VOCs are shown in Table S9 (C_1–C129). "Red", "dark-orange" and "yellow" colours indicate a high, average and low abundance, respectively. The abbreviations of the VOCs are shown in Table S5.

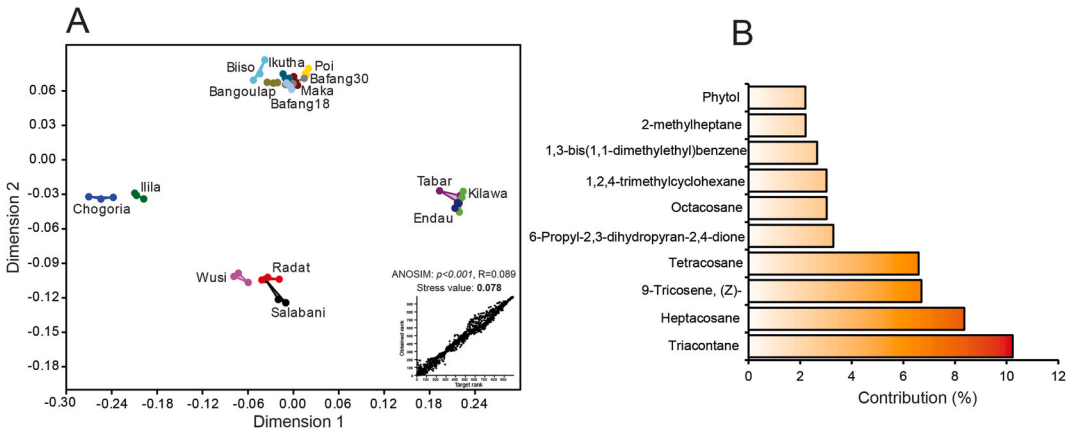

Fig. 6. Non-metric multidimensional scaling plot (NMDS) clustering the different honey samples based on the type of odorants they emit (**A**), and Bar graphs depicting the contribution of the 10 most abundant VOCs to the differentiation of the analysed honey samples (**B**).

this GC-MS analysis.

4. Conclusion

Overall, honeys from the Rift Valley (particularly Poi, Salabani and Mbechot) and Western (particularly Bangoulap) regions of Kenya and Cameroon, respectively, are of particular interest because they displayed the highest antioxidant (DPPH RSA of 41.52–43.81%) and antimicrobial (MIC (3.125–6.25% w/v) and MBC (6.25–12.5% w/v)) activities among the tested African honey types, which were like those of the Manuka 5+ honey. Thus, their potential to be used as natural sources of antimicrobial and antioxidant therapies needs to be further probed. Their high bioactivities were partly linked to their high levels of phenols (944.79–1047.53 mg GAE/100 g), flavonoids (770–970 mg QE/100 g), terpenoids (239.78–320.89 mg LE/100 g) and/or alkaloids (119.40–266.57 mg CE/100 g). This finding further supports the usefulness of these phytochemicals together with the 10 most abundant VOCs identified herein by GC-MS as potential markers for pinpointing the geographical origin of honey.

Funding

Sample collection in Kenya was funded by the European Union project through Kenya Agricultural and Livestock Research Organization (KALRO), Grant/Award Number: KALRO/CS APP/LOA No. March 2019; whereas sampling in Cameroon was funded by JRS Biodiversity Foundation, Grant Number: 70054.

Data availability statement

Data will be made available on request from the corresponding author.

CRediT authorship contribution statement

Nelly N. Ndungu: Writing – original draft, Methodology, Investigation. **Timothy M. Kegode:** Writing – original draft, Methodology, Investigation, Formal analysis, Data curation. **Justus K. Kurgat:** Writing – original draft, Methodology, Investigation, Formal analysis, Data curation. **Steve B.S. Baleba:** Writing – review & editing, Formal analysis. **Xavier Cheseto:** Writing – review & editing, Visualization, Validation, Formal analysis, Data curation. **S. Turner:** Writing – review & editing. **Geraud C. Tasse Taboue:** Writing – review & editing. **J.M. Kasina:** Writing – review & editing. **Sevgan Subramanian:** Writing – review & editing. **Beatrice T. Nganso:** Writing – review & editing, Writing – original draft, Methodology, Formal analysis, Data curation, Conceptualization, Supervision, Validation.

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.

Acknowledgements

The authors acknowledge all beekeepers for generously providing honey samples needed for this study: KALRO-Apicultural

Research Institute Baringo Kenya, KAMAKI Cooperative in Kitui, Kenya, and Coopérative avec Conseil d'administration de l'université des Montagnes (Coop-CA Udm), Bangangté, Cameroon. The authors further acknowledge the financial support for this research by the following organisations and agencies: the Swedish International Development Cooperation Agency (Sida); the Swiss Agency for Development and Cooperation (SDC); the Australian Centre for International Agricultural Research (ACIAR); the Norwegian Agency for Development Cooperation (Norad); the German Federal Ministry for Economic Cooperation and Development (BMZ); and the Government of the Republic of Kenya. The views expressed herein do not necessarily reflect the official opinion of the donors.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e30839.

References

[1] M.D. Mandal, S. Mandal, Honey: its medicinal property and antibacterial activity, Asian Pac. J. Trop. Biomed. 1 (2011) 154–160, https://doi.org/10.1016/S2221-1691(11)60016-6.
[2] S. Samarghandian, T. Farkhondeh, F. Samini, Honey and health: a review of recent clinical research, Pharmacogn. Res. 9 (2017) 121–127, https://doi.org/10.4103/0974-8490.204647.
[3] S. Bogdanov, C. Lüllmann, P. Martin, W. Von Der Ohe, H. Russmann, G. Vorwohl, L.P. Oddo, A.G. Sabatini, G.L. Marcazzan, R. Piro, C. Flamini, Honey quality and international regulatory standards: review of the international honey commission drafts for Codex Alimentarius and Eu Honey Standards, Bee World 80 (1999) 61–69, https://doi.org/10.1080/0005772X.1999.11099428.
[4] S. Bogdanov, T. Jurendic, R. Sieber, P. Gallmann, Honey for nutrition and health: a review, J. Am. Coll. Nutr. 27 (2008) 677–689, https://doi.org/10.1080/07315724.2008.10719745.
[5] P. Combarros-Fuertes, J.M. Fresno, M.M. Estevinho, M. Sousa-Pimenta, M.E. Tornadijo, L.M. Estevinho, Honey: Another alternative in the fight against antibiotic-resistant bacteria? Antibiotics 9 (2020) 1–21, https://doi.org/10.3390/antibiotics9110774.
[6] L. Pippinato, S. Blanc, T. Mancuso, F. Brun, A sustainable niche market: how does honey behave? Sustain. Times 12 (2020) 1–14, https://doi.org/10.3390/su122410678.
[7] M.C.B. Otero, L. Bernolo, Honey as functional food and prospects in natural honey production. Functional Foods and Nutraceuticals: bioactive Components, Formulations and Innovations (2020) 197–210, https://doi.org/10.1201/9781003415381-1.
[8] N.A. Albaridi, Antibacterial potency of honey, Int. J. Microbiol. 2019 (2019) 1–10, https://doi.org/10.1155/2019/2464507.
[9] I. Jerković, P.M. Kuś, Terpenes in honey: Occurrence, origin and their role as chemical biomarkers, RSC Adv. 4 (2014) 31710–31728, https://doi.org/10.1039/c4ra04791e.
[10] A.S. Ahmed, S.A. Sulaiman, A.A. Baig, M. Ibrahim, S. Liaqat, S. Fatima, S. Jabeen, N. Shamim, N.H. Othman, Honey as a potential natural antioxidant medicine: an insight into its molecular mechanisms of action, Oxid. Med. Cell. Longev. (2018) 1–19, https://doi.org/10.1155/2018/8367846.
[11] A. Bendini, L. Cerretani, L. Pizzolante, T.G. Toschi, F. Guzzo, S. Ceoldo, A.M. Marconi, F. Andreetta, M. Levi, Phenol content related to antioxidant and antimicrobial activities of Passiflora spp. extracts, Eur. Food Res. Technol. 223 (2006) 102–109, https://doi.org/10.1007/s00217-005-0150-7.
[12] V.C. Nolan, J. Harrison, J.E.E. Wright, J.A.G. Cox, Clinical significance of manuka and medical-grade honey for antibiotic-resistant infections: a systematic review, Antibiotics 9 (2020) 1–24, https://doi.org/10.1007/s00217-005-0150-7.
[13] A.S. Bazaid, A. Alamri, M.N. Almashjary, H. Qanash, A.A. Almishaal, J. Amin, N.K. Binsaleh, J.A. KraiemAldarhami, A. Alafnan, Antioxidant, anticancer, antibacterial, antibiofilm properties and gas chromatography and mass spectrometry analysis of Manuka honey: a Nature's Bioactive Honey, Appl. Sci. 12 (2022) 1–13, https://doi.org/10.3390/app12199928.
[14] C. Stefanis, E. Stavropoulou, E. Giorgi, C. Voidarou, T.C. Constantinidis, G. Vrioni, A. Tsakris, Honey's Antioxidant and antimicrobial properties: a bibliometric study, Antioxidants 12 (2023) (2023) 414–439, https://doi.org/10.3390/antiox12020414.
[15] M. Moyo, A.O. Aremu, J. Van Staden, Medicinal plants: an invaluable, dwindling resource in sub-Saharan Africa, J. Ethnopharmacol. 174 (2015) 595–606, https://doi.org/10.1016/j.jep.2015.04.034.
[16] N. Adgaba, A. Al-Ghamdi, D. Sharma, Y. Tadess, S.M. Alghanem, K.A. Khan, G.K.A. Mohamed, Physico-chemical, antioxidant and anti-microbial properties of some Ethiopian mono-floral honeys, Saudi J. Biol. Sci. 27 (2020) 2366–2372, https://doi.org/10.1016/j.sjbs.2020.05.031.
[17] G. Morroni, J.M. Alvarez-Suarez, A. Brenciani, S. Simoni, S. Fioriti, A. Pugnaloni, E. Giovanetti, Comparison of the antimicrobial activities of four honeys from three countries (New Zealand, Cuba, and Kenya), Front. Microbiol. 9 (2018) 1378–1387, https://doi.org/10.3389/fmicb.2018.01378.
[18] H.O. Mokaya, J.L. Bargul, J.W. Irungu, H.M.G. Lattorff, Bioactive constituents, in vitro radical scavenging and antibacterial activities of selected *Apis mellifera* honey from Kenya, Int. J. Food Sci. Technol. 55 (2020) 1246–1254, https://doi.org/10.1111/ijfs.14403.
[19] F. Khan, J. Hill, S. Kaehler, M. Allsopp, S. van Vuuren, Antimicrobial properties and isotope investigations of South African honey, J. Appl. Microbiol. 117 (2014) 366–379, https://doi.org/10.1111/jam.12533.
[20] J. Boateng, K.N. Diunase, Comparing the antibacterial and functional properties of cameroonian and Manuka honeys for potential wound healing-have we come full cycle in dealing with antibiotic resistance? Molecules 20 (2015) 16068–16084, https://doi.org/10.3390/molecules200916068.
[21] M.I. Zainol, K. Mohd Yusoff, M.Y. Mohd Yusof, Antibacterial activity of selected Malaysian honey, BMC Compl. Alternative Med. 13 (2013) 129–138, https://doi.org/10.1186/1472-6882-13-129.
[22] T.M. Kegode, J.L. Bargul, H.O. Mokaya, H.M.G. Lattorff, Phytochemical composition and bio-functional properties of *Apis mellifera* propolis from Kenya, R. Soc. Open Sci. 9 (2022) 211214–211227, https://doi.org/10.1098/rsos.211214.
[23] R Development Core Team, R, A Language and Environment for Statistical Computing, R Foundation for Statistical Computing, Vienna, Austria, 2021.
[24] Ø. Hammer, D.A.T. Harper, P.D. Ryan, Past: Paleontological statistics software package for education and data analysis, Palaeontol. Electron. 4 (2001) 1–9. http://palaeo-electronica.org.
[25] G.G. Duthie, S.J. Duthie, J.A.M. Kyle, Plant polyphenols in cancer and heart disease: implications as nutritional antioxidants, Nutr. Res. Rev. 13 (2000) 79–106, https://doi.org/10.1079/095442200108729016.
[26] Y. Ruiz-Navajas, M. Viuda-Martos, J. Fernández-López, J.M. Zaldivar-Cruz, V. Kuri, J.Á. Pérez-Álvarez, Antioxidant activity of artisanal honey from Tabasco, Mexico, Int. J. Food Prop. 14 (2011) 459–470, https://doi.org/10.1080/10942910903249480.
[27] V. Baltrušaitytė, V. Venskutonis, P.R. Čeksterytė, Radical scavenging activity of different floral origin honey and beebread phenolic extracts Vilma, Food Chem. 101 (2007) 502–514, https://doi.org/10.1016/j.foodchem.2006.02.007.
[28] P. Kačániová, M. Borotová, P. Galovičová, L. Kunová, S. Štefániková, J. Kowalczewski, P.Ł. Šedík, Antimicrobial and antioxidant activity of different honey samples from beekeepers and commercial Producers, Antibiotics 11 (2022) 1163–1189, https://doi.org/10.3390/antibiotics11091163.
[29] M. Lewoyehu, M. Amare, Comparative assessment on selected physicochemical parameters and antioxidant and antimicrobial activities of honey samples from selected districts of the Amhara and Tigray Regions, Ethiopia, Int. J. Food Sci. 2019 (2019) 1–10, https://doi.org/10.1155/2019/4101695.
[30] Z. Shakoori, E. Salaseh, A.R. Mehrabian, D.M. Tehrani, N.F. Dardashti, F. Salmanpour, The amount of antioxidants in honey has a strong relationship with the plants selected by honey bees, Sci. Rep. 14 (2024) 1–10, https://doi.org/10.1038/s41598-023-51099-9.

[31] A. Skadiņš, I. Labsvārds, K.D. Grava, A. Amirian, J. Tomsone, L.E. Ruško, J. Viksna, A. Bandere, D. Brangule, Antimicrobial and antibiofilm properties of Latvian honey against causative agents of wound infections, Antibiotics 12 (2023) 816–834.
[32] D. Pauliuc, F. Dranca, M. Oroian, Antioxidant activity, total phenolic content, individual phenolics and physicochemical parameters suitability for Romanian honey authentication, Foods 9 (2020) 306–328, https://doi.org/10.3390/foods9030306.
[33] A. Albu, C. Simeanu, I.M. Pop, A. Pui, D. Tarcău, S.-M. Cucu-Man, Selected characteristics of multifloral honeys from North-Eastern Romania, Agriculture (2023) 14, https://doi.org/10.3390/agriculture14010026.
[34] D. Cianciosi, T.Y. Forbes-Hernández, S. Afrin, M. Gasparrini, P. Reboredo-Rodriguez, P.P. Manna, J. Zhang, L. Bravo Lamas, S. Martínez Flórez, P. Agudo Toyos, J.L. Quiles, Phenolic compounds in honey and their associated health benefits: a review, Molecules 23 (2018) 2322–2342, https://doi.org/10.3390/molecules23092322.
[35] I. Smetanska, S.S. Alharthi, K.A. Selim, Physicochemical, antioxidant capacity and color analysis of six honeys from different origin, J. King Saud Univ. Sci. 33 (2021) 101447–101456, https://doi.org/10.1016/j.jksus.2021.101447.
[36] M. Al-Farsi, A. Al-Amri, A. Al-Hadhrami, S. Al-Belushi, Color, flavonoids, phenolics and antioxidants of Omani honey, Heliyon 4 (2018) 00874, https://doi.org/10.1016/j.heliyon.2018.e00874.
[37] H. Lakhmili, K. Warda, A. El-Abbassi, A. Hafidi, Antioxidant and anti-glycation activity of eight Moroccan honeys from different botanical origins, Discov. Food 4 (2024), https://doi.org/10.1007/s44187-024-00074-y.
[38] P.M. Davidson, J.N. Sofos, A.L. Branen, Antimicrobials in Food, third edition, 2005, https://doi.org/10.1201/9781420028737.
[39] Y. Cheung, M. Meenu, X. Yu, B. Xu, Phenolic acids and flavonoids profiles of commercial honey from different floral sources and geographic sources, Int. J. Food Prop. 22 (2019) 290–308, https://doi.org/10.1080/10942912.2019.1579835.
[40] C.E. Manyi-Loh, R.N. Ndip, A.M. Clarke, Volatile compounds in honey: a review on their involvement in aroma, botanical origin determination and potential biomedical activities, Int. J. Mol. Sci. 12 (2011) 9514–9532, https://doi.org/10.3390/ijms12129514.
[41] G.W. Zammit Young, R. Blundell, A review on the phytochemical composition and health applications of honey, Heliyon 9 (2023) e12507, https://doi.org/10.1016/j.heliyon.2022.e12507.
[42] J.M. Alvarez-Suarez, M. Gasparrini, T.Y. Forbes-Hernández, L. Mazzoni, F. Giampieri, The composition and biological activity of honey: a focus on manuka honey, Foods 3 (2014) 420–432, https://doi.org/10.3390/foods3030420.
[43] A.L. Becerril-sánchez, B. Quintero-salazar, O. Dublán-garcía, H.B. Escalona-buendía, Phenolic compounds in honey and their relationship with antioxidant activity, botanical origin, and color, Antioxidants 10 (2021) 1–23, https://doi.org/10.3390/antiox10111700.
[44] E. Bernklau, H.S. Arathi, Seasonal patterns of beneficial phytochemical availability in honey and stored pollen from honey bee colonies in large apiaries, J. Econ. Entomol. 116 (2023) 1069–1077, https://doi.org/10.1093/jee/toad096.
[45] C.Y. Wang, Y.W. Chen, C.Y. Hou, Antioxidant and antibacterial activity of seven predominant terpenoids, Int. J. Food Prop. 22 (2019) 230–238, https://doi.org/10.1080/10942912.2019.1582541.
[46] S. Maache, L. Zbadi, A.E. Ghouizi, N. Soulo, H. Saghrouchni, F. Siddique, B. Sitotaw, A.M. Salamatullah, H.A. Nafidi, M. Bourhia, B. Lyoussi, Antioxidant and antimicrobial effects of essential oils from two salvia species with in vitro and in silico analysis targeting 1AJ6 and 1R4U proteins, Sci. Rep. 13 (2023) 14038–14060, https://doi.org/10.1038/s41598-023-41178-2.
[47] K.G. Lee, T. Shibamoto, Antioxidant activities of volatile components isolated from Eucalyptus species, J. Sci. Food Agric. 81 (2001) 1573–1579, https://doi.org/10.1002/jsfa.980.
[48] H. Zengin, A.H. Baysal, Antibacterial and antioxidant activity of essential oil terpenes against pathogenic and spoilage-forming bacteria and cell structure-activity relationships evaluated by SEM microscopy, Molecules 19 (2014) 17773–17798, https://doi.org/10.3390/molecules191117773.
[49] N.E. Hachlafi, T. Aanniz, N.E. Menyiy, A.E. Baaboua, N.E. Omari, A. Balahbib, M.A. Shariati, G. Zengin, K. Fikri-Benbrahim, A. Bouyahya, *In vitro* and *in vivo* biological investigations of camphene and its mechanism insights: a Review, Food Rev. Int. 39 (2023) 1799–1826, https://doi.org/10.1080/87559129.2021.1936007.