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Antioxidant Potential of Stingless Bee Honey from Mangrove and Montane Vegetation Types in Tanzania

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Abstract

Stingless bee honey is a rich source of antioxidants and is highly regarded in traditional medicine for the treatment of various ailments. The antioxidant capacity of honey can be influenced by its origin and the floral resources used by bees. Consequently, this study evaluated the antioxidant potential of stingless bee honey from two vegetation types: montane and mangrove. The honey samples displayed remarkable antioxidant potential, with notable levels of total phenolic content (197.0–263.1 mg GAE/100 g), total flavonoid content (118.5–156.7 mg QE/100 g), ascorbic acid (25.1–30.0 mg/100 g), lycopene (2.8–3.7 mg/100 g), β-carotene (0.8–1.7 mg/100 g), DPPH (2,2-diphenyl-1-picrylhydrazyl) scavenging activity (68.5–73.6%), and ferric reducing antioxidant power (265.9–272.7 µmol Fe(II)/100 g). Notably, the antioxidant properties varied between vegetation types, with mangrove honey exhibiting significantly higher levels of total phenolic and flavonoid content ($p < 0.01$). These results underscore the therapeutic potential of stingless bee honey for managing oxidative stress and the associated diseases.

Keywords: stingless bee honey; antioxidants; Meliponini; phytochemicals; Tanzania

Introduction

Free radicals are byproducts of the body's metabolism that are created through oxidation reactions. These natural compounds include hydrogen peroxide, hydroxyl radicals and superoxide anion radicals. They can also be produced by external factors such as radiation, pollution, stress, processed food, and environmental toxins (Tuksitha et al. 2018). Free radicals can lead to chain reactions in the body, causing oxidative stress and potentially damaging cells and tissues. Many diseases including cancer, heart disease, hypertension, diabetes mellitus and atherosclerosis have been linked to the accumulation of free radicals in body tissues (Shamsudin et al. 2019).

Antioxidants are molecules that can neutralize free radicals, thereby inhibiting destructive chemical reactions in the body (Chan et al. 2017). The human body has an endogenous antioxidant defense system that employs both enzymatic and non-enzymatic antioxidants to remove excess free radicals and mitigate the harmful effects of these molecules (Patlevič et al. 2016). However, this defense system can become overwhelmed, particularly in situations where there is an excessive production of free radicals or when the body's natural antioxidant capacity is compromised. In such cases, the intake of additional antioxidants, either from synthetic sources or naturally occurring in foods, can help to supplement and support the body's antioxidant defenses (Juma et al. 2023).

Honey stands out as a promising solution for addressing various health challenges in

the contemporary world. It is a potent source of antioxidants, primarily due to its abundance of bioactive phytochemicals including phenolic acids, flavonoids, vitamin C, and carotenoids (Muruke 2014). The unique composition of honey makes it a valuable alternative to synthetic drugs, as it poses minimal risks of toxicity (Zainol et al. 2013). Additionally, its accessibility and affordability makes it a viable option for a natural remedy (da Cruz et al. 2020).

Research on the antioxidant properties of honey has primarily focused on honey produced by the western honey bee, *Apis mellifera*, of the tribe Apini (Hymenoptera, Apidae). However, a related group of bees, the stingless bees of the tribe Meliponini (Hymenoptera, Apidae), also produce honey that is highly valued. Studies conducted in East Africa highlight the significant role of stingless bee honey in traditional medicine for treating various ailments (Kiprono et al., 2022; Héger et al., 2023; Mduda et al., 2023b). Despite this, the therapeutic potential of stingless bee honey, particularly from species native to the Afrotropical region, remains largely underexplored. This study, therefore, investigated the antioxidant potential of honey produced by *Axestotrigona ferruginea*, the most widespread stingless bee species in Tanzania (Mduda et al. 2023b). Specifically, it aimed to compare the phytochemical content and antioxidant activity of honey samples collected from two vegetation types, namely montane and mangrove. The findings underscore the antioxidant potential of Tanzanian stingless bee honey, which can enhance its use in therapy alongside existing traditional knowledge.

Materials and Methods

Study area

This study was carried out in Tanzania, located in East Africa (Figure 1). Honey samples were collected in September 2023 from two locations representing distinct vegetation types. The first location was Siha district, situated in northern Tanzania and bordering Mount Kilimanjaro to the west. The elevation of the sampling sites ranged from 1,618 to 1,880 meters above sea level (m.a.s.l), characterized with montane vegetation (Thomas et al. 2022). This area exhibited high plant diversity, with prominent representation of two plant families, Asteraceae and Fabaceae, along with other less abundant families including Amaranthaceae, Rubiaceae, Vitaceae, Malvaceae, Celastraceae, and Solanaceae. The second location was Kibiti district, located in eastern Tanzania along the western Indian Ocean coast. Here, honey was collected from the mangrove forest within the Rufiji Delta, which is renowned for having the largest concentration of mangroves on the eastern coast of Africa. The vegetation in this area comprises six plant families: Sonneratiaceae, Sterculiaceae, Rhizophoraceae, Combretaceae, Meliaceae and Avicenniaceae (Monga et al. 2018). The pot-puncture method described in Mduda et al. (2023b) was employed to collect honey samples from *Axestotrigona ferruginea* colonies managed in wooden hives. A total of fourteen samples were collected, with seven hives sampled from each vegetation type. After harvesting, samples were filtered and stored at 4 °C pending analysis.

Determination of the levels of selected phytochemicals in stingless bee honey

Total phenolic content

The Folin-Ciocalteau method outlined by Singleton et al. (1999) was employed for the determination of total phenolic content (TPC). A 10% (w/v) honey-methanol solution was sonicated for 15 minutes followed by centrifugation at 9000 rpm. Subsequently, 0.5 mL of the supernatant was transferred to a separate tube and mixed with 2.5 mL of Folin-Ciocalteau phenol reagent and 2 mL of 7.5% Na_2CO_3 solution. This mixture was mixed thoroughly and permitted to settle at room temperature for 2 hours. Absorbance was read at 765 nm using Cary 60 UV/Vis spectrophotometer (Agilent Technologies). A calibration curve was created using gallic acid (99%, Glentham Life Sciences) solutions with concentrations ranging from 0.02 to 0.20 mg/mL. TPC was quantified by comparing the absorbance values of the honey samples to the calibration

curve and the results were expressed as gallic acid equivalents (GAE) per 100 grams of honey (mg GAE/100 g).

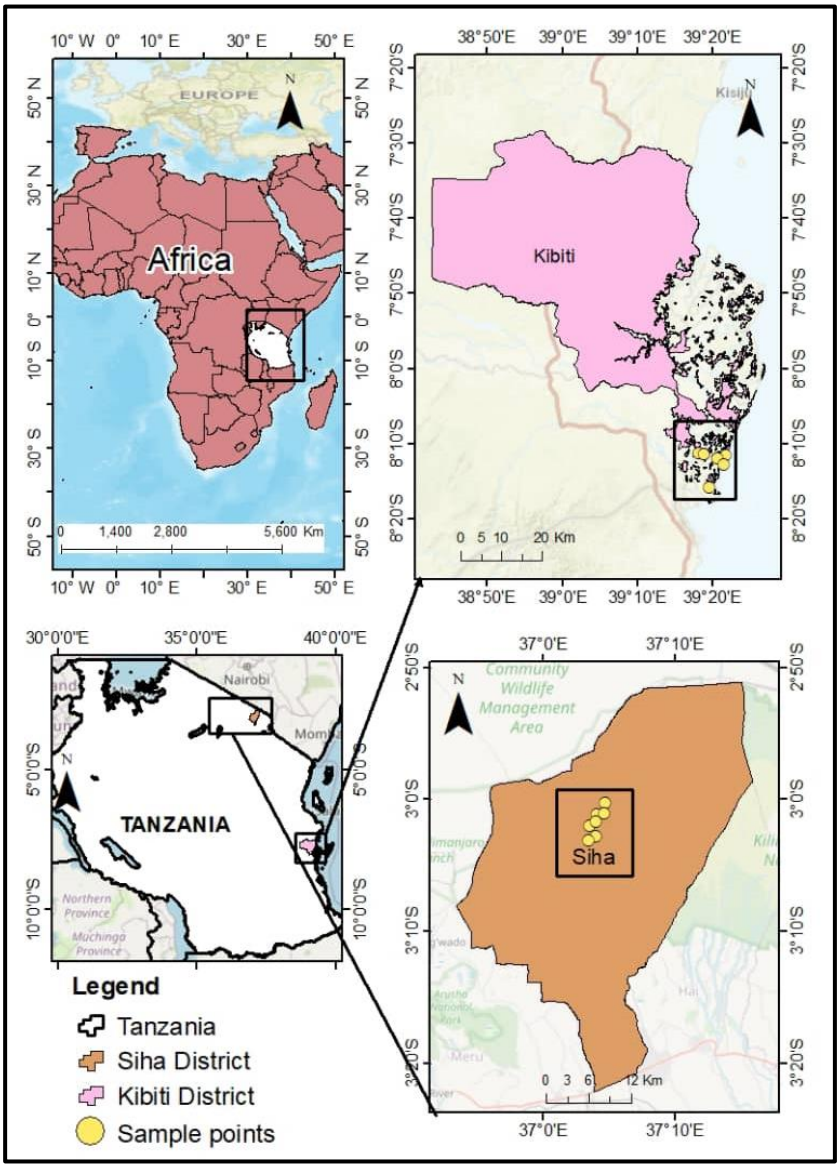

Figure 1: Map of the study area showing sample collection points in Siha and Kibiti districts, representing montane and mangrove vegetation, respectively.

Total flavonoid content

The total flavonoid content (TFC) in honey was determined using the method described by Zhishen et al. (1999). First, a sample solution was prepared by dissolving 5 grams of honey in 20 mL of distilled water. One mL of this solution was combined with 0.3 mL of 5% $NaNO_2$ and 4 mL of distilled water in a separate tube followed by the addition of 0.3 mL of 10% $AlCl_3$ after 5 minutes. The

mixture and allowed to settle for 1 minute followed by the addition of 2 mL of 1 M NaOH and 2.4 mL of distilled water. Cary 60 UV/Vis spectrophotometer (Agilent Technologies) was used to read the absorbance of the final mixture at 510 nm. A calibration curve was established using quercetin (98%, Glentham Life Sciences) concentrations ranging from 0.01 to 0.10 mg/mL. TFC was computed in quercetin equivalent per 100 grams of honey (mg QE/100 g).

Ascorbic acid

The ascorbic acid (AsA) content in honey was determined using the indophenol method described by Nielsen (2017). An indophenol solution was prepared by mixing sodium bicarbonate (42 g), distilled water (50 mL), and 2,6-dichlorophenol indophenol (50 mg), then diluting to a final volume of 200 mL, filtering, and storing in the dark. Three 50 mL conical flasks each containing 2 mL of a standard ascorbic acid solution (50 mg of standard ascorbic acid (99%, Sigma-Aldrich) dissolved in 50 mL of 5% metaphosphoric acid) were titrated with the indophenol solution until a pink color persisted for 5 seconds. Blank titrations were performed with 7 mL of 5% metaphosphoric acid against the indophenol solution. For the honey samples, 5 grams of honey were mixed with 25 mL of 5% metaphosphoric acid, filtered, and 7 mL of this solution was titrated similarly. The ascorbic acid content, expressed in milligrams per 100 grams of honey (mg/100 g), was calculated using the following formula:

$$Ascorbic\ acid = (X - B) \times \frac{V}{Y} \times \frac{F}{E} \times 100$$

Whereby, X = volume of indophenol solution for sample titration, B = average volume of indophenol solution for blank titration, V = volume (mL) of initial assay solution, Y = volume (mL) of sample aliquot titrated, F = weight (mg) of ascorbic acid equivalent to 1 mL of indophenol solution, and E = weight (g) of honey sample assayed.

Lycopene and β-carotene

Lycopene and β-carotene in honey were determined according to the procedure outlined in Ferreira et al. (2009). Initially, 100 mg of the sample were vigorously shaken with 10 mL of an acetone-hexane mixture (4:6) for 1 minute. The mixture was then filtered through Whatman No. 4 filter paper and the absorbance of the filtrate was measured at 453, 505, and 663 nm using Cary 60 UV/Vis spectrophotometer (Agilent Technologies). The concentrations of lycopene and β-carotene (in mg/100 g of honey) were calculated using the following equations:

$$\text{Lycopene} = 0.0458A_{663} + 0.372A_{505} - 0.0806A_{453}$$

$$\beta\text{-carotene} = 0.216A_{663} - 0.304A_{505} + 0.452A_{453}$$

Determination of the antioxidant activity of stingless bee honey

DPPH radical scavenging assay

The DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging activity was determined according to the method described by Chua et al. (2013). Initially, 0.75 mL of a sample solution (10 g of honey in 200 mL of methanol) was mixed with 1.5 mL of a 20 mg/L DPPH-methanol solution and incubated in the dark at room temperature for 15 minutes. The absorbance of the resulting mixture was read at 517 nm against a blank (0.75 mL honey solution combined with 1.5 mL methanol) using Cary 60 UV/Vis spectrophotometer (Agilent Technologies). Methanol (0.75 mL) was combined with 1.5 mL DPPH solution to make a control sample, while methanol was used as the blank. The DPPH scavenging activity calculated in percentage using the following formula:

$$DPPH\ scavaging\ activity = \frac{AC - AS}{AC} \times 100$$

Whereby, AC = absorbance of the control, and AS = absorbance of the sample.

Ferric reducing antioxidant power

The ferric reducing antioxidant power (FRAP) was determined based on the method described by Halvorsen et al. (2002). The FRAP reagent was prepared by combining 2.5 mL of a solution containing 10 mmol/L TPTZ in 40 mmol/L hydrochloric acid, 2.5 mL of 20 mmol/L iron (III) chloride, and 25 mL of 0.30 mol/L acetate buffer (pH 3.6).

Next, 4.5 mL of the FRAP reagent was mixed with 0.5 mL of the sample in a tube, followed by incubation at 37°C for 10 minutes. The absorbance was then measured at 595 nm using a Cary 60 UV/Vis spectrophotometer (Agilent Technologies). A calibration curve was generated using iron (II) sulfate solutions ranging from 0 to 1000 µmol, and the FRAP value was expressed in µmol Fe (II) per 100 g of honey.

Data analysis

All the studied parameters were measured in triplicates. The Shapiro-Wilk test was employed to assess data normality, revealing a normal distribution ($p > 0.05$). An independent samples t-test was conducted to compare the antioxidant properties of stingless bee honey from montane and mangrove vegetation types. Pearson's correlation coefficient was used to evaluate pairwise relationships among the measured parameters, specifically to determine the extent to which the antioxidant activity of honey was influenced by the levels of phytochemicals. Finally, principal component analysis was employed to classify the honey samples from the two vegetation types. Data analysis was carried out using PAleontological STatistics Software Version 4.03 (Hammer et al. 2001).

Results

Antioxidant potential of stingless bee honey

The antioxidant properties of stingless bee honey were evaluated using multiple parameters, and the findings are summarized in Table 1. Honey samples from both mangrove and montane vegetation types contained notable amounts of total phenolic and flavonoid content, with mangrove honey showing significantly higher concentrations ($p < 0.01$). Honey from the mangrove vegetation also had slightly higher levels of ascorbic acid, lycopene and β-carotene compared to montane honey, although these differences were not statistically significant ($p > 0.05$). Furthermore, no significant differences were observed in DPPH scavenging activity or ferric reducing antioxidant power (FRAP) between the two honey types ($p > 0.05$).

Table 1: Antioxidant properties of stingless bee honey from the montane and mangrove vegetation types

Assayed parameters	**Montane** (n = 7)	**Mangrove** (n = 7)	**Test statistics**	
	Mean ± SD	Mean ± SD	t	*p-value*
Total phenolic content (mg GAE/100 g)	197.0 ± 33.7[b]	263.1 ± 29.1[a]	3.9	*0.002*
Total flavonoid content (mg QE/100 g)	118.5 ± 18.5[b]	156.7 ± 21.5[a]	3.6	*0.004*
Ascorbic acid (mg/100 g)	25.1 ± 6.7[a]	30.0 ± 10.7[a]	1.0	*0.326*
Lycopene (mg/100 g)	2.8 ± 1.0[a]	3.7 ± 1.6[a]	1.3	*0.206*
β-Carotene (mg/100 g)	0.8 ± 0.7[a]	1.7 ± 1.3[a]	1.7	*0.119*
DPPH radical scavenging activity (%)	68.5 ± 3.6[a]	73.6 ± 5.9[a]	2.0	*0.075*
Ferric reducing antioxidant power (µmol Fe(II)/100 g)	265.9 ± 64.6[a]	272.7 ± 59.4[a]	0.2	*0.842*

Values with different superscripts in the same row are significantly different. n = number of samples, SD = standard deviation

Correlation among variables

The correlation matrix in Figure 2 displays the relationships between each pair of the assayed parameters. DPPH scavenging activity showed positive correlations with total phenolic content (TPC) (r = 0.82, $p = 0.000$), total flavonoid content (TFC) (r = 0.70, $p = 0.005$), ascorbic acid (r = 0.65, $p = 0.012$), and β-carotene (r = 0.65, $p = 0.013$). In contrast, ferric reducing antioxidant power (FRAP) was only correlated with TPC (r = 0.54, $p = 0.046$) and DPPH scavenging activity (r = 0.81, $p = 0.000$).

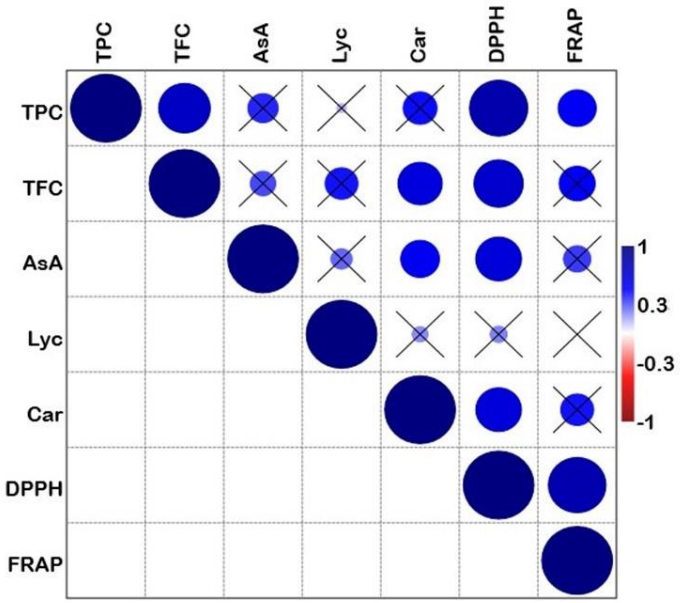

Figure 2: Correlation matrix showing pairwise Pearson's coefficients among variables. Crossed boxes (×) indicate correlations which are not statistically significant ($p > 0.05$). TPC = total phenolic content, TFC = total flavonoid content, AsA = ascorbic acid, Lyc = lycopene, Car = β-carotene, DPPH = DPPH radical scavenging activity, FRAP = ferric reducing antioxidant power

Classification of the honey samples based on vegetation types

A principal component analysis (PCA) plot was constructed using PC1 and PC2, which accounted for 84.1% of the total variance (Figure 3). The honey samples from the two vegetation types were separated along PC1, with montane honey skewing towards the negative side and mangrove honey towards the positive side. The biplot vectors indicate that mangrove honey contained relatively higher levels of total phenolic content (TPC), total flavonoid content (TFC), ascorbic acid, β-carotene, DPPH scavenging activity and ferric reducing antioxidant power (FRAP).

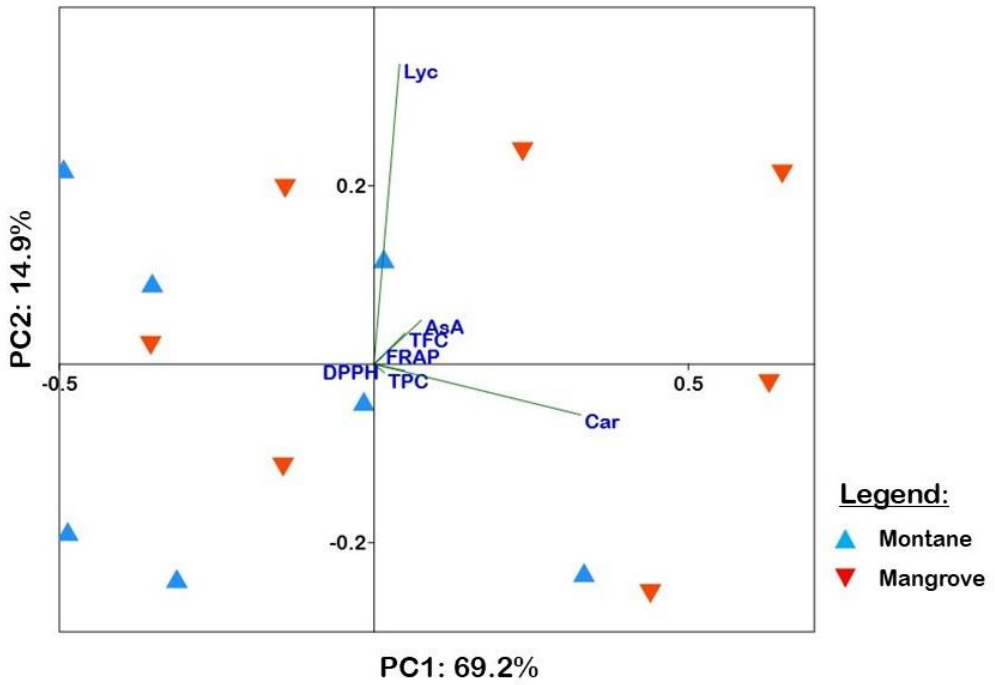

Figure 3: PCA plot for classifying stingless bee honey from the montane and mangrove vegetation types based on antioxidant properties. TPC = total phenolic content, TFC = total flavonoid content, AsA = ascorbic acid, Lyc = lycopene, Car = β-carotene, DPPH = DPPH radical scavenging activity, FRAP = ferric reducing antioxidant power.

Discussion

The studied honey samples had remarkable levels of total phenolic content (TPC) and total flavonoid content (TFC) (Table 1). These findings align with previous reports of TPC (46.8–365.2 mg GAE/100 g) and TFC (27.5–210.8 mg QE/100 g) in stingless bee honey from Kenya and Tanzania (Mokaya et al. 2022, Mduda et al. 2023a). The presence of these phytochemicals signifies the superior quality of the honey and is linked to diverse biofunctional properties. Phenolic acids and flavonoids are potent antioxidants, capable of counteracting free radicals and other reactive oxygen species, thereby shielding cells from oxidative damage (Bueno-Costa et al. 2016, Sousa et al. 2016). As such, these compounds play a crucial role in mitigating the risk of chronic diseases such as cardiovascular disease, cancer, hypertension, and diabetes (Shamsudin et al. 2019).

Ascorbic acid was also found at appreciable levels in the studied honey samples (Table 1). Previous research documented varying levels of ascorbic acid in stingless bee honey from Tanzania, ranging from 7.4 to 60.5 mg/100 g (Mduda et al. 2023a). Ranneh et al. (2018) recorded higher amounts of ascorbic acid (20–90 mg/100 g) in Malaysian stingless bee honey (Ranneh et al. 2018). Ascorbic acid reflects the amounts of vitamin C, a significant bioactive constituent in honey. Vitamin C is a potent antioxidant that rapidly scavenges reactive oxygen species (ROS), and enhances the antioxidant potency of honey (Majtan et al. 2020). Additionally, it is an essential nutrient for humans, responsible for collagen synthesis, cholesterol conversion, hormonal regulation and immune functioning (Chambial et al. 2013). Ascorbic acid is derived from the nectar collected by bees from flowers, and its concentration in honey depends on the botanical and

geographical origin, processing methods and storage conditions (Majtan et al. 2020).

Carotenoids are a minor component in honey, but they significantly contribute to its overall antioxidant capacity (Jimenez et al. 2016). Specifically, lycopene and β-carotene have been identified as powerful antioxidants, playing an important role in human biological systems (Muruke 2014). The levels of lycopene and β-carotene recorded in this study exceeded those previously reported for Malaysian stingless bee honey i.e. 0.1–0.2 mg/100 g and 0.2–0.3 mg/100 g for lycopene and β-carotene, respectively (Chan et al. 2017). Values comparable to our results were observed in honey samples from different country origins, with lycopene ranging from 0.5 to 1.2 mg/100 g, and β-carotene ranging from 0.9 to 2.7 mg/100 g (Smetanska et al. 2021). The carotenoid content in honey is determined by its botanical source and also contributes to the variations in honey color (Smetanska et al. 2021).

The antioxidant activity of honey was assessed based on its capacity to scavenge the DPPH free radical and its ability to reduce ferric ion (Fe^{3+}) to ferrous ion (Fe^{2+}). Honey samples from both vegetation types exhibited notable levels DPPH scavenging activity and ferric reducing antioxidant power (FRAP) (Table 1), indicating their high antioxidant potential. The level of DPPH scavenging activity observed in this study was within the range of 30.0–76.2% that was previously reported for Kenyan stingless bee honey (Mokaya et al. 2022), and exceeded that documented for stingless bee honey from Borneo (Tuksitha et al. 2018). Similarly, the studied honey samples displayed higher FRAP values compared to those reported for *Melipona* spp. (50–230 µmol Fe(II)/100 g) from Brazil (Braghini et al. 2020). Much higher FRAP values (180.6–512.1 µmol Fe(II)/100 g) were reported for Malaysian stingless bee honey (Shamsudin et al. 2019).

Phytochemicals such as polyphenols, ascorbic acid, and carotenoids play a crucial role in determining the antioxidant potency of honey (Bueno-Costa et al. 2016, Jimenez et al. 2016, Matjan et al. 2020). In this study, the antioxidant activity of stingless bee honey demonstrated significant correlations with TPC, TFC, ascorbic acid, and β-carotene (Figure 2). These results are consistent with previous findings by Muruke (2014) and Mduda et al. (2023a). Furthermore, the PCA plot indicates higher levels of phytochemicals and antioxidant activity in mangrove honey compared to montane honey (Figure 3). These differences likely stem from variations in the plant forage resources utilized by bees in the two vegetation types. These findings are consistent with previous studies that identified mangrove honey as a rich source of antioxidants, with significant pharmacological and nutraceutical potential (Islam et al. 2017, Basu and Cetzal-lx 2018). Future research should focus on identifying the specific plant species utilized by bees, as well as elucidating the bioactive compounds responsible for the observed antioxidant potency.

Conclusion

Stingless bee honey exhibited notable antioxidant potential, suggesting substantial therapeutic value. In particular, mangrove honey showed relatively higher levels of phytochemicals and antioxidant activity compared to montane honey. Further research is necessary to elucidate the mechanisms and bioactive compounds responsible for the observed activity, in order to fully validate the therapeutic potential of stingless bee honey.

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