Received: 19 July 2023 | Revised: 2 January 2024 | Accepted: 10 April 2024

DOI: 10.1002/jsf2.190

SHORT COMMUNICATION

JSFA Reports

Total phenolic and flavonoid content of *P. armata* honey and propolis produced in Bomet, Kisii and Maralal, Kenya

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Funding information
NORAD

Abstract

Background: Bee products are gaining interest in the field of research due to their biochemical and nutritive properties. Honey bee products have been researched extensively but little has been done in regards to stingless bees. There are many species of stingless bees including *Plebeina armata*, which are found in the Afrotropics. They are underground nesting and produce honey, propolis, wax, pollen and bee bread. These products are known to be rich in polyphenols that comprise of flavonoids and phenolic. In our study, we analysed colorimetrically the total flavonoid, phenolic content and radical scavenging activity of honey ($n = 22$) and propolis ($n = 25$) from Bomet, Kisii and Maralal in Kenya.

Results: Honey and propolis had total flavonoid content of 12.00–22.67 mg QE/100 g and 288.15–944.76 mg QE/100 g while total phenolic content was 87.01–239.93 mg GAE/100 g and 524.14–1225.01 mg GAE/100 g, respectively. In considerations to the regions, Maralal had the highest phenolic and flavanoid content followed by Bomet and Kisii was the least. The same trend was observed in the radical scavenging activity. Except for the total flavonoid content in honey, the difference was significant ($p < 0.05$).

Conclusion: The polyphenol content of both honey and propolis of *P. armata* are equally affected by geographical location as a result of different vegetation. They are good source of antioxidants, which can be utilized in diet due to their radical scavenging properties.

KEYWORDS
Afrotropics, Flavanoids, phenolics, *Plebeina*, stingless bee

INTRODUCTION

In many cultures, insects and their products are an invaluable source of food. Bee products, honey and propolis are among those that are gaining unmatched interest owed to their rich chemical composition that has priceless contribution to the human health and nutrition.[1] Honey is made up of sugars, water and other compounds such phytochemicals, vitamins, minerals and proteins all derived from the nectar and exudation of plant by the bees.[2,3] Propolis is a resinous mixture comprising of resins and gums from various plants, salivary bee secretions and wax.[4] Like honey, propolis chemical variability is due to its plant origin and different geographic locations of the source plants. This explains the diversity in the chemical composition of propolis.[5] Honey has long been utilized with studies indicating that its biological activities such as anti-inflammatory, antioxidant and antimicrobial actions are as a result of polyphenols. Which are a distinct group of phytocompounds from the secondary metabolism of plants and comprises of phenolic acids, flavonoids and phenolic.[6,7] Propolis has also been utilized as a remedy for various maladies in folk medicine and food industry possessing infinite potential for application in human and veterinary medicine.[8] This also makes them an alternative source of food and nutraceuticals.

Polyphenols are found in plants playing protective role against pathogen.[9,10] In food, they impart properties such as flavour and

bioactivities.[11] Foods rich in polyphenols are thought to offer protection against onset of some diseases such as cancer, diabetes, neurodegenerative diseases and cardiovascular diseases due to their antioxidant properties.[12]

Stingless bees have a salient characteristic in that they store their honey in pots.[13] They also utilize propolis as a major material in nest construction that offers protection against intruders to the nest.[14] Stingless bees are diverse and in Kenya, there are six genera that have been reported and *Plebeina* is among them. In this genera, we have *Plebeina armata*, which is an underground-nesting and usually modifies cavities dug by the termites.[15,16] Studies on honey and propolis of stingless bees especially from Africa and particular Kenya are scarce and many reports are on honey bee. In our study, we aimed at quantification of total flavonoid content (TFC), total phenolic of content and radical scavenging activity of honey and propolis of *P. armata* from three different regions in Kenya.

MATERIALS AND METHODS

Chemicals

Aluminium chloride ($AlCl_3$), sodium nitrite ($NaNO_2$), gallic acid, quercetin, sodium carbonate (Na_2CO_3), Folin–Ciocalteu's reagent, sodium hydroxide (NaOH) and ethanol were all of analytical grade and purchased from Sigma-Aldrich (Kobian Kenya Ltd.).

Sample collection

Honey ($n = 22$) and propolis (25) samples were collected from their natural environment in three different regions namely Bomet, Kisii and Maralal. The underground nests in the form of a round mould (Figure 1) were dug out and the honey collected under sterile conditions into sterile sample collection tubes. Propolis was also collected using sterile forceps and then wrapped in aluminium foil. They were transported in a cooler box to the lab where they were prepared for analysis.

Honey preparation

For the analysis, 10% (w/v) honey solution was prepared by dissolving 1 g of honey in 10 mL of distilled water.

Hydroalcohol preparation of propolis extracts

For propolis extracts, 5% (w/v) of the extract was prepared by crushing the propolis into powder in liquid nitrogen. The extract was then prepared by dissolving 0.5 g of the powder in 10 mL of 50% ethanol (v/v), and the solution was vortexed and left for 72 h. The resulting solution was centrifuged at 4000 rpm for 5 min and the supernatant collected for analysis.

FIGURE 1 Underground nest of *P. armata* when it was dug out (*Photo by T. Kegode*).

Total flavonoid content

Aluminium chloride ($AlCl_3$) colorimetric assay was used to determine the TFC[17] with minor modifications. In brief, to 1 mL of honey solution, 4 mL of distilled water was added and then 0.3 mL of 5% (w/v) sodium nitrite was introduced. The mixture was left for 5 min before adding 0.3 mL of 10% (w/v) aluminium chloride followed by 2 mL of sodium hydroxide after 1 min. The solution was then topped up with 2.4 mL of distilled water, and the absorbance of the solution read at 510 nm against a blank with all other components except the sample. These readings were then compared with those of a standard curve of quercetin (20–200 μg/mL) to calculate the TFC in 100 g of honey expressed as mg of quercetin equivalents (mg QE/100 g honey). The above procedure was repeated for propolis, and the results were expressed as (mg QE/100 g propolis).

Total phenolic content

Folin–Ciocalteu colorimetric method was used to determine the total phenolic content,[18] with minor modifications. Briefly, 5 mL of 0.2 N Folin–Ciocalteu reagent was added to 1 mL of the honey solution and left for 5 min before adding 4 mL of 75 g/L sodium carbonate. The mixture was then incubated in the dark and at room temperature for 2 h. The absorbance readings were read at 760 nm against a blank and compared to the gallic acid standard (0–250 μg/mL) to calculate the total phenolic content in 100 g of honey, which was expressed as mg of gallic acid equivalents (mg GAE/100 g honey). The same procedure was repeated for propolis and results expressed as (mg GAE/100 g propolis).

Determination of radical scavenging activity

To determine radical scavenging activity of the honey and propolis extracts, DPPH (1,1-diphenyl-2-picrylhydrazyl) assay was carried.[19] Briefly, DPPH ethanolic solution was prepared by adding 20 mg of DPPH into 1 L of absolute ethanol (20 mg/L). To 3 mL of this solution,

1.5 mL of propolis extract was added and the absorbance at 517 nm measured after 15 min of incubation at 25°C in the dark using a spectrophotometer (Biospec, Bartlesville, USA). Quercetin was used as a positive control in which each sample was assayed in three times, and the mean was obtained to calculate the percentage of radical scavenging activity, using the following formula:

$$\%\textbf{Radical Scavenging Activity} = [(\textbf{control absorbance} - \textbf{sample absorbance})/\textbf{control absorbance}] \times \textbf{100}$$

Data analysis

The R-Software version 4.2.2 software was used to compute all statistical analyses. To compare the total phenols and flavonoids phytochemical contents among the honey and propolis samples, we ran a Kruskal–Wallis test followed by post-hoc Dunn's test after confirming that the data for the parameters were not normally distributed and the variances were not homogeneous using the Shapiro–Wilk test ($p < 0.05$).

RESULTS

The TFC of honey ranged from 12.00 ± 1.65 to 22.67 ± 4.73 mg QE in 100 g. Bomet had the highest amount while Kisii had the least. The TPC of honey ranged from 87.01 ± 8.24 to 239.93 ± 24.00 mg GAE in 100 g with Maralal having the highest total phenolic and Kisii with the least (Table 1). The TFC of propolis ranged from 222.15 ± 189.32 to 944.76 ± 374.13 mg QE in 100 g with Maralal having the highest amounts and Kisii the least. The total phenolic content of propolis ranged from 454.60 ± 29.42 to 1225.01 ± 203.46 mg GAE. The total phenolic content was higher in both honey and propolis compared to the TFC.

The radical scavenging activity of honey and propolis displayed a similar pattern. Samples from Maralal had the highest activity while those from Kisii had the least (Figure 2). Propolis had higher activity than honey from all the regions.

DISCUSSION

The two hive products contain flavonoids and phenolics in varying quantities as shown in the Table 1. These belong to a group of compounds known as polyphenols, which are secondary metabolites of plants.[20] In the products, they originate from the nectar, plant exudates and resins that bees collect while foraging.[21] The chemical composition of honey and propolis is dependent on many factors such as bee species, vegetation and geographical location.[22] This explains the difference we see in the different regions. Maralal has hot and dry climate, Kisii has wet and cold climate while Bomet has hot and wet climate, which further expounds the observed variations in the chemical composition. Other researchers have reported different total phenolics and flavonoids content of these bee products from different countries (Table 2).

The TFC in honey in our study was lower than that recorded by Mokaya et al.[23] of 28.7–73.0 mg QE/100 g, and TPC was higher 54–214 mg GAE/100 g. In comparison to Brazil stingless bee honey, TFC of 6.67–27.22 mg QE/100 g is within the range but TPC of 16.30–62.33 GAE/100 g[24] is lower, while for Thailand TFC of 0.2–1.5 mg QE/100 g and TPC of 10–55 mg GAE/100 g[25] is lower compared to our results.

The TFC and TPC of propolis in our study compared to Indian stingless bee propolis 09.63–48.60 µg GAE/mg and 0.30–4.93 µg QE/mg[26] are lower and within the range of Indonesian stingless bee propolis of 0.76–3.39 mg/g QE and 10.00–28.65 mg/g GAE.[27]

We reported higher flavonoid and phenolic content in propolis than honey, which is in line with what has been reported earlier.[10] Among the two compounds, phenolic is more compared to the flavanoids in all the reports including ours.

Bee products are diverse in their chemical composition leading to their strong health-promoting properties such as the antioxidant

TABLE 1 Averages ± SEM of the total phenolic and flavonoid contents of honey and propolis from the different regions (mg/100 g).

Hive product	Honey		Propolis	
Region	TFC (mg QE/100 g)	TPC (mg GAE/100 g)	TFC (mg QE/100 g)	TPC (mg GAE/100 g)
Bomet	22.67 ± 8.41[a]	102.45 ± 29.96[a]	406.67 ± 58.69[ab]	586.58 ± 21.41[a]
Kisii	12.00 ± 1.65[a]	87.01 ± 8.24[a]	222.15 ± 189.32[a]	454.60 ± 29.42[a]
Maralal	21.00 ± 4.73[a]	239.93 ± 24.00[b]	944.76 ± 374.13[b]	1225.01 ± 203.46[b]
p-value	0.197	0.008	0.001	0.002

Note: Column with different letters are significantly different for $p < 0.05$ by Dunn's test.
Abbreviations: GAE, gallic acid equivalent; QE, quercetin equivalent; TFC, total flavonoid content; TPC, total phenolics content.

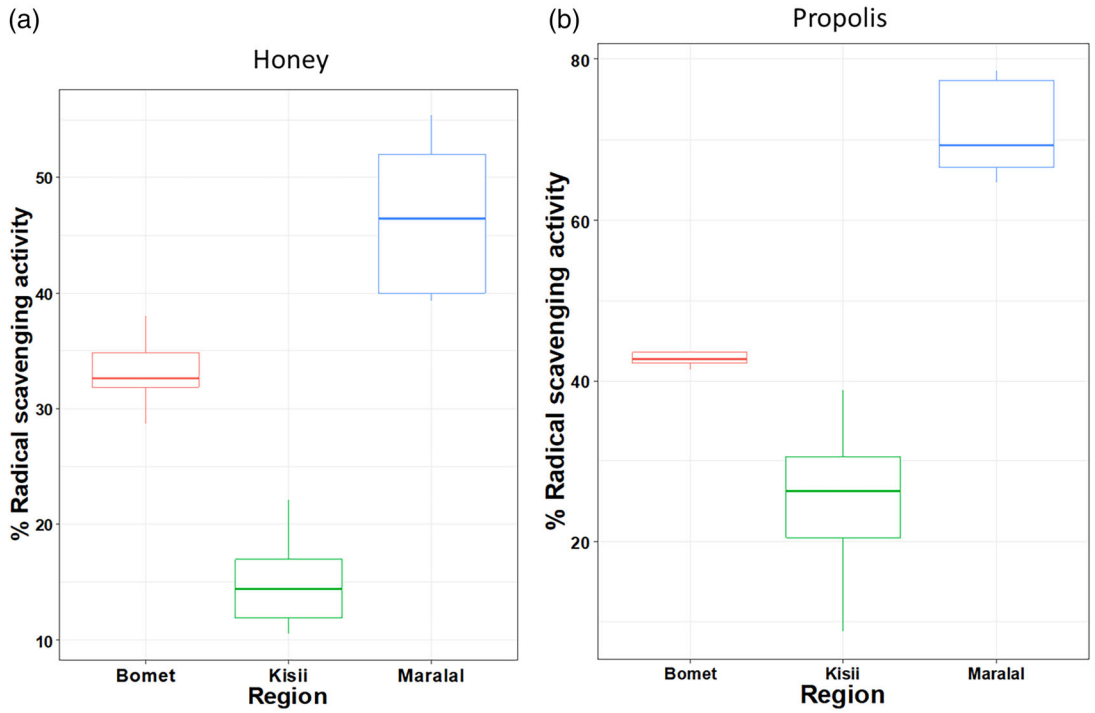

FIGURE 2 Radical scavenging activity of honey and propolis of *P. armata*.

TABLE 2 Total phenolics and flavonoid content of honey and propolis from other countries.

Flavonoids (quercetin equivalents)	Polyphenols (gallic acid equivalents)	Product	Country
10.70–25.71 mg/100 g	52.64–74.62 mg/100 g	*Honey*	Malaysia[39]
13.5 mg/100 g	4.47 mg/100 g	*Honey*	Malaysia[40]
3.74 mg/100 g	3.39 mg/100 g	*Honey*	
9.98 mg/100 g	6.74 mg/100 g	*Honey*	
28.7–73.0 mg /100 g	54–214 mg /100 g	*Honey*	Kenya[23]
6.67–27.22 mg/100 g	16.30–62.33 GAE/100 g	*Honey*	Brazil[20]
0.2–1.5 mg/100 g	10–55 mg /100 g	*Honey*	Thailand[25]
0.030–0.493 g/100 g	0.963–4.860 g /100 g	*Honey*	India[22]
0.76–3.39 mg/ 100 g	10.00–28.65 mg/100 g	*Honey*	Indonesia[23]
6.67–27.22 mg /100 g	16.30–62.33 mg/100 g	*Propolis*	Brazil[24]
299.4 mg/g	2192.7 mg /g	*Propolis*	Brunei[31]
275.2 mg/g	2391.0 mg /g	*Propolis*	
275.9 mg/g	2151.9 mg /g	*Propolis*	
3.42–22.83 mg/100 g	200.06–847.73 ± mg/100 g	*Propolis*	Cameroon[32]
4.8–9.8 mg/100 g	1.05–1.8 mg/100 g	*Propolis*	India[33]
102.2–3324.4 mg /100 g	522.6–3711.8 mg/100 g	*Propolis*	Kenya[19]
58.34–87.5 mg/g	167.6–246.3 mg/g	*Propolis*	Mexico[34]
45.15 mg /g	192 mg/g	*Propolis*	Morocco[35]
35.64–62.04 mg/g	150.05 to 197.14 mg/g	*Propolis*	Poland[36]
522.71 ± 11.45 mg/g	314.36 ± 3.65 mg/g	*Propolis*	Turkey[37]
300.1 mg/100 g	667.6 mg/100 g	*Propolis*	Venezuela[38]

activity[28] owing to their ability to scavenge free radicals.[29] The radical scavenging activity can be quantified calorimetrically. The chemical diversity influence their properties and biological activity and has been used to characterize the geographical origin, hence the difference in the radical scavenging of the products from the different regions.[30]

CONCLUSION

From our study, both honey and propolis of *P. armata* have considerable amounts of flavonoids and phenolic and exhibit radical scavenging activity. Geographical location has effect on their chemical compositions as this directly affects the vegetation type. Honey and propolis from the stingless bee can be utilized source of dietary antioxidants owed to their polyphenol composition.

ACKNOWLEDGMENTS

The authors acknowledge Norwegian Agency for Development Cooperation (NORAD) for funding the project. We are grateful to icipe for all facilitation provided for the accomplishment of this research. We also gratefully acknowledge the support of the International Centre of Insect Physiology and Ecology (*icipe*) core funding provided by the United Kingdom's Foreign, Commonwealth, and Development Office (FCDO); the Swedish International Development Cooperation Agency (Sida); the Swiss Agency for Development and Cooperation (SDC); the Federal Democratic Republic of Ethiopia and the Government of the Republic of Kenya.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

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How to cite this article: Kegode TM, Mokaya HO, Kiatoko N. Total phenolic and flavonoid content of *P. armata* honey and propolis produced in Bomet, Kisii and Maralal, Kenya. JSFA Reports. 2024;4(4):191–6. https://doi.org/10.1002/jsf2.190