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Original Article

Chemical Analysis of Polyphenolic Content and Antioxidant Screening of 17 African Propolis Samples Using RP-HPLC and Spectroscopy

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Abstract

Background and objectives: Propolis is a resinous material produced by honeybees. Its chemical composition is highly complex and varies significantly depending on geographic region and season. This intrinsic variability presents challenges to the standardization and quality control of propolis. This study aimed to evaluate the chemical composition, total phenolic content, and antioxidant potential of propolis collected from seventeen geographical regions across Africa.

Methods: A reverse-phase high-performance liquid chromatography (RP-HPLC) method coupled with a photodiode array detector was used for analysis of propolis samples. The flavonoid and phenolic contents of the samples were determined using colorimetric and *Folin-Ciocalteu* methods. Antioxidant capacity was assessed using the 2,2-diphenyl-1-picrylhydrazyl assay.

Results: Five flavonoids (naringenin, pinocembrin, galangin, chrysin, and quercetin), one flavonoid glycoside (rutin), six phenolic acids (caffeic acid, *p*-coumaric acid, cinnamic acid, chlorogenic acid, ferulic acid, and gallic acid), and an aromatic ester - caffeic acid phenethyl ester were simultaneously detected and quantified using RP-HPLC with an ACE-5 C18 column (250 mm × 4.6 mm i.d., 5 µm) and photodiode array detector detector. The reference standards showed good linearity with regression coefficients (R^2) ranging from 0.96 to 0.99. For precision, repeatability, and stability studies, the relative standard deviation for all reference standards was below 2.5%. The 2,2-diphenyl-1-picrylhydrazyl assay yielded EC_{50} values ranging from 17.6 ± 0.39 to 0.16 ± 0.001 mg/mL.

Conclusions: RP-HPLC method for the simultaneous quantification of thirteen reference standards will serve as a reliable tool for the standardization and quality evaluation of propolis. The flavonoid and phenolic contents are key contributors to the antioxidant activity of propolis and reflect local plant biodiversity and bee–plant interactions within the ecosystem.

Introduction

Researchers have shown growing interest in propolis over the past few years due to its wide range of therapeutic potential. Propolis is a resinous material produced by bees from a combination of beeswax and plant exudates. Its chemical composition is influenced by various factors including geographical location, local flora, and the type of bees collecting propolis.[1] With the advent of hyphenated analytical techniques, more than 800 different phytochemicals have been reported in propolis from several parts of the world.[2,3] This chemical variability poses significant challenges to the standardization and quality control of raw propolis.

Polyphenols are the most abundant constituents in propolis, mainly represented by flavonoids, phenolic acids, and their esters.[4] These compounds are largely responsible for propolis's diverse biological activities, including antioxidant, antimicrobial, anti-inflammatory, and immunomodulatory effects.[5] Consequently, they have been identified as biomarkers for the standardization of raw propolis.[6]

The complex nature of propolis presents challenges in estab-

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lishing consistent quality assessment protocols. This is a significant factor limiting its universal acceptability as an authenticated drug or health-promoting product in international food and healthcare markets. The literature suggests that a multiple-marker, compound-targeted approach which enables the characterization of different propolis types would be appropriate to ensure consistent quality.[1] Different analytical methods have been used to analyse the chemical profiles of propolis from various regions.[7–10] Among these, chromatographic methods such as liquid chromatography offer valuable insights into the origin of the samples. As a result, many researchers recommend prioritizing these techniques as key analytical tools for the standardization of propolis.[1]

The chemical composition of propolis has been extensively studied across numerous geographical locations worldwide. Although propolis has been used widely in Africa for decades, scientific research on its chemical composition remains limited to a few comprehensive studies.[11] In South African propolis, the chemical profiles of some raw samples resemble those of temperate region poplar propolis, while others show similarities to eastern Mediterranean propolis, particularly due to the significant presence of diterpenoids.[11,12] Triterpenes, alk(en)ylphenols, alk(en)ylresorcinols, and monoterpenes have been reported in North-Western Cameroonian propolis.[13] A study by Zhang *et al*.[14] on Nigerian propolis identified prenylated isoflavonoids similar to those in Brazilian red propolis, along with a notable abundance of stilbenoids. Sawaya *et al*.[8] also reported the presence of pinocembrin in African propolis samples. Biological studies investigating antioxidant, antimicrobial, and anti-inflammatory activities have been conducted on African propolis.[15] For example, antibacterial activities have been reported for Cameroonian propolis,[16] while novel anti-trypanosomal flavanonol and alkylresorcinol compounds have been identified in Libyan propolis.[17]

The resemblance of African propolis to poplar propolis has informed the selection of chemical markers for analyzing samples from Malawi, Nigeria, Tanzania, and Zambia in the present study. The aim of this study was to perform the simultaneous quantification and chemical mapping of 17 African propolis samples using a reverse-phase (RP) high-performance chromatography (HPLC) couples with photodiode array detector. A simple and validated analytical method will be essential for identifying the chemical diversity of propolis across the African region. Another objective was to determine the phenolic and antioxidant profiles specifically using 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay in the raw African propolis via ultraviolet (UV) spectroscopy.

Materials and methods

Chemicals

Ferulic acid (Acros Organics, USA), cinnamic acid (Merck, Germany), caffeic acid, p-coumaric acid, quercetin, gallic acid, naringenin, pinocembrin, galangin, rutin, chlorogenic acid (Sigma-Aldrich, Germany), caffeic acid phenethyl ester (CAPE), chrysin (AbCam, UK), and DPPH (Sigma-Aldrich, UK) were used for the analysis. Solvents such as methanol, formic acid, absolute ethanol (Fisher Scientific, UK), and water were of analytical grade.

Raw propolis samples

Propolis samples were collected from Malawi, Nigeria, Tanzania, and Zambia between September and December 2016 from various locations (see Fig. 1). The samples were coded based on the region and country of collection. Samples with no specific locality were coded as "unknown," as shown in Table 1. The texture and color of the propolis samples varied, reflecting the diverse flora across the regions (Fig. 1). Samples were stored in a cool, dark environment until analysis.

Preparation of raw propolis samples for analysis

One hundred milligrams of each propolis sample were extracted via sonication at 50°C for 30 m using 10 mL of 70% ethanol. After cooling to room temperature, the extracts were filtered into 10 mL volumetric flasks. The final volume was adjusted to 10 mL with 70% ethanol and centrifuged at 3,500 rpm for 5 m. The supernatant was filtered using Whatman No. 1 filter paper and stored in a refrigerator until analysis. For RP-HPLC analysis, the samples were further filtered through a 0.45 µm HPLC syringe filter, and 10 µL of each sample was injected into the HPLC system.

Preparation of standard solutions for HPLC calibration curves

Stock solutions (10 mg/mL) of caffeic acid, p-coumaric acid, ferulic acid, gallic acid, chlorogenic acid, rutin, quercetin, cinnamic acid, naringenin, pinocembrin, chrysin, CAPE, and galangin were prepared in HPLC-grade methanol. Each standard solution was injected at different concentrations (10 µL/injection) to generate calibration curves.

RP-HPLC analysis

Analysis was conducted using a Chromaster (Hitachi) HPLC system equipped with an autosampler (5260), pump (5160), column oven (5310), and photodiode array detector detector (5430). An ACE-5 C18 column (250 mm × 4.6 mm i.d., 5 µm) with a security guard cartridge was used. The mobile phase consisted of methanol (solvent A) and 0.1% formic acid (solvent B), with the following gradient at a flow rate of 1 mL/m: 65% B, 0 m; 50% B, 8 m; 40% B, 15 m; 35% B, 25 m; 20% B, 40 m; 10% B, 60 m and 10% B, 70 m. A 10-m equilibration time was allowed between runs. The chromatograms were monitored at 290 nm.[6] The method's precision was assessed by repeating the analysis of three concentrations of standard solutions, calculating the relative standard deviation of retention time and area under the curve for standards to assess precision, repeatability, and stability.[6] The stability of standard solutions was evaluated over 24 h at room temperature.

Method accuracy was determined based on previously cited literature.[6] Briefly, known amounts of each standard were added to 1 mL of an already-analysed propolis sample and analysed in triplicate. The total amount of each compound was determined from its calibration curve, and the recovery percent was calculated using the formula:

$$\text{Recovery (\%)} = \frac{\text{Amount found} - \text{Amount contained}}{\text{Amount found}} \times 100$$

Total phenolic content (TPC) and total flavonoid content (TFC) determination

The phenolic content of African propolis samples was assessed as TPC and TFC using the *Folin-Ciocalteau* method and the Jurd and Geissmann colorimetric method, respectively, as described in the literature.[18–20] TPC results are expressed as milligrams of gallic acid equivalent per gram of raw propolis (mg gallic acid (GAE)/g), and TFC results as milligrams of quercetin equivalent per gram of raw propolis (mg quercetin (QE)/g).

DPPH radical-scavenging activity of propolis samples

Hydrogen-donating activity was measured based on the direct

Fig. 1. Geographical locations of samples: Figure representing the collection site and color of propolis. Google map presentation, where the red dot represents propolis collection sites. Samples with no specific known locality are not shown on the map. Google Maps were accessed on 09/05/2019. Pictures of African propolis are shown as: (a) MAL-BLA (Blantyre (Southern), Malawi); (b) MAL-MAT (Matete, Malawi); (c) NIG-BEN (Benue state, Nigeria); (d) NIG-CRO (Cross River state Nigeria); (e) NIG-JOS (Jos Platue state, Nigeria); (f) NIG-KOG (Kogi state, Nigeria); (g) NIG-NAS-1 (Nassarava state, Nigeria); (h) NIG-NAS-2 (Nassarava state, Nigeria); (i), NIG-NIG (Niger state, Nigeria); (j) NIG-OND (Ondo state, Nigeria); (k) NIG-UNK (Unknown, Nigeria); (l) NIG-ADA (Adamawa state, Nigeria); (m) TAN-MOS (Mosh, Tanzania); (n) TAN-PEM; (o) TAN-UNK-1 (Unknown, Tanzania); (p) TAN-UNK-2 (Unknown, Tanzania); (q) ZAM-MIO (Miombo forest, Zambia).

Table 1. Countries and regions of the African propolis samples collection

Country	Region	Code
Malawi	Blantyre (Southern)	MAL-BLA
Malawi	Matete	MAL-MAT
Nigeria	Adamawa state	NIG-ADA
Nigeria	Benue state	NIG-BEN
Nigeria	Cross River state	NIG-CRO
Nigeria	Jos Plateau state	NIG-JOS
Nigeria	Kogi state	NIG-KOG
Nigeria	Nassarava state	NIG-NAS-1
Nigeria	Nassarava state	NIG-NAS-2
Nigeria	Niger state	NIG-NIG
Nigeria	Ondo state	NIG-OND
Nigeria	Unknown	NIG-UNK
Tanzania	Moshi	TAN-MOS
Tanzania	Pemba iceland	TAN-PEM
Tanzania	Unknown	TAN-UNK-1
Tanzania	Unknown	TAN-UNK-2
Zambia	Miombo forest	ZAM-MIO

hydrogen donation to the DPPH radical, following a previously reported method.[21] Propolis samples were screened by calculating the percentage inhibition of DPPH using the following equation:

$$\text{DPPH \%} = \frac{\text{Absorbance of DPPH} - \text{Absorbance of extracts} + \text{DPPH}}{\text{Absorbance of the blank}} \times 100$$

The sample concentration (EC_{50}, mg/mL) that scavenges 50% of DPPH radicals was used to express the antioxidant activity of the sample. The EC_{50} values were calculated from the dose-response curve plotting scavenging activity (%) against the concentration of each respective sample.[22]

Results

RP-HPLC analysis of African propolis samples

An RP-HPLC gradient method was developed to ensure that the most complex patterns of peaks in the test sample solutions were well resolved. The RP-HPLC method was optimized by varying different analytical parameters, such as flow rate, mobile phase composition, and gradient, among others. Under the chromatographic conditions mentioned above, the peak of each marker was well resolved, indicating that the method was highly selective. Intensity versus retention time chromatograms for reference standards are shown in Fig. 2. Calibration curves for these reference standards were constructed across a range of 0.001–0.5 µg/mL (Table 2). The UV spectra with absorption maxima of all the reference standards and their relative retention times are shown in Figure 2. Linear regression was constructed against the ratio of intensity to the concentration (µg/mL) of the standards (Table 2). R^2 values ranging from 0.9651 to 0.9999 were observed, indicating the linearity of the calibration curves for all standards. Linearity within the investigated concentration ranges was observed in their respective linear ranges.

Considering the complexity of the propolis composition and the number of detected peaks, the precision is reasonable. In the stability and repeatability studies, the percent relative standard deviation of compounds displayed a range from 0.4–2.1 and 0.5–2.4, respectively (Table 2). The results showed that the standards were stable at room temperature for 24 h, and the developed method was sufficiently effective for the routine analysis of propolis.

The most complex region of the raw sample chromatograms was between three and forty minutes, where peaks eluted very close to each other. This demonstrates the complexity of propolis analysis, making precise quantification a challenge.

In the propolis sample chromatograms, peaks corresponding to the reference standards were identified by comparing retention times and UV spectra under the same operating conditions. Additionally, sample solutions were spiked with reference standards to assist in confirming peak identity. The individual standards quantified (µg/g) in the propolis samples are shown in Table 3.

Determination of total phenolic and flavonoid content

The results of TPC and TFC of 17 African propolis samples are presented in Table 4.

DPPH radical-scavenging activity of propolis samples

The antioxidant potential of the African propolis samples is presented as (sample concentration that scavenges 50% of DPPH radicals) EC_{50} values in Table 4.

Discussion

RP-HPLC analysis of African propolis samples

Among the thirteen marker compounds, naringenin was found to be present in most of the samples. Ferulic acid and CAPE were absent in all analyzed African propolis samples. Gallic acid was only observed in samples from Kogi and Nassarawa state regions in Nigeria. Chlorogenic acid was detected only in Malawian samples. A wide variation in the composition of the propolis samples was observed based on the reference standards and the number of peaks in the chromatograms. The variation in the chemical profile of African propolis is related to the type of plant species surrounding the beehives at the collection sites.[23] The RP-HPLC study shows that propolis from Malawi, Tanzania, and Zambia contained a greater proportion of marker compounds compared to Nigerian propolis.

Malawian propolis showed the presence of caffeic acid, p-coumaric acid, ferulic acid, quercetin, cinnamic acid, naringenin, pinocembrin, chrysin, and galangin (Fig. 3). Standards such as gallic acid, chlorogenic acid, rutin, and CAPE were either not detectable or absent.

The standards identified in propolis samples from Nigeria were gallic acid, caffeic acid, p-coumaric acid, rutin, quercetin, cinnamic acid, naringenin, pinocembrin, and chrysin (Fig. 4). Standards such as chlorogenic acid, ferulic acid, CAPE, and galangin were either not detectable or absent in the Nigerian propolis. Samples from the Adamawa and Unknown regions in Nigeria contained only naringenin, samples from the Jos Plateau state region contained only cinnamic acid, while the Niger state sample contained only caffeic acid. A sample from the Moshi region in Nigeria contained only quercetin among the tested reference standards. In contrast to all analyzed African propolis samples, the Pemba Island sample did not contain any of the thirteen marker compounds. Furthermore, pinocembrin was observed to

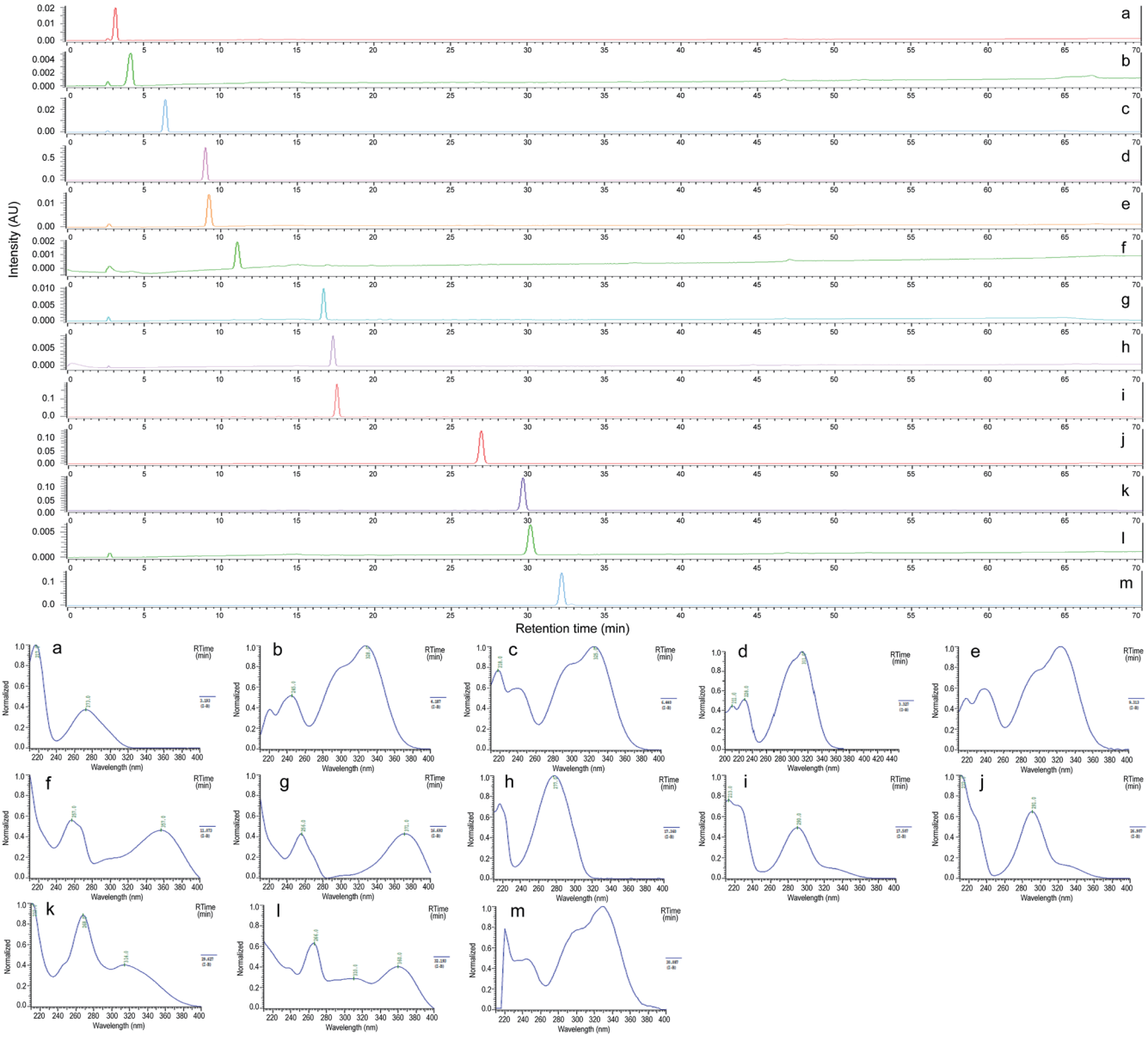

Fig. 2. Chromatograms of standard materials: The relative chromatograms of reference standards and their UV spectra with retention times in minutes. (a) Gallic acid (R_t 3.19 m, λmax 217, 273 nm); (b) Chlorogenic acid (R_t 4.49 m, λmax 245, 327 nm); (c) Caffeic acid (R_t 6.40 m, λmax 218, 245, 325nm); (d) p-Coumaric acid (R_t 9.05 m, λmax 227, 310 nm); (e) Ferulic acid (R_t 9.31 m, λmax 254, 324 nm); (f) Rutin (R_t 11.17 m, λmax 257, 357 nm); (g) Quercetin (R_t 16.69 m, λmax 256, 371 nm); (h) Cinnamic acid (R_t 17.36 m, λmax 277 nm); (i) Naringenin (R_t 17.50 m, λmax 290 nm); (j) Pinocembrin (R_t 26.29 m, λmax 290 nm); (k) Chrysin (R_t 29.26 m, λmax 268, 314 nm); (l) CAPE (R_t 30.08 m, λmax 328 nm); (m) Galangin (R_t 32.19 m, λmax 266, 310, 360 nm). AU, absorbance unit; CAPE, caffeic acid phenethyl ester; UV, ultraviolet.

be a prominent component in the Nigerian samples from Cross River state and Kogi.

Chromatograms of propolis samples from Tanzania (Fig. 5) showed the presence of caffeic acid, p-coumaric acid, quercetin, rutin, and naringenin, while other standards were either present at non-detectable levels or absent.

Analysis of Zambian propolis revealed the presence of caffeic acid, p-coumaric acid, cinnamic acid, naringenin, chrysin, and galangin, whereas other reference standards were either present at non-detectable levels or absent (Fig. 6).

It is worth noting that, despite being biomarkers for European and Brazilian propolis, ferulic acid and CAPE were absent in all 17 African propolis samples investigated, suggesting that unique floral sources surround the beehives that produced the propolis samples in this study.[24,25]

There are many peaks eluting at the later part of the chromatogram, suggesting the non-polar nature of the chemicals. Although the aim of this study was limited to the simultaneous quantification of markers, TPC and TFC, and antioxidant activity, further studies to identify these chemicals will help determine their chemical nature and predict the biological potential of African propolis.

Table 2. Regression equations with R^2 values, linearity, precision, stability, and repeatability of the RP-HPLC method

Standard	Equation of the calibration curve	Determination coefficient (R^2)	Linearity range (µg/mL)	Precision RSD (%)	Stability RSD%	Repeatability RSD%	Accuracy	
							Mean recovery (%)	Mean RSD (%)
Ferulic acid	y = 730,425 x + 8,016.8	0.9811	0.001–0.05	±2.2	0.8	1.0	100.57	1.97
Cinnamic acid	$y = 1 \times 10^6$ x + 176.2	0.9997	0.01–0.3	±4.3	0.8	0.7	100.60	1.20
Caffeic acid	y = 577,684 x + 504.34	0.9801	0.05–0.5	±1.7	1.1	1.3	100.48	1.25
p-coumaric acid	$y = 2 \times 10^6$ x + 98.9	0.9999	0.01–0.05	±1.9	1.5	0.5	100.37	1.69
Quercetin	$y = 1 \times 10^6$ x + 15,370	0.9651	0.01–0.3	±1.6	0.9	1.6	100.25	1.01
Gallic acid	y = 464,738 x + 728.3	1	0.05–0.25	±1.3	0.4	0.5	100.42	1.24
Naringenin	$y = 9 \times 10^6$ x + 12,307	0.9996	0.01–0.10	±6.5	1.0	0.8	100.39	1.10
Pinocembrin	$y = 1 \times 10^5$ x + 1,492.3	0.9998	0.01–1.00	±5.1	1.2	1.3	100.51	1.65
Galangin	$y = 4 \times 10^5$ x + 6,009.3	0.9947	0.01–0.30	±4.5	1.2	1.4	100.44	1.23
Rutin	y = 113,682 x + 2,769.5	0.9849	0.05–0.25	±3.9	0.6	2.4	100.33	1.48
Chlorogenic acid	y = 369,530 x + 7,578.5	0.9823	0.05–0.25	±3.2	0.7	0.9	100.4	1.3
CAPE	y = 606,110 x + 129.1	1	0.01–0.05	±4.1	2.1	2.1	100.5	1.23
Chrysin	$y = 8 \times 10^5$ x + 1,982.3	0.9999	0.005–0.05	±3.8	0.7	1.9	100.44	1.43

y, area under the curve; x, concentration (µg/mL). CAPE, caffeic acid phenethyl ester; HPLC, high-performance chromatography; RP, reverse-phase; RSD, relative standard deviation.

Table 3. Levels of reference standards estimated in African propolis samples

	Amount of standard compound per gram of propolis (µg/g)																
Standard	MAL-BLA	MAL-MAT	NIG-ADA	NIG-BEN	NIG-CRO	NIG-JOS	NIG-KOG	NIG-NAS-1	NIG-NAS-2	NIG-NIG	NIG-OND	NIG-UNK	TAN-MOS	TAN-PEM	TAN-UNK-1	TAN-UNK-2	ZAM-MIO
Ferulic acid	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent
Cinnamic acid	510.88	Absent	Absent	Absent	Absent	620.02	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	1, 830.08
Caffeic acid	1, 260.1	1, 950.11	Absent	4, 370.5	Absent	Absent	Absent	Absent	520.37	50.28	50.48	Absent	Absent	Absent	Absent	40.67	160.73
p-coumaric acid	Absent	100.82	Absent	Absent	Absent	Absent	70.8	Absent	Absent	Absent	Absent	Absent	Absent	Absent	100. 35	100.56	200.36
Quercetin	Absent	800.29	Absent	800.06	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	800. 15	Absent	Absent	1, 400.75	Absent
Gallic acid	Absent	Absent	Absent	Absent	Absent	Absent	1, 500.98	660. 02	1, 460.92	Absent	Absent	Absent	Absent	Absent	*	Absent	Absent
Naringenin	140.97	Absent	2, 910.85	210.45	2, 090.7	Absent	310.13	Absent	140.1	Absent	140. 45	200. 07	Absent	Absent	Absent	180.25	160.63
Pinocembrin	220.94	Absent	Absent	Absent	84, 140.13	Absent	25, 560.25	190. 83	Absent	Absent	Absent	Absent	Absent	Absent	Absent	200.48	Absent
Galangin	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	70
Rutin	Absent	240.62	Absent	Absent	Absent	Absent	60.62	Absent	Absent	Absent	Absent	Absent	Absent	Absent	140.3	740.64	Absent
Chlorogenic acid	12, 230.87	2, 840	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent
CAPE	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent
Chrysin	90.31	Absent	Absent	Absent	220.05	Absent	70.91	Absent	Absent	Absent	50.77	Absent	Absent	Absent	Absent	Absent	280.13

Table 4. TPC, TFC, and antioxidant activities of African propolis samples

Code	TPC (mg GAE/g)	TFC (mg QE/g)	DPPH EC_{50} (mg/mL)*
MAL-BLA	7.01 ± 0.04	15.50 ± 0.06	2.4 ± 0.02
MAL-MAT	15.09 ± 0.02	40.84 ± 0.03	0.39 ± 0.01
NIG-ADA	7.48 ± 0.04	42.84 ± 0.03	2.1 ± 0.04
NIG-BEN	41.09 ± 0.08	228.51 ± 0.04	0.16 ± 0.001
NIG-CRO	26.66 ± 0.12	54.75 ± 0.19	0.79 ± 0.001
NIG-JOS	4.62 ± 0.07	11.84 ± 0.09	5.2 ± 0.06
NIG-KOG	59.29 ± 0.05	323.40 ± 0.08	0.22 ± 0.001
NIG-NAS-1	56.98 ± 0.05	307.66 ± 0.03	0.17 ± 0.002
NIG-NAS-2	7.82 ± 0.04	11.49 ± 0.09	3.9 ± 0.03
NIG-NIG	30.60 ± 0.11	27.65 ± 0.04	0.34 ± 0.004
NIG-OND	8.28 ± 0.04	8.40 ± 0.12	7.4 ± 0.05
NIG-UNK	10.47 ± 0.03	14.81 ± 0.07	1.3 ± 0.01
TAN-MOS	3.02 ± 0.11	4.41 ± 0.06	17.6 ± 0.39
TAN-PEM	27.05 ± 0.12	7.83 ± 0.13	6.5 ± 0.3
TAN-UNK-1	25.47 ± 0.13	9.74 ± 0.11	3.0 ± 0.02
TAN-UNK-2	4.11 ± 0.08	63.49 ± 0.02	6.1 ± 0.04
ZAM-MIO	2.73 ± 0.12	168.37 ± 0.06	0.72 ± 0.004

*Control (ascorbic acid, 0.004 ± 0.000004). DPPH, 2,2-diphenyl-1-picrylhydrazyl; EC_{50}, sample concentration that scavenges 50% of DPPH radicals; GAE, gallic acid; QE, quercetin; TFC, total flavonoid content; TPC, total phenolic content.

Determination of total phenolic and flavonoid content

Flavonoids are the most abundant phenolic compounds in propolis. The biological potential of propolis, such as its antioxidant activity, relies on the composition of its phenolic compounds.[26] The chemical profile of propolis varies according to location, climate, time of year, bee species, and surrounding flora.[23] In terms of TPC, Zambian propolis presented the lowest phenolic content (2.73 ± 0.12 mg GAE/g), while Nigerian propolis from Kogi state (NIG-KOG) presented the highest polyphenol content (59.29 ± 0.05 mg GAE/g). The flavonoid content results indicated that the highest flavonoid value was found in sample NIG-KOG from Nigeria, with a value of 323.40 ± 0.08 mg QE/g of propolis, while the lowest amount was observed in sample TAN-MOS from Moshi Tanzania, with a value of 4.41 ± 0.06 mg QE/g. The results of the phenolic profile study align well with the RP-HPLC analysis data.

The phenolic content in NIG-NIG from Niger state, Nigeria and the sample TAN-MOS from Moshi Tanzania suggests higher antimicrobial potential, as high phenolic acid content is associated with increased antimicrobial activity, while higher flavonoid content correlates with antioxidant activity. As observed in Table 4, samples with the highest TFC exhibited the lowest EC_{50} values, suggesting their higher antioxidant potential. Thus, this simultaneous analysis study has the potential to provide insights into the biological potential of propolis samples.

DPPH radical-scavenging activity of propolis samples

Propolis has been studied *in vivo* for its antioxidant potential and its ability to relieve oxidative stress by scavenging free radicals, which are associated with many diseases, such as cardiovascular disorders, diabetes, and inflammation.[27,28] Propolis samples showed moderate scavenging activity against DPPH free radicals, with EC_{50} values ranging from 17.6 ± 0.39 to 0.16 ± 0.001 mg/mL, in comparison to the control. Samples NIG-BEN (Beneu state), NIG-NAS-1 (Nassarava state), and NIG-KOG (Kogi state) from Nigeria showed the highest antioxidant potential (EC_{50} values of 0.16 ± 0.001, 0.17 ± 0.002, and 0.22 ± 0.001, respectively) among the 17 African samples analyzed. The samples with the maximum DPPH radical scavenging activity were those rich in polyphenols and flavonoids, as presented in Table 4. The results of our study agree with previous investigations on African propolis.[29]

Although 17 samples were investigated in the current study, they may not entirely represent the chemical diversity of all African propolis. Africa is an enormous continent with a broad range of flora, which could significantly influence propolis chemical composition. In addition, the samples may have been collected from areas that are more accessible or convenient, leading to regional bias.

RP-HPLC is a reasonable analytical method, however, it may

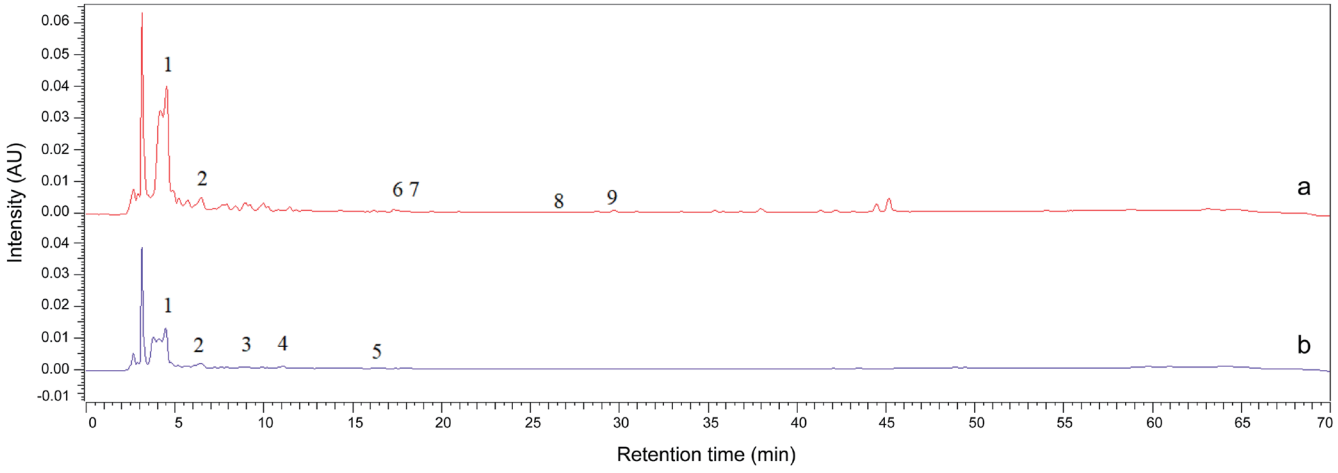

Fig. 3. Chromatograms for raw propolis samples from Malawi with identified standards. (a) MAL-BLA (Blantyre (Southern), Malawi); (b) MAL-MAT (Matete, Malawi). 1. Chlorogenic acid; 2. Caffeic acid; 3. p-Coumaric acid; 4. Rutin; 5. Quercetin; 6. Cinnamic acid; 7. Naringenin; 8. Pinocembrin; 9. Chrysin. AU, absorbance unit.

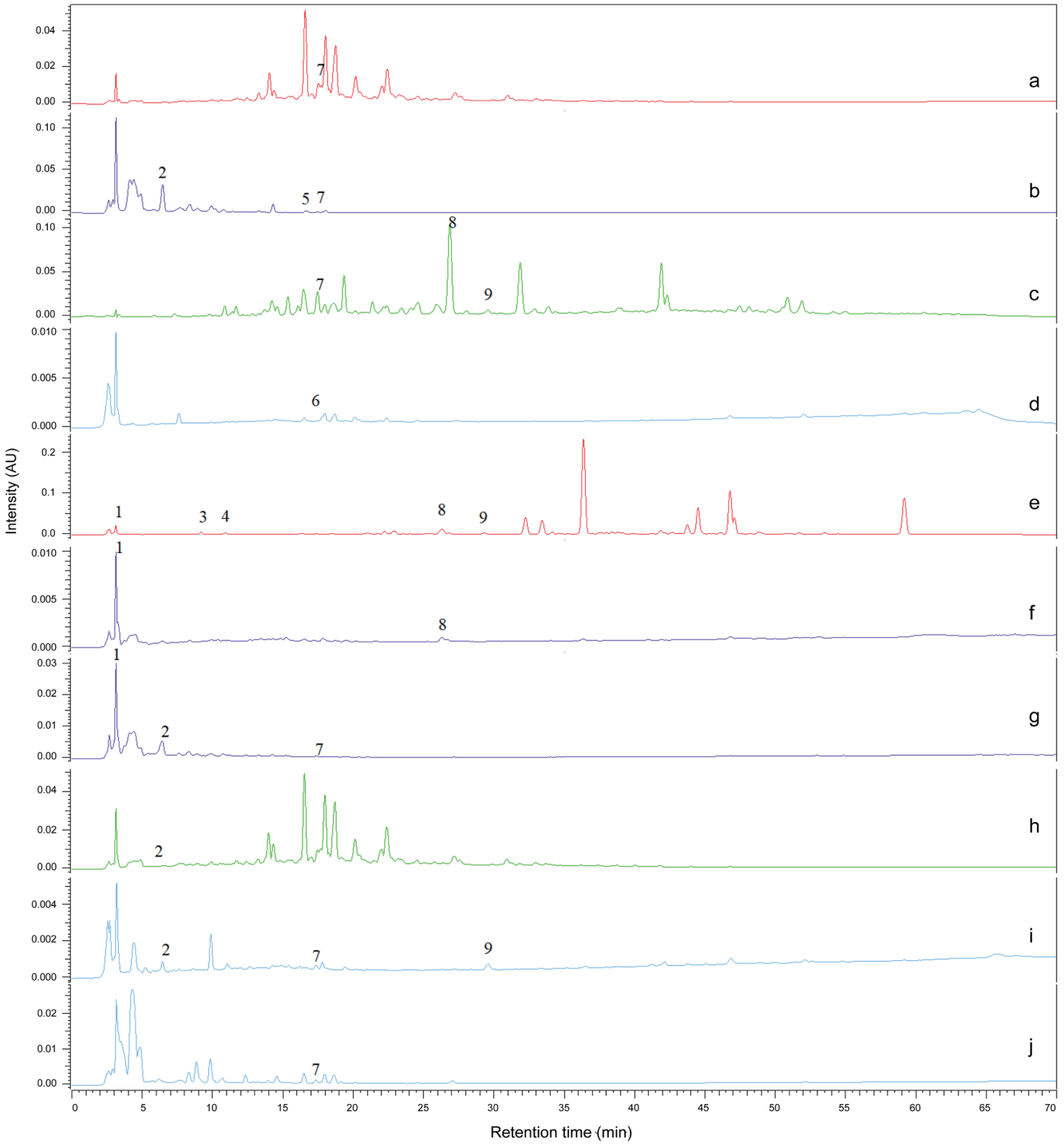

Fig. 4. Chromatograms for Nigerian propolis samples with identified standards. (a) NIG-ADA (Adamawa state, Nigeria); (b) NIG-BEN (Benue state, Nigeria); (c) NIG-CRO (Cross River state Nigeria); (d) NIG-JOS (Jos Platue state, Nigeria); (e) NIG-KOG (Kogi state, Nigeria); (f) NIG-NAS-1 (Nassarava state, Nigeria); (g) NIG-NAS-2 (Nassarava state, Nigeria); (h) NIG-NIG (Niger state, Nigeria); (i) NIG-OND (Ondo state, Nigeria); (j) NIG-UNK (Unknown, Nigeria). 1. Gallic acid; 2. Caffeic acid; 3. p-Coumaric acid; 4. Rutin; 5. Quercetin; 6. Cinnamic acid; 7. Naringenin; 8. Pinocembrin; 9. Chrysin. AU, absorbance unit.

not be able to detect all polyphenolic compounds, especially those present in very low concentrations. Unknown or novel polyphenols might remain undetected or unidentified. Hyphenation of RP-HPLC with other techniques such as mass spectroscopy or nuclear magnetic resonance could assist in detecting trace compounds as well as identifying unknown compounds in raw propolis samples.

In this study, only one antioxidant assay was used, which might not capture the full antioxidant potential of the samples. The full

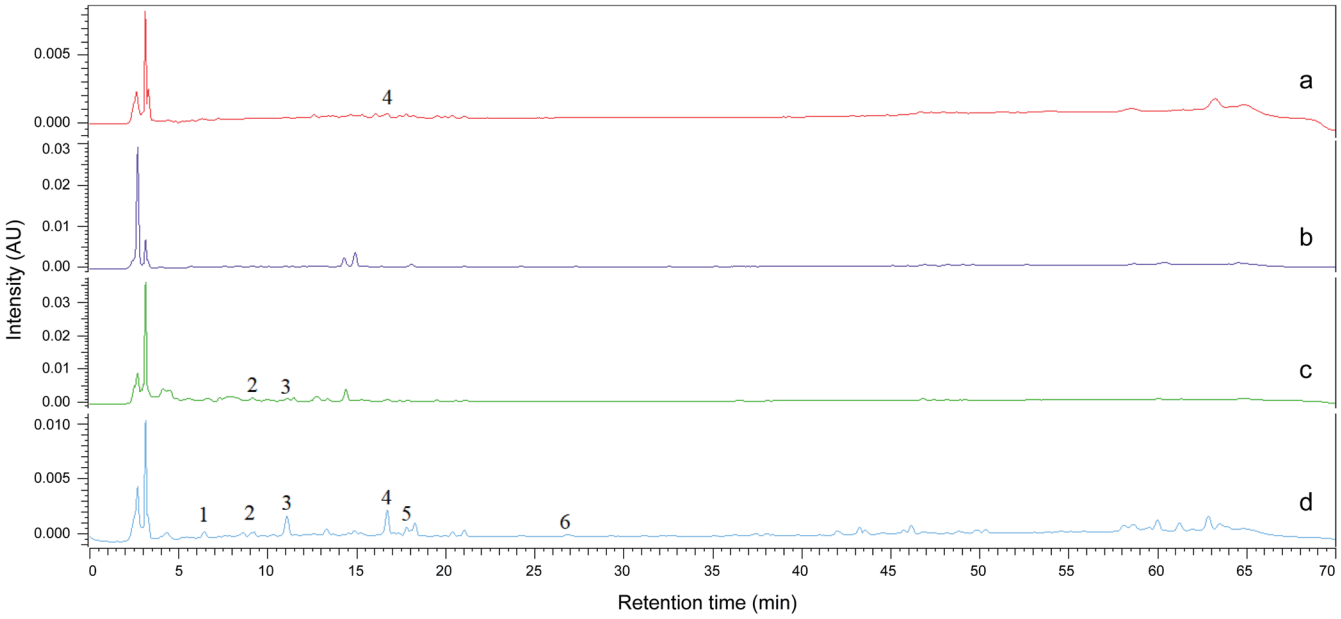

Fig. 5. Chromatograms of Tanzanian propolis samples with identified reference standards. (a) TAN-MOS (Moshi); (b) TAN-PEM (Pemba Iceland); (c) TAN-UNK-1 (Unknown region); (d) TAN-UNK-2 (Unknown region). 1. Caffeic acid; 2. p-Coumaric acid; 3. Rutin; 4. Quercetin; 5. Naringenin. AU, absorbance unit.

antioxidant potential of the samples needs to be confirmed by other *in-vitro* antioxidant assays as well as *in-vivo* evaluations.

Conclusions

This study conducted a simultaneous analysis of flavonoids, phenolic acids, esters, and glycoside compounds in 17 raw propolis samples from Africa. The results indicated that the Nigerian samples were particularly rich in polyphenols and flavonoids compared to those from Tanzania, Malawi, and Zambia. Sample NIG-KOG from Nigeria showed the highest content of phenolic compounds (323.40 ± 0.08 mg QE/g and 59.29 ± 0.05 mg GAE/g for flavonoids and phenolic acid contents, respectively) among the 17 samples. Additionally, the antioxidant potential assay demon-

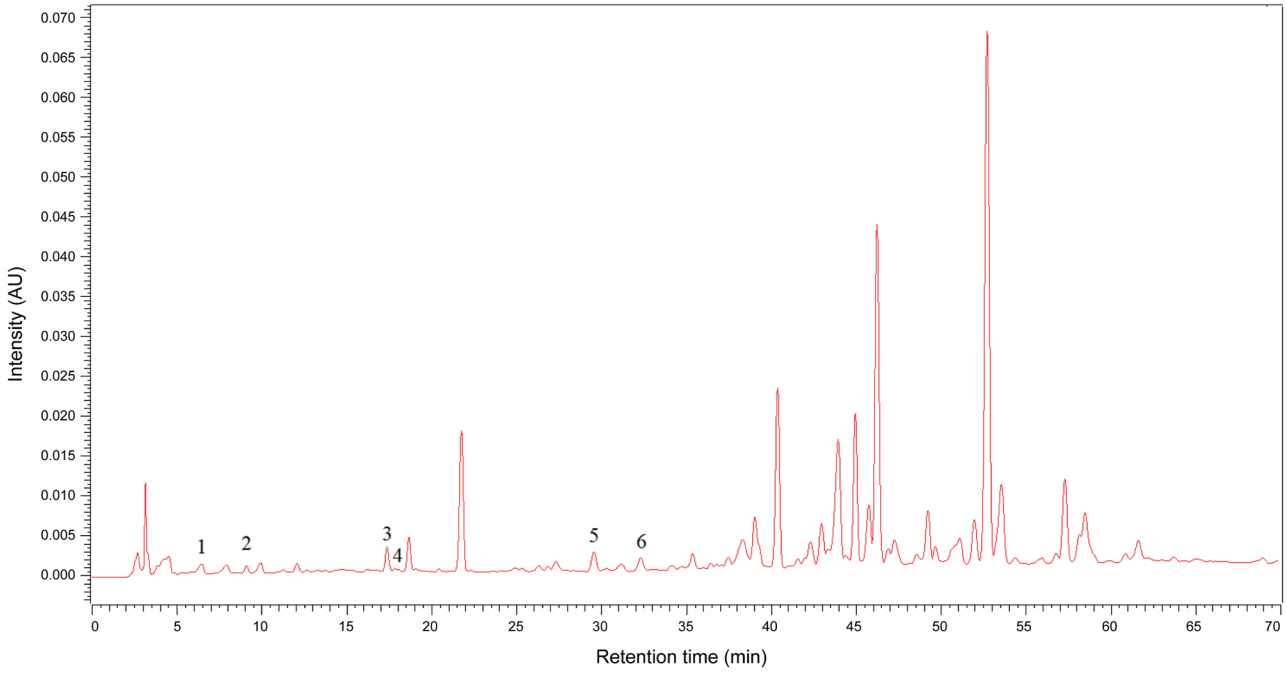

Fig. 6. Chromatogram for Zambian propolis sample (ZAM-MIO) from Miombo forest with identified standards. 1. Caffeic acid; 2. p-Coumaric acid; 3. Cinnamic acid; 4. Naringenin; 5. Chrysin; 6. Galangin. AU, absorbance unit.

strated moderate free radical scavenging activity of the African propolis samples against DPPH, with EC_{50} values ranging from 17.6 ± 0.39 to 0.16 ± 0.001 mg/mL. Notably, naringenin, a flavonoid compound, was detected in most of the samples, suggesting its potential use as a quality indicator for African propolis. However, further detailed investigations involving a larger number of African propolis samples are required to validate whether naringenin can reliably be considered as a quality indicator.

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Conflict of interest

The authors confirm that there are no known conflicts of interest.

Author contributions

Study concept, study design, analytical study performance (SK), writing of the manuscript (SK, GM), review, guidance of the writing (BK), design and performance of the total polyphenol content and antioxidant experiments (GM), sample collection, funding acquisition, research program (JF), project leadership, supervision, and final proofreading of the manuscript (AP). All authors have approved the final version and publication of the manuscript.

Data sharing statement

All data generated or analyzed during this study are included in this published article.

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