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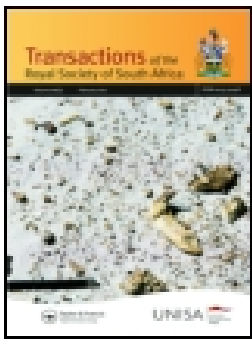
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Therapeutic potential and physicochemical standardisation of Lesotho propolis based on geographical location and botanical sources: a pilot study in Mphahlele district

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We evaluated the therapeutic potential and physicochemical characteristics of propolis samples collected from three councils, namely Khoelenya (F03), Lithipeng (F04) and Thaba-mokhele (F05), in the Mphahlele district, Lesotho. The aim of this study was to investigate the relationship of the therapeutic potential, physicochemical characteristics and colour variation to the geographical location and botanical sources of the collected propolis samples. The collected samples presented remarkable colour variation, ranging from green (25%) to brown (58%) to grey (17%). The highest antioxidant activity was observed in green-coloured propolis samples collected from Lithipeng F04P01 and Khoelenya F03P01, with 2,2-diphenyl-1-picryl-hydrazyl-hydrate (DPPH) half maximal inhibitory concentration (IC₅₀) values of 0.21 and 0.23 mg/mL, respectively. The antioxidant activity (DPPH IC₅₀) did not correlate completely with the total polyphenolic content ($R^2 = 0.1733$) and total flavonoid content ($R^2 = 0.4836$). Moreover, the highest antimicrobial activity was observed on grey propolis collected from Thaba-mokhele, F05P04 and F05P03, with minimum inhibitory concentration of 3.13 mg/mL for both samples, especially against *Staphylococcus aureus*. The qualitative phytochemical analysis detected the presence of polyphenols, alkaloids and flavonoids in all collected samples. Thus, our findings could lead to the formulation of a "local" Lesotho type of propolis that could be used as an official medicine. This could be a big marketing advantage for the Lesotho pharmaceutical and beekeeping industries.

Keywords: green propolis; grey propolis; antioxidant activity; antibacterial activity; phytochemicals

INTRODUCTION

Bees (*Apis mellifera* L.) collect resins with antimicrobial properties from resinous plant species and deposit them into their hives for propolis (bee glue) production (Hlokoane *et al.*, 2022a). The applications of propolis for skin treatment and healing of wounds and ulcers has been documented since ancient times (Dantas Silva *et al.*, 2017; Silva-Beltrán *et al.*, 2021). In fact, examples and proof of ancient use of propolis for therapeutic purposes are found in biblical records (Castaldo and Capasso, 2002; Silva-Beltrán *et al.*, 2021). Propolis began to be industrially incorporated into food and pharmaceutical products, especially for topical applications, in the 1980s (Silva-Beltrán *et al.*, 2021). The pharmacologically active chemicals in propolis are flavonoids and phenolic acids as well as their esters (Castaldo and Capasso, 2002). The composition of propolis is exceptionally diverse and depends on geographic location and botanical origins (Wilson *et al.*, 2013; Hlokoane *et al.*, 2022a).

There are no documented studies on propolis from Lesotho; however, a diversity of propolis products is available in the

Lesotho market, especially for topical applications, such as skin creams, and as oral medication for treatment of upper respiratory infections. According to Ristivojević *et al.* (2015), there are significant differences in the chemical composition of propolis samples originating from different geographic and climatic zones; thus, it is crucial to reliably characterise each type of propolis. In our previous study (Hlokoane *et al.*, 2022a), we tried to identify the botanical origins of Lesotho propolis and reported that *Eucalyptus* spp., *Populus nigra* L. and *Salix babylonica* L. are the probable sources. These plant species flower during the spring season, the time interval when bees are able to cut small parts of the vegetative apices of young leaves and buds to liberate resins from trichomes and ducts for propolis production.

Herein, we report our efforts towards an evaluation of the therapeutic potential and physicochemical characterisation of our propolis samples, and thus towards the formulation of a "local" Lesotho type of propolis that could be used as an official medicine. In this pilot study, we evaluated the

therapeutic potential and physicochemical characteristics of propolis samples collected from different sampling points across three selected community councils in Mhale's Hoek district. The therapeutic potential was determined by analysing antioxidant activity (AA), total polyphenolic content (TPC), total flavonoid content (TFC) and antimicrobial properties. Furthermore, the physicochemical characteristics were determined by qualitative analysis of phytochemicals, pH, and metallic and acid radical impurities. The study also established the relationship of the therapeutic potential, physicochemical characteristics and colour variation to the botanical sources of the collected propolis samples. To the best of our knowledge, this is the first report of the therapeutic potential and physicochemical characterisation of propolis from Lesotho.

MATERIALS AND METHODS

Sampling

Propolis samples were collected from three councils (Khoelenya (F03), Lithipeng (F04) and Thaba-mokhele (F05)) in Mhale's Hoek district in April 2021 (Figure 1). Stratified random sampling was employed, whereby random points were created using the Geographic Information System (GIS) software across the three councils. A total of 12 sampling points/areas with differing vegetation were selected. Colonies in the selected areas were in good health. In all colonies the hive technology was Langstroth and hive management practices included hive inspection, pest and disease control, supering and honey harvesting, and colonisation. The propolis samples were collected together with information about the dominant plant species around the hives, and the sample colour variations were identified (Table 1). The samples were cleanly scraped from the propolis traps, packaged in plastic bottles and given identification codes based on the community council, the first letter of the hive product and the sample number. For example, in the code F03P01, F03 is the code for the Khoelenya community council, P is the first letter in propolis and 01 is the first sample collected. The labelled samples were stored at -4°C until extraction.

Extract preparation

The extracts were prepared using a procedure described by Bankova *et al.* (2019), with modifications. Samples were ground into powder to achieve a maximum particle size of 1.0 mm. Then, 5 g of each powdered sample was added into 70% ethanol (1:30 w:v) and kept at room temperature for 24 h with frequent agitation. The resulting suspension was filtered at room temperature using a Whatman No. 1 filter paper. The procedure was repeated with the residue trapped on the filter paper, extracting the residue again under the same conditions. The combined filtrate was concentrated under reduced pressure at 50°C using a rotary evaporator to obtain propolis extracts. The extracts were stored in labelled air-tight scintillation vials and refrigerated at -4°C before analysis.

2,2-diphenyl-1-picryl-hydrazyl-hydrate (DPPH) radical scavenging activity

The 2,2-diphenyl-1-picryl-hydrazyl-hydrate (DPPH) radical scavenging activity of extracts was performed according to the previously reported procedure by Hlokoane *et al.* (2022b), with slight modifications. The reaction mixture

contained 1.0 mL of 0.1 mM DPPH solution in methanol and 3.0 mL of propolis extracts at various concentrations. After 30 min incubation at 37°C , the absorbances were measured at 517 nm using an ultraviolet (UV)-visible spectrophotometer (Apex Scientific UV-Visible Spectroscopy, South Africa) against the corresponding blank solution. L-ascorbic acid served as a positive control. The ability of the extracts and/or ascorbate standard to scavenge DPPH radicals was calculated using the equation below:

$$\text{DPPH radical scavenging activity (\%)} = \left(\frac{\text{Acont} - \text{Atest}}{\text{Acont}} \right) \times 100\%$$

Acont = Absorbance of the negative control

Atest = Absorbance in the presence of the extract or positive control

The DPPH half maximal inhibitory concentration (IC_{50}) values were generated using Microsoft Excel by plotting the exact concentration versus the percentage inhibition of DPPH free radicals.

Determination of TPC

The TPC of extracts was determined using the Folin-Ciocalteu method reported by Graikini *et al.* (2019), with slight modifications. The reaction mixture contained 3.16 mL of distilled water, 0.04 mL of appropriately diluted propolis extracts and 0.2 mL of Folin-Ciocalteu reagent. After shaking and resting for 1 min, 0.6 mL of sodium carbonate (20% w/v in distilled water) was added, and the sample was vortexed and stored in the dark for 120 min. Absorbance of the samples was measured at 750 nm using the UV-visible spectrophotometer and the final results were expressed as mg gallic acid equivalents (GAE) per g of dry propolis weight.

Determination of TFC

The TFC was determined using the published method of Graikini *et al.* (2019), with modifications. The reaction mixture contained 0.5 mL aliquots of appropriately diluted propolis extracts, 500 μL AlCl_3 reagent (2% [w/v] AlCl_3 in 5% [v/v] acetic acid in methanol) and 700 μL of 5% (v/v) acetic acid in methanol. The mixtures were allowed to stand for 30 min at room temperature and absorbances were obtained at 415 nm using deionised water as a blank solution. The TFC was calculated from a calibration curve, constructed with quercetin as the calibration standard, and results are expressed as mg quercetin equivalents (QE) per gram of dry propolis weight.

Antimicrobial activity

The antimicrobial activities of extracts were determined against three microorganisms, namely *Staphylococcus aureus* ATCC25923, *Escherichia coli* ATCC23922 and *Pseudomonas aeruginosa* ATCC27853, using well diffusion assay according to the procedure previously reported by Hlokoane *et al.* (2022b), with slight modifications. Briefly, Mueller Hinton agar plates, inoculated with 100 μL of bacterial suspensions, having wells 6 mm in diameter filled with 20 μL of 100 mg/mL propolis extracts, were incubated for 18–24 h at 37°C . The antibacterial activities were measured based on the diameter expressed in mm of the clear zone on the wells. Ciprofloxacin (1 μg /disc) served as a positive control. The minimum inhibitory concentrations (MIC) were determined using a

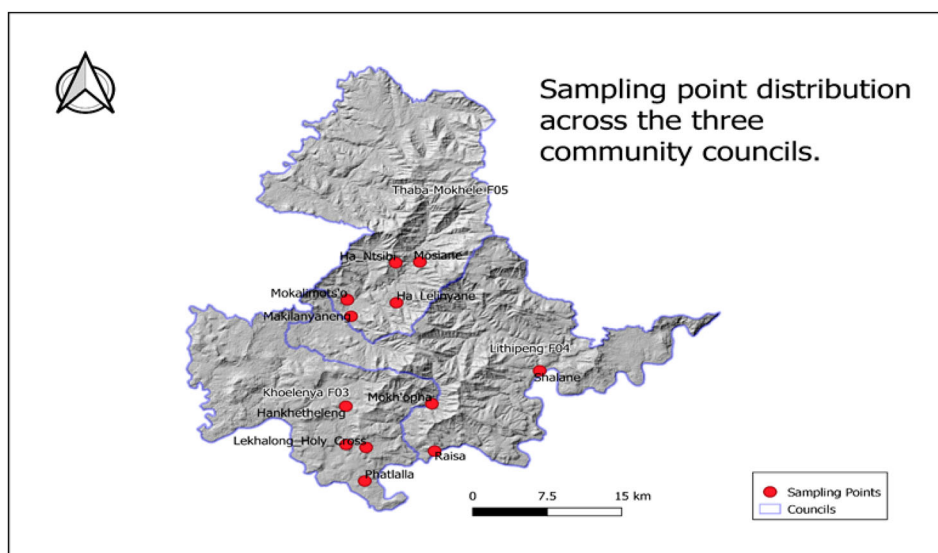


Figure 1. Distribution of sampling points across the three community councils in Mohale's Hoek district, Lesotho.

broth macro-dilution method (Hlokoane *et al.*, 2022b). The 6 mL tubes, each containing 1 mL of Mueller Hinton broth, 1 mL of diluted propolis extract and 20 μ L of bacterial suspension, were incubated at 37°C for 24 h. The MIC was the lowest concentration of the extract that completely inhibited the bacterial growth in the tube. Furthermore, the minimum bactericidal concentration (MBC) was determined by incubating the Mueller Hinton agar plates, streaked with the mixtures from each of the MIC assay negative wells, at 37°C for 24 h. The lowest concentration that resulted in no visible cell growth was defined as the MBC value.

Qualitative phytochemical screening, pH and limit tests for acid radical and metallic impurities

Qualitative phytochemical screening was conducted to determine the presence of various active phytochemicals from the extracts using the method previously described by Hlokoane *et al.* (2022b). The pH and limit tests for acid radical and metallic impurities were performed according to the procedures previously reported by Mautsoe *et al.* (2021).

Statistics

All assays were done in triplicate and results are expressed as the mean \pm Standard Deviation. Microsoft Excel was used to compute IC_{50} values for the antioxidant activities and to evaluate the correlation between phenolic compounds and antioxidant activity. Statistical comparisons were performed with one-way analysis of variance, and $P < .05$ was regarded statistically significant.

RESULTS

Colour variations

Lesotho (Supplementary material, Figure S1), divided into 10 districts, is a small landlocked mountainous country (30 648 km²) with a temperate climate that lies more than 1400 metres above sea level in the Southern Africa region (Hlokoane *et al.*, 2022a). A total of 12 propolis samples were collected from 12 sampling points (Figure 1) across three community councils in Mohale's Hoek district with diverse

vegetation as summarised in Table 1. As shown in Table 1, propolis samples presented remarkable colour variation, ranging from green (25%) to brown (58%) to grey (17%) (also see Table S1), and the most dominant plant species were *Agave americana* L. and *Diospyros lycioides* Desf., followed by *Rhamnus prinoides* L'Her, *Searsia erosa* (Thunb.) Moffett and *Populus × canescens* (Aiton) Sm.

Antioxidant activities

The antioxidant activities were different among the 12 propolis extracts (Figure 2A). However, the extracts all scavenged DPPH free radicals in a concentration-dependent manner (also see Supplementary material, Table S2). Samples F04P01 and F03P01 had quite high antioxidant capacities, as their inhibition percentages were 99.17 and 95.17%, respectively, at a concentration of 0.50 mg/mL. A lower IC_{50} value indicates a high potency for antioxidant activity. As shown in Table 2, extracts F04P01, F03P01 and F05P02 had lower IC_{50} values of 0.21, 0.23 and 0.25 mg/mL, respectively (also see Figure S2), indicating high potency for antioxidant activity. Sample F05P02 showed the highest TPC of 84.50 mg/g GAE, while F03P05 showed the lowest TPC of 19.36 mg/g GAE (Table 2). On the other hand, F03P01 showed the highest TFC of 100.00 mg/g QE, while F05P03 showed the lowest TFC of 0.20 mg/g QE. However, as shown in Figure 2B and C, the antioxidant activity (DPPH IC_{50}) did not correlate fully with the TPC ($R^2 = 0.1733$) or TFC ($R^2 = 0.4836$).

Antimicrobial activities

The antimicrobial activities of the samples are presented in Table 2. According to Table 2, all 12 extracts exhibited antibacterial activity against all test organisms, one Gram positive and two Gram negative, at a concentration of 100 mg/mL. In general, the extracts exhibited strong antibacterial activities against the Gram-positive microorganism *Staphylococcus aureus*. In particular, F05P04 and F05P03 showed the highest inhibition zone diameters of 38.06 ± 0.89 and 33.83 ± 1.34 mm, respectively, and the lowest minimum inhibitory concentration of 3.13 mg/mL for both samples, indicating good activity. Moderate and weak activities were observed against

Table 1. Sampling points, dominant plant species and colour variations of propolis from three community councils in Mohale's Hoek district, Lesotho.

Community councils	Sampling points	Propolis	Dominant plant species	Colour variations
Khoelenya (F03)	Holly cross	F03P01	<i>Agave Americana</i> L., <i>Searsia burchellii</i> (Sond. Ex Engl.) Moffett and <i>Eucalyptus</i> spp.	Greenish yellow
	Lekhalong Holly cross	F03P02	<i>Schinus molle</i> L., <i>Searsia erosa</i> (Thunb.) Moffett and <i>Punica granatum</i> L.	Dark brown
	Mokh'opha	F03P03	<i>Rhamnus prinoides</i> L'Her, <i>Leucosidea sericea</i> Eckl. and Zeyh and <i>Diospyros lycioides</i> Desf.	Greenish brown
	Phatlalla	F03P04	<i>Eucalyptus</i> spp., <i>Prunus persica</i> (L.) Batsch and <i>Asparagus larinicus</i> Burch.	Dark brown
	Hankhetheleng	F03P05	<i>Populus nigra</i> L., <i>Prunus armeniaca</i> Thunb. and <i>Opuntia ficus-indica</i> (L.) Mill.	Light brown
Lithipeng (F04)	Shalane	F04P01	<i>Agave Americana</i> L., <i>Rhamnus prinoides</i> L'Her and <i>Diospyros lycioides</i> Desf.	Light green
	Raisa	F04P02	<i>Prunus persica</i> (L.) Batsch, <i>Diospyros lycioides</i> Desf. and <i>Agave Americana</i> L.	Dark brown
Thaba-mokhele (F05)	Mosiane	F05P01	<i>Rhamnus prinoides</i> L'Her, <i>Leucosidea sericea</i> Eckl. and Zeyh, and <i>Searsia erosa</i> (Thunb.) Moffett	Orange-brown
	Ha Ntsibi	F05P02	<i>Rosa canina</i> L., <i>Populus × canescens</i> (Aiton) Sm. and <i>Helianthus annuus</i> L.	Light green
	Ha Lelinyane	F05P03	<i>Populus × canescens</i> (Aiton) Sm., <i>Helianthus annuus</i> L. and <i>Searsia erosa</i> (Thunb.) Moffett	Dark grey
	Makilanyaneng	F05P04	<i>Agave Americana</i> L., <i>Helianthus annuus</i> L. and <i>Schinus molle</i> L.	Light grey
	Mokalimots'o	F05P05	<i>Prunus persica</i> (L.) Batsch, <i>Populus × canescens</i> (Aiton) Sm. and <i>Diospyros lycioides</i> Desf.	Greenish brown

two Gram-negative microorganisms, *Escherichia coli* and *Pseudomonas aeruginosa*, respectively (Table 2).

Phytochemical screening, pH and limit tests for acid radical and metallic impurities

As shown in Table 3, upon qualitative phytochemical screening, polyphenols, alkaloids and flavonoids were detected in all propolis extracts. On the other hand, saponins and phlobatanins were absent from all extracts. The pH analysis indicated that the extracts had a pH range of 5.32–8.00 (Table 2); 42% of extracts passed the limit tests for chlorides, 33% passed the limit tests for sulphates and 67% had total heavy metals less than 20 ppm. These results are presented in Table S2.

DISCUSSION

Colour variation

The colour of propolis varies considerably depending on its geographical origin and plant sources (Ristivojević *et al.*, 2015). Propolis samples collected in this study presented remarkable colour variation, ranging from green to brown to grey. Green and brown propolis have been well studied and documented in the literature (Salatino *et al.*, 2005; Ristivojević *et al.*, 2015). However, grey propolis is rarely documented. The grey propolis was collected from Thaba-mokhele (Ha Lelinyane and Makilanyaneng), the sampling points dominated by *Populus × canescens* (Aiton) Sm., *Helianthus annuus* L., *Searsia erosa* (Thunb.) Moffett, *Agave Americana* L., *Helianthus annuus* L. and *Schinus molle* L. plant species. Although the contribution of these plant species in the production of grey propolis cannot be assumed, there is evidence that propolis from temperate regions contains exudates of apical buds of *Populus* species, especially *P. nigra* L. and *Populus × canescens* (Aiton) Sm. (Salatino *et al.*, 2021).

Antioxidant activities

Antioxidants protect against oxidative stress by scavenging and removing or neutralising reactive species (Frezza *et al.*, 2013). DPPH is a purple stable organic radical, which changes to pale yellow when it captures an electron or a free radical species (Hernandez Zarate *et al.*, 2018; Kurek-Górecka *et al.*, 2022). The DPPH radical scavenging activity assay is commonly used to determine the antioxidant activities of natural products such as propolis. In this study, 12 propolis extracts scavenged DPPH free radicals in a concentration-dependent manner. The green propolis, F04P01 and F03P01, showed quite high antioxidant capacities of 99.17 and 95.17% as well as IC₅₀ values of 0.21 and 0.23 mg/mL, respectively.

The pharmacologically active elements in propolis are flavonoids and phenols (Özkök *et al.*, 2021). However, in our laboratory, the antioxidant activity (DPPH IC₅₀) did not correlate fully with the TPC ($R^2 = 0.1733$) and TFC ($R^2 = 0.4836$). Although, this study is the first to document antioxidant activity of Lesotho propolis, the same kinds of observations have been made by studies conducted in other countries as well (Cabral *et al.*, 2012; Teixeira *et al.*, 2010). It is likely that the flavonoids and polyphenols are not the only compounds responsible for antioxidant activities of the studied propolis samples.

Antimicrobial activities

The majority of biological studies on propolis have produced promising findings indicating that propolis could be beneficial in combating important health problems (Silva-Beltrán *et al.*, 2021). This study revealed that grey propolis extracts, F05P04 and F05P03, had the highest antibacterial activities, with diameter of inhibition zone of 38.06 ± 0.89 and 33.83 ± 1.34 mm, respectively, and MIC of 3.13 mg/mL

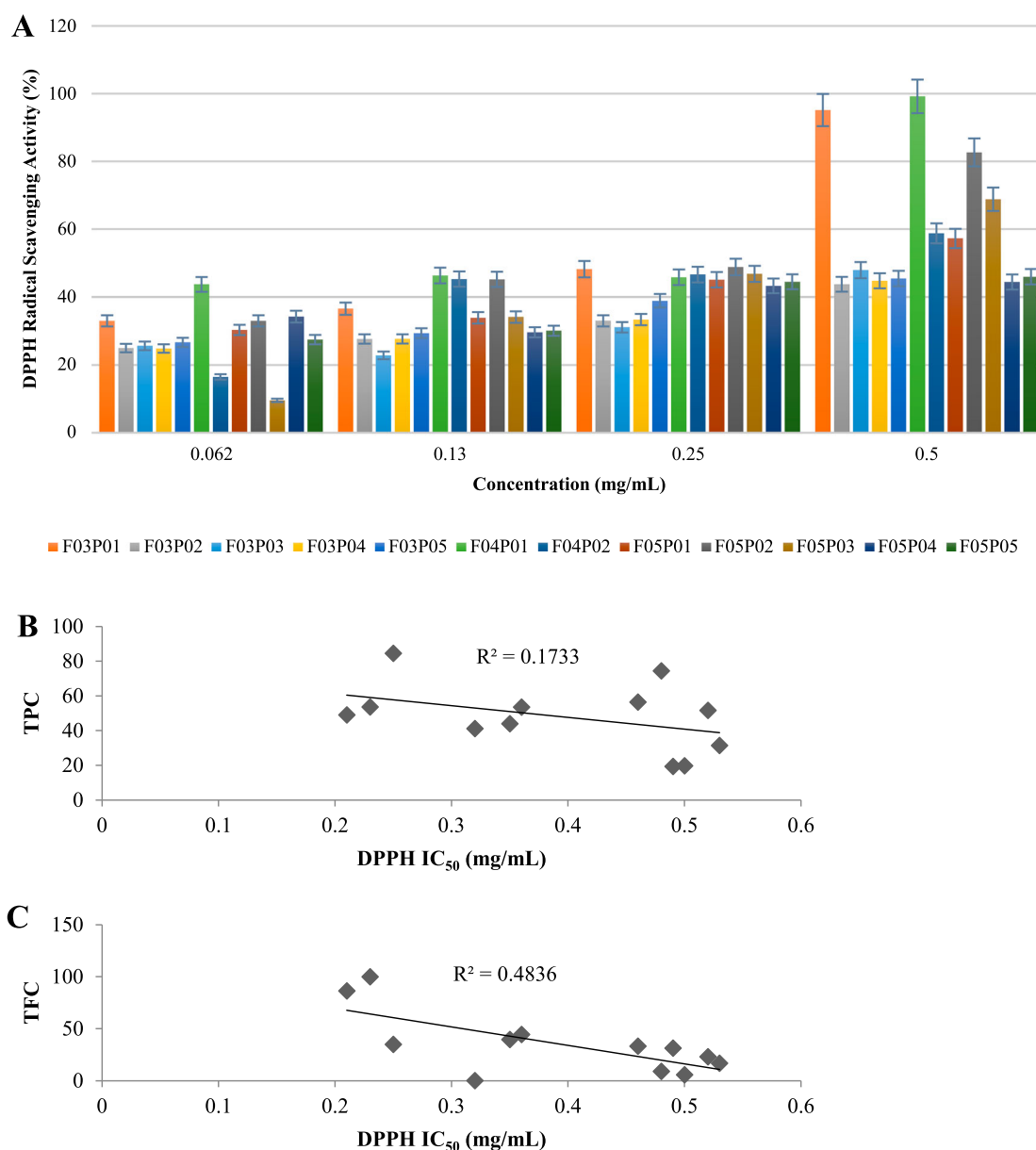


Figure 2. Antioxidant activity. (A) 2,2-diphenyl-1-picryl-hydrazyl-hydrate (DPPH) radical scavenging activity; (B) correlation between DPPH IC₅₀ and total polyphenolic content (TPC); and (C) correlation between DPPH IC₅₀ and total flavonoid content (TFC) of the propolis samples from Mohale's Hoek district, Lesotho.

for both samples against *S. aureus*. The activity of green propolis, F04P01, was equivalent to that of F05P03 against the same microorganism. Moderate to weak activities were observed against two Gram-negative microorganisms. These findings are in line with those reported by Silva-Beltrán *et al.* (2021), who found that Brazilian and Mexican propolis samples showed higher activity against the Gram-positive strains than against the Gram-negative strains. Silva-Beltrán *et al.* (2021) also found that green propolis had broader antimicrobial activity than brown propolis.

Phytochemical screening, pH and limit tests for acid radical and metallic impurities

The qualitative phytochemical analysis detected the presence of polyphenols, alkaloids and flavonoids in all the propolis extracts. Other phytochemicals such as tannins, terpenoids,

quinones, reducing sugars and sterols, which could be related to the diversity of geographical locations and plant sources, were detected. These findings are in line with those reported by Mulyati *et al.* (2020). Propolis can be polluted directly or indirectly by metals via different sources such as bees, air, water, plants and soil (Silva-Beltrán *et al.*, 2021). In this study, sulphates, chlorides and heavy metals detected were beyond prescribed limits for some extracts (for prescribed limits refer to Mautsoe *et al.*, 2021). Therefore, these findings suggest that the toxicological profile of propolis samples should be established before administration to humans.

CONCLUSION

The propolis samples analysed in this study were collected from 12 sampling points across three community councils in Mohale's Hoek district with diverse vegetation. Our

Table 2. Radical scavenging activity by 2,2-diphenyl-1-picryl-hydrazyl-hydrate (DPPH), total polyphenolic content (TPC), total flavonoid content (TFC), antimicrobial activity and pH of propolis extracts.

Propolis	DPPH IC ₅₀ (mg/mL)	TPC (mg/g) in GAE	TFC (mg/g) in QE	<i>Staphylococcus aureus</i>			<i>Escherichia coli</i>			<i>Pseudomonas aeruginosa</i>			pH
				DI (mm)	MIC (mg/ mL)	MBC (mg/ mL)	DI (mm)	MIC (mg/ mL)	MBC (mg/ mL)	DI (mm)	MIC (mg/ mL)	MBC (mg/ mL)	
F03P01	0.23	53.64	100.00	29.81 ± 1.09	6.25	>6.25	26.69 ± 1.50	6.25	>6.25	12.99 ± 0.12	50.00	>50.00	7.02
F03P02	0.53	31.42	16.98	17.85 ± 0.57	12.50	>12.50	18.61 ± 0.60	12.50	>12.50	14.20 ± 0.30	50.00	>50.00	7.09
F03P03	0.50	19.72	5.83	14.59 ± 1.12	12.50	>12.50	13.86 ± 1.02	25.00	>25.00	13.52 ± 0.50	50.00	>50.00	6.86
F03P04	0.52	51.61	23.21	27.88 ± 1.15	6.25	>6.25	26.48 ± 1.50	6.25	>6.25	18.9 ± 0.66	25.00	>25.00	6.24
F03P05	0.49	19.36	31.43	28.04 ± 0.57	6.25	>6.25	11.33 ± 1.12	25.00	>25.00	11.29 ± 1.10	50.00	>50.00	7.87
F04P01	0.21	49.04	86.54	33.45 ± 1.33	3.13	>3.13	20.37 ± 0.50	12.50	>12.50	20.48 ± 0.60	25.00	>25.00	6.55
F04P02	0.35	43.97	39.74	23.64 ± 0.13	6.25	>6.25	12.82 ± 1.10	25.00	>25.00	10.71 ± 0.56	50.00	>50.00	6.93
F05P01	0.36	53.56	44.51	32.30 ± 1.02	3.13	>3.13	26.22 ± 0.59	6.25	>6.25	16.19 ± 0.33	50.00	>50.00	6.83
F05P02	0.25	84.50	35.00	27.94 ± 1.21	6.25	>6.25	16.04 ± 1.50	12.50	>12.50	13.16 ± 0.36	50.00	>50.00	7.58
F05P03	0.32	41.15	0.20	33.83 ± 1.34	3.13	>3.13	22.11 ± 0.29	12.50	>12.50	11.91 ± 1.02	50.00	>50.00	5.32
F05P04	0.48	74.39	9.15	38.06 ± 0.89	3.13	>3.13	21.54 ± 1.73	12.50	>12.50	19.62 ± 0.60	25.00	>25.00	8.00
F05P05	0.46	56.43	33.33	27.06 ± 0.59	6.25	>6.25	22.09 ± 1.33	12.50	>12.50	16.07 ± 1.32	25.00	>25.00	6.84

Values are mean ± Standard Deviation of three replications. GAE: gallic acid equivalents, QE: quercetin equivalents, IC₅₀: half maximal inhibitory concentration, DI: diameter of inhibition zone, MIC: minimum inhibitory concentration, and MBC: minimum bactericidal concentration.

Table 3. Qualitative phytochemical screening of propolis extracts.

Phytochemical class	F03P01	F03P02	F03P03	F03P04	F03P05	F04P01	F04P02	F05P01	F05P02	F05P03	F05P04	F05P05
Tannins	–	–	–	–	–	+	–	+	–	+	–	–
Polyphenols	+	+	+	+	+	+	+	+	+	+	+	+
Alkaloids	+	+	+	+	+	+	+	+	+	+	+	+
Saponins	–	–	–	–	–	–	–	–	–	–	–	–
Terpenoids	+	+	+	+	+	+	–	+	–	+	+	+
Phlobatannins	–	–	–	–	–	–	–	–	–	–	–	–
Sterols	+	+	–	–	–	–	–	–	–	–	–	–
Quinones	–	+	+	+	+	–	+	–	–	–	–	+
Flavonoids	+	+	+	+	+	+	+	+	+	+	+	+
Reducing sugars	+	+	+	–	+	–	+	+	+	–	–	+

+: present; –: absent.

hypothesis, that there is a relationship of the therapeutic potential, physicochemical characteristics and colour variation with the geographical location and botanical sources of collected propolis samples, was confirmed. Thus, our findings could lead to the formulation of a “local” Lesotho type of propolis that could be used as an official medicine. This could be a big marketing advantage for the Lesotho pharmaceutical and bee-keeping industries.

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Disclosure statement

No potential conflict of interest was reported by the authors.

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Supplementary material

Supplemental data Figures S1 and S2, and Tables S1 and S2 for this article can be accessed at <http://dx.doi.org/10.1080/0035919X.2022.2163000>.

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