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# Chemical profile and *in vitro* bioactivity of tropical honey from Mauritius

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### ABSTRACT

**Objective:** To investigate into the antimicrobial and antioxidant potential of six varieties of honey from Mauritius.

**Methods:** Six samples [commercial (processed, syrup-flavoured and ginger) and unifloral (eucalyptus, litchi and longan)] were assessed as traditionally used. The disc diffusion method was used against five microbial strains. Antioxidant capacity was determined using eight assays: trolox equivalent antioxidant capacity (TEAC), ferric reducing antioxidant power, iron chelating, hydroxyl radical (OH<sup>•</sup>), 2,2-diphenyl-1-picryl-hydrazyl-hydrate free radical scavenging assay, hypochlorous acid (HOCl), nitric oxide (NO<sup>•</sup>) and 2,2'-azinobis(3-ethylbenzothiazoline-6-sulphonic acid) diammonium salt radical scavenging assay. The total phenolic (TPC) and flavonoid content (TFC) were determined to delineate any correlation with any observed bioactivity.

**Results:** Longan honey showed the highest antimicrobial activity while processed and raw eucalyptus honeys showed moderate activity. TEAC ranged from (0.95±0.04) to (1.80±0.03) mmol trolox equivalents/100 g while ferric reducing antioxidant power varied between (160.77±1.02) to (170.50±1.84) mmol trolox equivalents/100 g. Strongest iron chelating activity was recorded for processed honey [IC<sub>50</sub>=(4.51±0.34) mg/mL] while eucalyptus and longan honeys had the highest OH<sup>•</sup> scavenging activity [IC<sub>50</sub>=(31.24±0.75) mg/mL and (31.30±0.85) mg/mL respectively]. Ginger honey had the lowest IC<sub>50</sub> [(2.77±0.79) mg/mL] against NO<sup>•</sup> while longan honey was most active against HOCl<sup>•</sup> [IC<sub>50</sub>=(21.67±1.09) mg/mL] and processed honey showed the lowest IC<sub>50</sub> against 2,2'-azinobis(3-ethylbenzothiazoline-6-sulphonic acid) diammonium salt radical [(90.44±2.48) mg/mL]. TPC ranged between (54.03±0.99) to (77.37±1.01) mg gallic acid equivalent/100 g and TFC between (4.55±0.06) to (11.80±0.20) µg rutin equivalent/100 g. Pearson correlation established strong correlations between antioxidant assays and TPC (TEAC:  $r=0.9453$ ; iron chelating:  $r=-0.6964$ ; OH<sup>•</sup>:  $r=-0.6150$ ; 2,2-diphenyl-1-picryl-hydrazyl-hydrate:  $r=-0.7439$ ) and TFC (HOCl<sup>•</sup>:  $r=-0.7509$ ; NO<sup>•</sup>:  $r=0.8678$ ).

**Conclusions:** The current study has provided important baseline data on local honey which can be further exploited as a functional food.

## 1. Introduction

Honey has a long history of use as a natural sweetener as well as a complementary and alternative medicine in several ancient civilisations for the treatment of several medical conditions[1,2]. While the use of honey as a component of modern folk-medicine has long-standing use, it has recently acknowledged a renewed global interest with the advent of apitherapy as an alternative

branch of medicine involving the use of honey and honey-based products[3,4]. Honey is an excellent source of natural antioxidants which can potentially be effective in minimising the incidence of cardiovascular problems, ocular disorders, declining immune system as well as in the treatment and management of gastric sores and skin wounds[5-7]. Additionally, the potential of honey as a functional food for health promotion is predominantly dependent on its floral origin as well as geographical location which will influence its composition and thus, its antioxidant profile[8,9]. Moreover, several therapeutic properties associated with honey have been mostly attributed to its polyphenol content and various flavonoids.

Several studies have also observed the antimicrobial

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properties of honey in inhibiting the development of a wide spectrum of bacteria, fungi, protozoa and viruses<sup>[10,11]</sup>. The antimicrobial properties of honey have been linked to its low water activity, low pH, hydrogen peroxide concentration, presence of enzymes glucose oxidase as well as non-peroxide flavonoids<sup>[8,12]</sup>. The antimicrobial properties of honey has recently known increasing interest in medicine as an alternative antibacterial therapy, especially with the emergence of antibiotic-resistant pathogens as no honey resistance, adverse and harmful effects that have yet been identified<sup>[13]</sup>.

Honey has been reported to be an essential component of the traditional medicine in Mauritius. Daily oral administration of honey was indicated by the local population for the treatment of cough and fever<sup>[14]</sup>. Additionally, honey is the second most commonly used additive in the preparation of traditional remedies to decrease their bitterness or to improve the taste of the product to promote enhanced patients' compliance<sup>[14–16]</sup>.

While numerous studies have previously assessed and established the antioxidant and antimicrobial capacities of honey, local honey varieties has not been thoroughly investigated. Recently, a study has reviewed the physicochemical and antimicrobial properties of Mauritian processed and raw honeys which were observed to show significantly wide antimicrobial spectrum<sup>[16]</sup>. The present study was therefore aimed at investigating the antioxidant activities as well as the antimicrobial properties of six different local honeys, namely processed honey, syrup flavoured honey, ginger honey, eucalyptus honey, litchi honey and longan honey.

## 2. Materials and methods

### 2.1. Sample collection

Six honey samples originating from the Republic of Mauritius (Mauritius and Rodrigues) were collected in 2012. Samples of easily accessible local brands of honey were purchased from a local commercial source. Eucalyptus honey was obtained from an apiculture site in Rodrigues Island. The remaining samples were pure raw honeys obtained directly from apiculture sites which were certified by an apicultural officer of the Ministry of Agriculture. Following collection, the honey samples were stored in sterilised, air tight glass jars at room temperature in the dark during the whole period of the study. The commercially obtained samples (processed and syrup flavoured honeys), were assumed to be multifloral in nature since the origin was not specified by the manufacturer. Ginger honey denoted a commercial processed multifloral honey containing ginger extract while eucalyptus (*Eucalyptus tereticornis*), litchi (*Litchi chinensis*) and longan (*Dimocarpus longan*) honeys were

unifloral honeys.

### 2.2. Preparation of samples

The stock solutions of honey (w/v) were freshly prepared in sterile distilled water prior to conducting each assay which was then filtered through Whatman No. 1 filter paper to remove any solid particle. The stock solutions were further diluted to appropriate concentrations according to the different requirements of the assays.

### 2.3. Antimicrobial assay

Samples were diluted in sterile distilled water to different concentrations [12.5%, 25% and 50% (w/v)]. The 100% honey was referred to as undiluted honey. The standard isolates and strains of microorganisms, viz. *Escherichia coli* (ATCC 25922) (*E. coli*), *Pseudomonas aeruginosa* (ATCC 27853) (*P. aeruginosa*), *Staphylococcus aureus* (ATCC 29213) (*S. aureus*), *Candida albicans* (ATCC 10231) (*C. albicans*) and *Aspergillus niger* (ATCC 16404) (*A. niger*) were used as test organisms for both disc diffusion and the determination of minimum inhibitory concentration (MIC).

### 2.4. Disc diffusion method

The disc diffusion method described by Ansari and Alexander<sup>[11]</sup> and Kinoo *et al.*<sup>[16]</sup> was used with minor modifications. Ampicillin and nystatin at 10 µg were used for tested bacteria and fungi respectively. Sterile distilled water was added on paper disc and was used as negative control. All the inoculated Petri dishes were incubated for 24 h at 37 °C for the bacteria, 48 h at 37 °C for *C. albicans* and 24 h at ambient temperature in the dark for *A. niger*. Each plate was then examined for any zone of inhibition (mm).

### 2.5. MIC

MIC was determined based on methods used by Kinoo *et al.*<sup>[16]</sup> and Basson and Grobler<sup>[17]</sup> with slight modifications. Positive controls, streptomycin, gentamycin and nystatin at 20 mg/mL were used for bacteria and fungi respectively while sterile distilled water was used as negative control. The test was performed in triplicate. About 40 µL of a 0.2 mg/mL iodinitrotetrazolium violet indicator was added to each well and was incubated for 20 min. A red colour indicated the growth of microorganisms recorded and the well concentration was considered as the MIC.

### 2.6. Antioxidant assays

In order to establish the complete antioxidant profile of the local samples, eight standard and widely used antioxidant assays have been used to delineate the antioxidant potential of the samples.

### 2.6.1. Trolox equivalent antioxidant capacity (TEAC) assay

TEAC method was used to assess the capacity of hydrogen-donating compounds to scavenge the preformed radical cation 2,2'-azinobis(3-ethylbenzothiazoline-6-sulphonic acid) diammonium salt radical (ABTS<sup>•+</sup>) relative to that of the standard antioxidant Trolox<sup>®</sup>[18]. The radical cation was generated as described previously between ABTS (0.5 mmol/L) and activated MnO<sub>2</sub> (1 mmol/L) in phosphate buffer solution (PBS) (0.1 mol/L, pH 7)[18,19]. TEAC was calculated with respect to a trolox standard curve and the results were expressed in mmol trolox equivalent/100 g (mmol TE/100 g) honey.

### 2.6.2. Ferric reducing antioxidant power (FRAP) assay

The FRAP assay was adapted from Benzie and Strain[20] with some modifications and this method indicated the reducing ability of the samples. Stock solutions used for the assay comprised 300 mmol/L acetate buffer (pH 3.6), 10 mmol/L 2,4,6-tripyridyl-s-triazine solution in hydrochloric acid (40 mmol/L) and 20 mmol/L ferric chloride (FeCl<sub>3</sub>·6H<sub>2</sub>O). FRAP working solution was freshly prepared by mixing 25 mL acetate buffer, 2.5 mL 2,4,6-tripyridyl-s-triazine solution and 2.5 mL ferric chloride solution prior to equilibrating the mixture at 37 °C before use. Honey samples (150 µL) were added to 2850 µL FRAP solution and absorbance was read at 593 nm (Thermofisher scientific Genesys G10S) following incubation for 30 min in the dark at ambient temperature. A calibration curve of trolox was used to evaluate the reducing power and results were expressed in mmol/L TE/100 g honey. The test was carried out in triplicate.

### 2.6.3. Iron (II) chelating activity assay

The method described by Ramful *et al.* was modified to assess the chelating activity of the various samples on iron (II) ions[21]. The reaction mixture consisted of 200 µL of sample at various concentrations and 50 µL of 0.5 mmol/L ferrous chloride tetrahydrate (FeCl<sub>2</sub>·4H<sub>2</sub>O). The total volume of the reaction mixture was made up to 1 mL with water. The reacting solution was incubated for 5 min at room temperature, after which 50 µL of ferrozine (2.5 mmol/L) was added. The resulting purple colouration was read at 562 nm (Thermofisher scientific Genesys G10S). The control consisted of the reaction solution, without the samples or the positive control, ethylene diamine tetraacetic acid (EDTA). The chelating activity was calculated as followed:

$$\text{Chelating activity (\%)} = \frac{\text{Abs}_{\text{control}} - \text{Abs}_{\text{extract}}}{\text{Abs}_{\text{control}}} \times 100$$

### 2.6.4. Hydroxyl radical (OH<sup>•</sup>) scavenging assay

Hydroxyl scavenging potential of the various samples was determined through its ability to oxidised deoxyribose as described by Thirunavukkarasu *et al.* with minor modifications[22]. The reacting mixture comprised hydrogen peroxide (15 µmol/L), ascorbic acid (3 mmol/L), iron chloride (3 mmol/L), EDTA (3 mmol/L), honey samples in varying concentrations along with 2-deoxy-D-ribose (2.58 mmol/L)

in PBS (pH 7.4) to a final volume of 1 mL. Reaction mixtures were incubated for 30 min at 37 °C. Absorbance was then measured at 532 nm (Thermofisher scientific Genesys G10S) and results were expressed as percentage inhibition of deoxyribose oxidation by the various samples.

### 2.6.5. 2,2-diphenyl-1-picryl-hydrazyl-hydrate) free radical scavenging (DPPH) assay

DPPH assay was used to assess the antioxidant activity of the compound being tested by determining their capacity to scavenge the free stable DPPH radical as described by Parekh *et al.*[23]. Stock concentrations of the samples and the standard antioxidant prepared in methanol at appropriate concentrations were placed in a 96-well microtitre plate where a serial dilution was performed in methanol. DPPH (200 µL) was added to make a final volume of 300 µL per well. The plate was incubated for 30 min at 37 °C. Absorbance was then measured at 517 nm (Labsystems Multiskan MS: 35200 7847). The analyses were carried out in triplicates and a dose response curve was plotted and the IC<sub>50</sub> (concentration of sample required to scavenge 50% of DPPH radical) were determined as described below[24]:

$$\text{Inhibition (\%)} = \frac{\text{Abs}_{\text{control}} - \text{Abs}_{\text{extract}}}{\text{Abs}_{\text{control}}} \times 100$$

### 2.6.6. Hypochlorous acid (HOCl) scavenging assay

This assay measured the chlorination of taurine as described by Ramful *et al.*[21]. The reaction mixture consisted of 100 µL HOCl (600 µmol/L), 100 µL taurine (150 mmol/L) and 100 µL honey samples at different concentrations for a final volume of 1 mL of PBS (pH 7.4). The reacting solution was thoroughly mixed and incubated for 10 min at room temperature, following which 100 µL potassium iodide (20 mmol/L) was added. Absorbance was measured spectrophotometrically against a reference (where 100 µL PBS replaced the honey sample and corresponded to occurrence of 100% HOCl) at 350 nm (Thermofisher scientific Genesys G10S). Results were expressed as the percentage of HOCl inhibition and the IC<sub>50</sub> was determined for each sample.

### 2.6.7. Nitric oxide (NO) radical scavenging assay

NO, readily generated by sodium nitroprusside in aqueous solution at physiological pH, reacts with oxygen producing nitrite ions which can be determined by using Griess Illosvoy reagent, with naphthylethylenediamine dihydrochloride (0.1% w/v) in place of 5% 1-naphthylamine[25,26]. The absorbance of the pink-coloured chromophore was measured at 540 nm (Thermofisher scientific Genesys G10S) against corresponding blank solutions.

### 2.6.8. 2,2'-azinobis (3-ethylbenzothiazoline-6-sulphonic acid) diammonium salt radical (ABTS<sup>•+</sup>) scavenging assay

The method described by Re *et al.* was used with slight modifications[27]. ABTS radical cation (ABTS<sup>•+</sup>) was produced by reacting ABTS stock solution (7 mmol/L) with 2.45 mmol/L

potassium persulphate. Honey samples (10 µL) were prepared using serial dilution in ethanol and were allowed to react with 1 mL of the diluted ABTS solution and absorbance was recorded at 734 nm (Thermofisher scientific Genesys G10S) after 6 min. Ascorbic acid was used as positive control. All determinations were carried out in triplicate.

### 2.7. Chemical profile

A preliminary phytochemical screening of the aqueous honeys solutions such as alkaloids, saponins, phenolic compounds, anthraquinones, steroids and flavonoids was performed using modified standard methods described by Mahomoodally *et al.*[28] and Chethana *et al.*[29].

### 2.8. Determination of total phenolic and flavonoids content

Total phenolic content (TPC) was assessed according to Folin–Ciocalteu method and the test was carried out in triplicate[26]. TPC was calculated with respect to a gallic acid standard curve and was expressed in mg of gallic acid equivalent (GAE)/100 g honey.

Total flavonoid content (TFC) was established as described by Amaeze *et al.*[26]. Total flavonoids were calculated with respect to rutin standard curve and the results expressed in µg rutin equivalent (RE)/100 g honey.

### 2.9. Statistical analysis

Results were presented as mean±SD in tables and figures with error bars. Microsoft Excel 2010 was used for statistical analysis and to generate charts owing to its more professional appearance. ANOVA single factor

( $P=0.05$ ) and least significant difference (LSD) tests were used to compare the data. Differences between means ( $P<0.05$ ) were regarded as statistically significant. The correlation between total phenolic and antioxidant capacity was determined by Pearson correlation coefficient. Correlation coefficient values were interpreted as suggested by Pallant[30]. The range was as follows:  $r=-0.10$  to  $-0.29$  and  $r=0.10$  to  $0.29$  denoted weak correlation,  $r=-0.30$  to  $-0.49$  and  $r=0.30$  to  $0.49$  indicated moderate, while  $r=-0.50$  to  $-1.00$  and  $r=0.50$  to  $1.00$  showed strong correlation.

## 3. Results

### 3.1. Antimicrobial assays

Results shown in Table 1 depicted zones of inhibition obtained following disc diffusion assay of undiluted honeys. No activity was observed against *E. coli*. Only undiluted longan honey inhibited growth of *C. albicans* [ $8.00\pm 0.00$  mm] while both litchi and longan honey inhibited growth of *S. aureus* [inhibition zones of  $(7.33\pm 0.58)$  mm and  $(9.00\pm 1.73)$  mm respectively]. The positive control ampicillin (10 µg) showed zone of inhibition only for *E. coli* and *S. aureus*. It was observed that ampicillin had a significantly ( $P<0.05$ ) higher activity than the honey samples against *E. coli* and *S. aureus*. However, processed, syrup flavoured, eucalyptus and ginger honeys showed significantly higher activity against *P. aeruginosa* compared to the positive control. For *C. albicans*, only longan honey showed a significantly ( $P<0.05$ ) lower activity compared to the positive control (nystatin).

**Table 1**

Mean zones of inhibition for disc diffusion for undiluted honey.

Isolates	Disc diffusion (mm)							LSD
	Standard	Processed	Syrup flavoured	Ginger	Eucalyptus	Litchi	Longan	
Bacteria <sup>1</sup> EC	21.67±3.79	8.67±1.15	–	11.67±1.53	7.33±0.58	–	9.33±0.58	$P<0.05^*$
PA	–	10.33±1.15	7.67±0.58	–	8.67±0.58	–	9.33±0.58	$P<0.05^*$
SA	26.33±0.58	–	–	–	–	7.33±0.58	9.00±1.73	$P<0.05^*$
Fungi <sup>2</sup> CA	30.33±0.58	–	–	–	–	–	8.00±0.00	$P<0.05^*$
AN	23.00±1.00	–	–	–	–	–	–	$P<0.05^*$

Results are expressed as mean±SD. EC: *E. coli*; PA: *P. aeruginosa*; SA: *S. aureus*; CA: *C. albicans*; AN: *A. niger*; –: No zone of inhibition. <sup>1</sup>: Ampicillin (10 µg) was used as the positive standard against bacteria. <sup>2</sup>: Nystatin (10 µg) was used as the positive standard against fungi. \*: Values are significantly different ( $P<0.05$ ) from respective positive control.

**Table 2**

Minimum inhibitory concentration of the different honeys against the microbial isolates.

Isolates	Percentage (%) (w/v)						mg/mL	
	Processed	Syrup flavoured	Ginger	Eucalyptus	Litchi	Longan	Gentamycin	Streptomycin
EC	12.5 <sup>a</sup>	–	25.0 <sup>a</sup>	25.0 <sup>a</sup>	–	12.5 <sup>a</sup>	N/A	0.1563
PA	25.0 <sup>a</sup>	25.0 <sup>a</sup>	–	50.0 <sup>a</sup>	–	50.0 <sup>a</sup>	5.0	N/A
SA	–	–	–	–	50.0 <sup>a</sup>	50.0 <sup>a</sup>	N/A	0.1563
CA	–	–	–	–	–	12.5 <sup>a</sup>	2.5	N/A

EC: *E. coli*; PA: *P. aeruginosa*; SA: *S. aureus*; CA: *C. albicans*; AN: *A. niger*. N/A: Not applicable; –: Honey samples not tested in MIC determination. a: Value significantly higher ( $P<0.05$ ) from respective positive control for respective microorganisms.

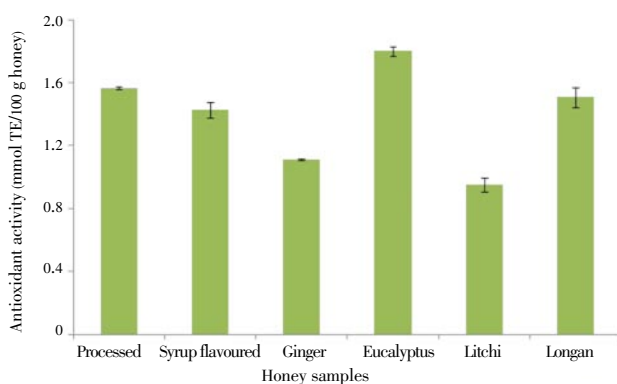
### 3.1.1. MIC

The MIC was based on the lowest concentration which was shown to effectively inhibit microbial growth. MIC for *A. niger* was not calculated since no zone of inhibition was observed in the disc diffusion assay (Table 2). The positive control showed higher activity against their respective microorganisms compared to tested honeys. Based on the data obtained from the MIC, it can be deduced that the most susceptible microorganisms to the six honeys analysed was *E. coli*, with the order of susceptibility being *E. coli*=*P. aeruginosa*>*S. aureus*>*C. albicans*.

## 3.2. Antioxidant capacity

### 3.2.1. TEAC assay

Results obtained from the assay are illustrated in Figure 1. All results were expressed as mmol TE/100 g honey. The TEAC values ranged from (0.950±0.044) to (1.800±0.029) mmol TE/100 g honey with eucalyptus honey having the highest TEAC values and litchi honey having the lowest. The activity in decreasing order was: eucalyptus honey>processed honey>longan honey>syrup flavoured honey>ginger honey> litchi honey. A significant difference in the TEAC was observed between the six samples ( $P<0.05$ ). LSD noted a significant difference between all samples, except between processed and longan honeys.

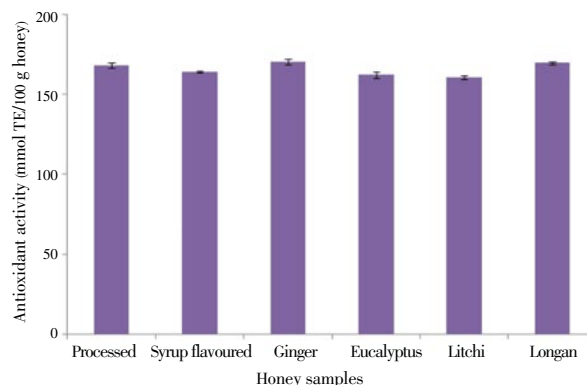


**Figure 1.** Antioxidant activity of different honey samples assessed by TEAC assay.

### 3.2.2. FRAP assay

The FRAP values for the honeys samples ranged from (160.77±1.02) to (170.50±1.84) mmol TE/100 g (Figure 2). Litchi

honey showed the lowest FRAP value while ginger honey had the highest one. There was a significant difference ( $P<0.05$ ) between FRAP activity of the tested honeys. LSD confirmed the significant differences between the samples except between ginger and longan honeys, ginger and processed honeys, and longan and processed honeys.



**Figure 2.** Antioxidant activity of different honey samples assessed by FRAP assay.

### 3.2.3. Iron (II) chelating, OH, DPPH, HOCl, NO and ABTS radical scavenging assays

IC<sub>50</sub> values obtained for iron chelating activity ranged from (4.51±0.34) to (14.31±1.56) mg/mL, with processed honey having the highest chelating potential and litchi honey having the weakest potential (Table 3). Ginger and eucalyptus honeys had similar IC<sub>50</sub> values. There was a significant difference ( $P<0.05$ ) between the chelating activities of the tested honeys. LSD established significant difference between the samples and EDTA.

The IC<sub>50</sub> values ranged from (31.24±0.75) to (46.75±0.95) mg/mL with eucalyptus honey having the highest potential and syrup flavoured honey having the lowest potential as an inhibitor of deoxyribose oxidation (OH<sup>•</sup> scavenging assay). A significant difference was confirmed between positive control and honeys as well as between honeys except between syrup flavoured and processed honeys, processed and longan honeys, and longan and processed honeys. Samples were observed to be weak scavengers of DPPH compared to the positive control ascorbic acid (Table 3). Most of the honey samples had low % inhibition values and thus IC<sub>25</sub> was evaluated. The 25% inhibition of DPPH for the assayed honey ranged from (15.850±0.890) to (47.430±

**Table 3**

Antioxidant properties of honey samples.

Sample	Iron chelating (IC <sub>50</sub> )	OH <sup>•</sup> (IC <sub>50</sub> )	DPPH <sup>•</sup> (IC <sub>25</sub> )	HOCl <sup>•</sup> (IC <sub>50</sub> )	NO <sup>•</sup> (IC <sub>50</sub> )	ABTS <sup>•+</sup> (IC <sub>50</sub> )
Processed	4.510±0.340 <sup>a</sup>	31.84±1.13 <sup>a</sup>	23.420±1.400 <sup>a</sup>	47.730±0.410 <sup>a</sup>	5.620±0.970 <sup>a</sup>	90.440±2.480 <sup>a</sup>
Syrup flavoured	8.220±0.750 <sup>a</sup>	46.75±0.95 <sup>a</sup>	15.850±0.890 <sup>a</sup>	68.850±1.230 <sup>a</sup>	4.960±0.490 <sup>a</sup>	94.470±0.580 <sup>a</sup>
Ginger	7.700±0.220 <sup>a</sup>	41.34±1.14 <sup>a</sup>	47.430±1.030 <sup>a</sup>	36.440±0.440 <sup>a</sup>	2.770±0.790 <sup>a</sup>	92.390±2.570 <sup>a</sup>
Eucalyptus	7.170±0.340 <sup>a</sup>	31.24±0.75 <sup>a</sup>	24.140±3.380 <sup>a</sup>	24.030±0.830 <sup>a</sup>	6.760±0.980 <sup>a</sup>	93.300±2.420 <sup>a</sup>
Litchi	14.310±1.560 <sup>a</sup>	46.64±0.29 <sup>a</sup>	32.230±0.900 <sup>a</sup>	26.510±0.920 <sup>a</sup>	37.050±1.070 <sup>a</sup>	92.150±1.070 <sup>a</sup>
Longan	9.060±0.750 <sup>a</sup>	31.30±0.85 <sup>a</sup>	27.230±2.400 <sup>a</sup>	21.670±1.090 <sup>a</sup>	47.300±1.210 <sup>a</sup>	92.790±2.070 <sup>a</sup>
α-tocopherol <sup>1</sup>	0.070±0.002 <sup>b</sup>	0.34±0.01 <sup>c</sup>	0.140±0.003 <sup>d</sup>	0.140±0.003 <sup>d</sup>	0.140±0.003 <sup>d</sup>	0.140±0.003 <sup>d</sup>

Results are expressed as mean±SD (mg/mL) (n=3). Positive control used in assay <sup>b</sup>EDTA, <sup>c</sup>α-tocopherol, <sup>d</sup>Ascorbic acid. <sup>a</sup>: Values are significantly higher ( $P<0.05$ ) from positive control.



1.030) mg/mL as compared to ascorbic acid [ $IC_{25}$  of (0.010±0.001) mg/mL].

Longan honey had the highest HCl scavenging potential followed by eucalyptus honey at (21.670±1.090) and (24.030±0.830) mg/mL, respectively. Syrup flavoured honey had the weakest scavenging potential with an  $IC_{50}$  value of (68.850±1.230) mg/mL. Ascorbic acid was used as the positive control for this assay and had an  $IC_{50}$  of (0.120±0.002) mg/mL. A significant difference ( $P<0.05$ ) in scavenging capacity was observed between the six honey samples.

The  $IC_{50}$  values for the NO radical scavenging assay obtained for this assay were between (2.770±0.790) and (47.300±1.210) mg/mL. Ginger honey was the most powerful scavenging sample and longan honey being the least potent scavenger of NO radical (Table 3). Ascorbic acid, used as a positive control, exhibited a 50% inhibition at (0.120±0.002) mg/mL. The six honey tested were ranked as followed: ginger honey>syrup flavoured honey>processed honey>eucalyptus honey>litchi honey>longan honey. There was a significant difference in the scavenging potential of the six honey sample ( $P<0.05$ ).

The  $IC_{50}$  for the ABTS radical scavenging assay ranged from (90.440±2.480) mg/mL to (94.470±0.580) mg/mL. The positive control, ascorbic acid, had an  $IC_{50}$  of (0.140±0.003) mg/mL. LSD performed ( $P<0.05$ ) exhibited significant difference in the scavenging potential of the six honey samples and the positive control.

### 3.3. Chemical profile

The six samples were screened for the presence of major classes of secondary metabolites, namely alkaloids, saponins, phenolic compounds, anthraquinones, flavonoids and steroids (Table 4). The presence of anthraquinones was noted in processed, syrup flavoured, ginger and eucalyptus honey with prominent amount observed in syrup flavoured honey. Steroids were found to be absent in all the six samples. The alkaline test indicated the presence of flavonoids in all honeys with more prominent amounts in litchi and longan honeys.

**Table 4**

Phytochemical screening of the aqueous honeys.

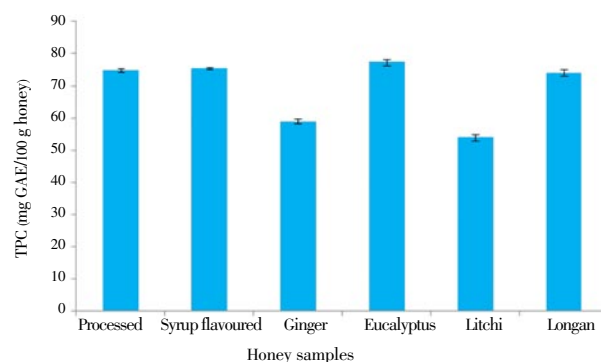
Analysis samples	H1	H2	H3	H4	H5	H6
Alkaloids	+++	++	+++	+++	–	+
Saponins	–	–	–	–	+	+
Phenolics	+	+	+	+++	+	++
Anthraquinones	+	++	+	+	–	–
Steroids	–	–	–	–	–	–
Flavonoids	+	+	++	+	+++	+++

H1: Processed honey; H2: Syrup flavoured honey; H3: Ginger honey; H4: Eucalyptus honey; H5: Litchi honey; H6: Longan honey. –: Absence; +: Presence; ++: Prominent presence; +++: Very prominent presence.

### 3.4. TPC

All the samples analysed showed moderate to high

phenolic contents ranging from (54.03±0.99) to (77.37±1.01) mg GAE/100 g honey, with eucalyptus honey having the highest level of phenols (Figure 3). Processed and syrup flavoured honey had similar phenolic content while longan honey had a slightly lower amount. Ginger and litchi honey had the lowest total phenolic levels with (59.06±0.68) and (54.03±0.99) mg GAE/100 g honey. There was a significant difference between the TPC of the six analysed honeys ( $P<0.05$ ) which was confirmed by LSD value except for syrup flavoured and processed honey, syrup flavoured and longan honeys, and processed and longan honeys.

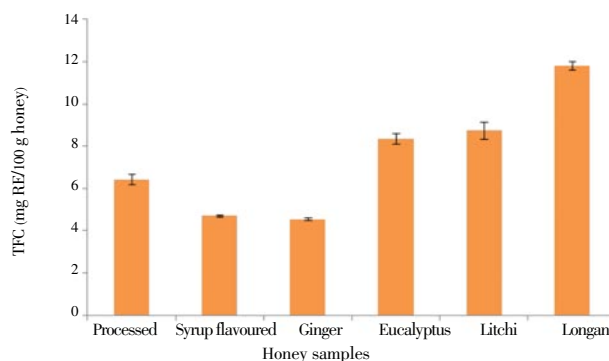


**Figure 3.** TPC of the six samples.

Results were expressed as mean±SD ( $n=3$ ).

### 3.5. TFC

The TFC varied from (4.55±0.06) to (11.80±0.20) mg RE/100 g honey, with ginger honey having the lowest value and longan honey having the highest TFC (Figure 4). Litchi honey had the second highest flavonoid level [(8.75±0.39) mg RE/100 g honey] followed by eucalyptus honey [(8.36±0.25) mg RE/100 g honey], processed honey [(6.44±0.26) mg RE/100 g honey] and syrup flavoured honey [(4.72±0.06) mg RE/100 g honey]. The flavonoid content was higher in raw honeys. Significant difference was observed in the TFC among the six samples ( $P<0.05$ ). LSD was performed since a significant difference was confirmed except for syrup flavoured and ginger honey.



**Figure 4.** TFC of the six samples.

Results expressed as mean±SD ( $n=3$ ).

### 3.6. Correlation between phenolic content and antioxidant capacity

Pearson correlation coefficient was used to determine the

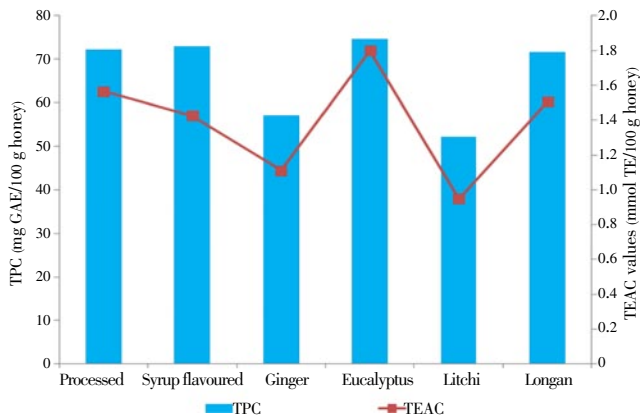
strength of correlation between TPC, TFC and antioxidant capacity (Table 5) and summarised in Figures 5–10.

Strong positive correlation was observed between TPC and TEAC ( $r=0.9453$ ) as well as between TFC and NO<sup>•</sup> scavenging assay ( $r=0.8678$ ). Strong negative correlations were noted between TPC and iron chelating ( $r=-0.6964$ ), OH<sup>•</sup> ( $r=-0.6150$ ), DPPH<sup>•</sup> ( $r=-0.7439$ ) and TFC and scavenging HOCl assay ( $r=-0.7509$ ). A poor correlation was observed in both TPC and TFC and the remaining assays. Strong correlations observed between the polyphenols and the antioxidant assays.

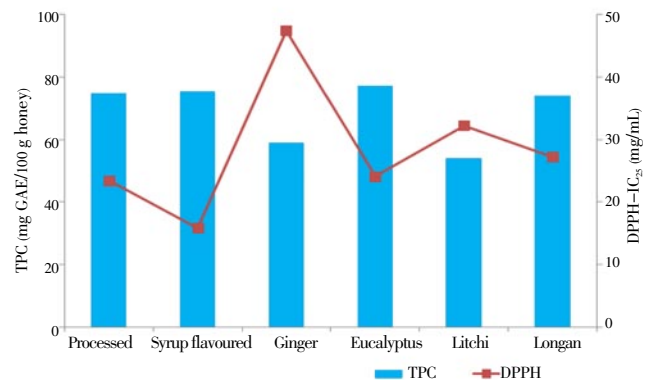
**Table 5**

Correlation between TPC, TFC and antioxidant activity.

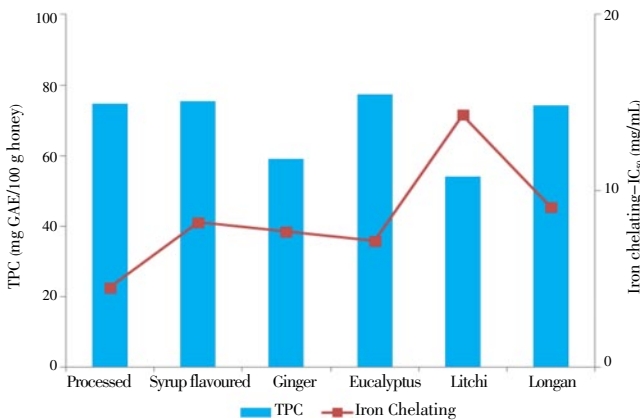
Assay	TPC	TFC
TEAC	0.9453	0.1934
FRAP	0.1164	-0.0658
Iron chelating	-0.6964	0.3539
OH <sup>•</sup>	-0.6150	-0.4874
DPPH	-0.7439	-0.1328
HOCl	0.2685	-0.7509
NO <sup>•</sup>	-0.2396	0.8678
ABTS <sup>•+</sup>	0.2302	-0.0784



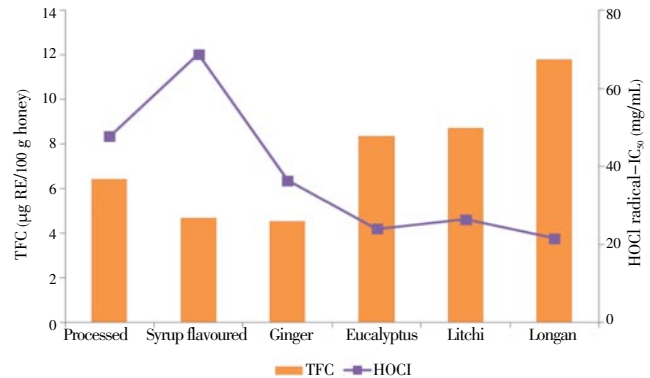
**Figure 5.** Relationship between TPC and antioxidant capacity as assessed by TEAC assay.



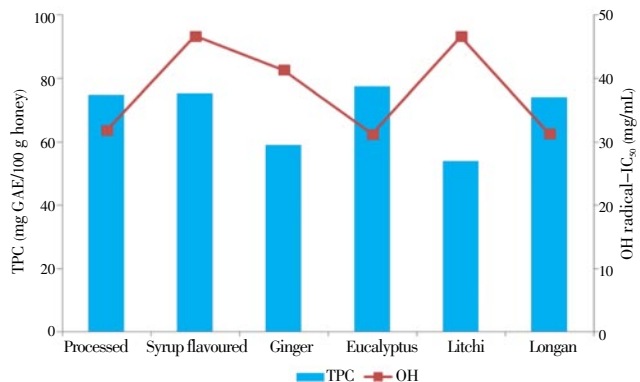
**Figure 8.** Relationship between TPC and antioxidant capacity as assessed by DPPH radical scavenging assay.



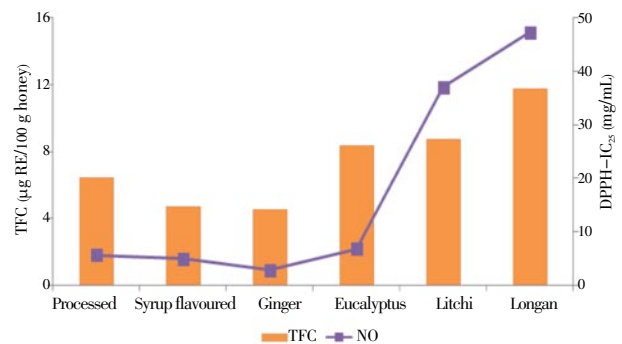
**Figure 6.** Relationship between TPC and antioxidant capacity as assessed by iron (II) chelating activity.



**Figure 9.** Relationship between TFC and antioxidant capacity as assessed by HOCl radical scavenging assay.



**Figure 7.** Relationship between TPC and antioxidant capacity as assessed by OH radical scavenging assay.



**Figure 10.** Relationship between TFC and antioxidant capacity as assessed by NO radical scavenging assay.

#### 4. Discussion

Honey has a long history of use which has experienced a resurgence of interest as a functional food owing to its numerous health benefits<sup>[31–33]</sup>. In the present study, the antimicrobial potential revealed that undiluted samples inhibited the growth of several microorganisms. Undiluted ginger, eucalyptus and longan honeys inhibited the growth of *E. coli* while processed honey achieved the same result at halved concentration. Undiluted honeys except ginger and litchi honeys exhibited antibacterial property against *P. aeruginosa*. However, they were found to be ineffective against *S. aureus* except for litchi and longan honeys. Longan honey was the only active sample against *C. albicans*. However, none of the tested honeys were able to inhibit growth of *A. niger*. Undiluted ginger honey was found to possess a weaker antibacterial potential, inhibiting only *E. coli* but not *P. aeruginosa* and *S. aureus*. This difference could be explained by different concentration of ginger extract present in the local honey compared to previous studies. Moreover, a weak overall activity of Mauritian honey against *S. aureus*, with growth inhibition occurring in litchi and longan honey only, was observed which was similar to results reported by Lusby *et al.*<sup>[34]</sup>. Additionally, Chauhan *et al.* has previously reported the zone of inhibition of raw honeys for *S. aureus* which was similar to the values obtained for the raw honeys (litchi and longan honeys)<sup>[1]</sup>. However, compared to the zone of inhibition for processed honeys obtained by Chauhan *et al.*<sup>[1]</sup>, the processed honeys in the present study showed no inhibition potential against *S. aureus*. Additionally, it was found that longan honey was the only sample to prevent the spread of *C. albicans*. Our findings are similar to a study by Lusby *et al.* where it was established that the growth of *C. albicans* was not inhibited by honey<sup>[34]</sup>.

Mohapatra *et al.* studied the MIC for both raw and processed methanol extracts of honey against several bacterial strains<sup>[35]</sup>. It was observed that raw and processed honeys had MIC values for *E. coli* and *P. aeruginosa* which were much lower to those obtained in the present study. These differences in activity could be attributed to the different solvents used for sample preparation where in the previous study the honeys were treated in methanol while sterile distilled water was used for the current study. It was observed that the MIC values for manuka and multifloral honeys against *S. aureus* were lower than MIC obtained for multifloral honeys in the present work<sup>[36]</sup>. Contrarily to our study, Boukraâ and Bouchegrane noted the antifungal properties of two multifloral honeys against *C. albicans*<sup>[37]</sup>. The differences observed between previous studies and the assayed Mauritian honeys could be potentially due to factors such as different antioxidant profiles as well as

hydrogen peroxide concentrations.

Therefore, it can be concluded that the assayed Mauritian honeys had generally a weaker antimicrobial activity compared to previous studies. Differences in the antibacterial and antifungal potency of the different honeys could be attributed to diverse floral source, geographical and seasonal variations along with processing and storage factors<sup>[38,39]</sup>. Extraction and dilution procedures could have affected the outcome of the assays as demonstrated by the significantly larger zones of inhibition or lower MIC values with methanol, ethanol and ethyl acetate extracts of raw and processed honeys<sup>[35]</sup>.

In a study conducted by Vit *et al.*<sup>[6]</sup>, it was established that the average antioxidant activity of unifloral honeys was higher than mean TEAC value of the present study. Conversely, South African honeys had an antioxidant activity range which was similar to the range of the current work<sup>[40,41]</sup>. FRAP is a direct assay commonly used for the determination of the antioxidant activity of various compounds since it assesses the potential of a sample to reduce ferric compound to their ferrous state<sup>[40]</sup>. However, compared to Malaysian and Bangladeshi honeys, Mauritian honeys had a smaller FRAP activity range<sup>[42,43]</sup>. The FRAP value of litchi honey was higher in the present study compared to Bangladeshi litchi honey<sup>[43]</sup>.

OH<sup>•</sup> is produced from hydroxide peroxide and superoxide anion when metal ions namely iron and copper are present. Moreover, it has the highest reactivity along with having the most elevated oxidative potential<sup>[44,45]</sup>. OH<sup>•</sup> adversely interact with biological molecules namely amino acids, lipids, proteins and nucleic acid particularly thiamine compounds forming hydroxycyclohexadienyl radical which can further be degraded in the presence of oxygen<sup>[44,45]</sup>. It was found that eucalyptus honey had the highest hydroxyl scavenging potential and syrup flavoured honey the lowest activity.  $\alpha$ -tocopherol, used as a positive control, exhibited a scavenging power which was much higher than that observed in the assayed honeys.

The percentage DPPH scavenging activity of commercial Indian honeys was higher than that of locally tested honeys<sup>[46,47]</sup>. In another study<sup>[5]</sup>, it was observed that multifloral honeys had an IC<sub>50</sub> range lower than that observed in Mauritian multifloral specimens. Therefore, the honeys analysed in the current study had a lower free radical scavenging power compared to Slovenian multifloral honeys. Silva *et al.* had greater DPPH scavenging activity with ethanolic, methanolic honey extract and pure honey compared to the current study<sup>[48]</sup>. Serem and Bester established the antioxidant potential of honey using DPPH where the IC<sub>50</sub> of eucalyptus honey was (1.44±0.30)  $\mu$ mol TE/g and (2.52±0.40)  $\mu$ mol TE/g and litchi (1.15±0.50)  $\mu$ mol TE/g<sup>[41]</sup>. Thus, compared to these results, local eucalyptus and litchi



honeys were weaker DPPH scavengers.

To our best knowledge, no previous study has assessed the HOCl scavenging activity of honey. The present study has showed that the local honeys showed weak to moderate HOCl scavenging activity with unifloral honeys being the most powerful ones. Moreover, it was also established that the honeys assessed had good to moderate activities as NO scavengers with litchi and longan honeys being the least potential ones. These findings are similar to the results obtained by Uthurry *et al.* who observed that multifloral honeys exhibited more potent scavenging activity for the reactive NO<sup>•</sup> assay[49].

The honey analysed were observed to be powerful iron chelators compared to honeys assayed by Ita who established the poor metal chelating activity of honey[50]. Compared to Nigerian honey, local honeys were also high in active compounds which strongly bind to metal ions. However, the lack of information on these assays on honey makes any comparison with the present study difficult. Contrarily to Isla *et al.* who observed high ABTS<sup>•+</sup> antioxidant activity[51], all the honeys analysed in the present study possessed weak activity against ABTS<sup>•+</sup>. Additionally, other studies observed a high ABTS<sup>•+</sup> antioxidant activity in multi-source honeys[9,48]. A possible explanation is that the tested honeys contained inferring components which reacted with the ABTS radical.

The TPC of the honeys assayed was found to be similar to those previously reported for Algerian honeys and Burkina Fasan honey[41]. However, a study reported that the polyphenol content of unisource honeys was much lower than the TPC of the present study[6]. Additionally, it was observed that the TPC content of unifloral Cuban honeys was higher than the Mauritian monofloral samples[8]. Eucalyptus honey had the highest level of phenols which was found to be similar to one of the study carried by Serem and Bester[41]. However, litchi honey was observed to have significantly lower TPC compared to the results reported by Serem and Bester for agricultural litchi[41]. Bertoncelj *et al.* observed that multifloral honeys TPC which was higher than those levels of processed and syrup flavoured honeys[5]. However, a study conducted on commercial Indian honeys found that the TPC range was comparable the TPC of the commercial processed and syrup flavoured honeys in the present study[47].

The TFC values for the currently assayed were low compared to those observed in Malaysian honeys[42]. A study conducted by Serem and Bester established the TFC for litchi honey and agricultural eucalyptus honeys were higher than in the current study[41]. However, it was observed that local unprocessed honeys namely eucalyptus, litchi and longan honeys had a higher flavonoids content compared to South Eastern Nigerian raw honeys[52].

In the current study, the correlation analysis showed a very strong correlation between TPC and TEAC for the six analysed honeys. Thus, it can be inferred that the total phenols of honey contribute substantially to its TEAC potential. Similarly, Brazilian honeys showed a strong positive association between TPC and ABTS radical[53]. Additionally Alvarez–Suarez *et al.*[8] and Serem and Bester[41] observed strong positive correlation between TPC and TEAC. Serem and Bester observed a positive correlation between TFC and TEAC[41]. A study conducted by Lianda *et al.* noted a strong association between TFC and ABTS radical[53]. However, a very weak positive correlation was established between TFC and TEAC in our study.

Bertoncelj *et al.* observed an elevated correlation coefficient between TPC and FRAP[5], thereby establishing phenols as the major compounds which accounts for the FRAP potential of honey. Their findings are in accordance with previous research where similar association has been established[8,47,54]. However, a weak association had been established between the TPC and FRAP. Likewise, the honeys analysed had a very low negative association between TFC and FRAP implying that there is potentially no association between TFC and FRAP activity or a very poor inverse relationship unlike findings of Lianda *et al.* where TFC was moderately associated with FRAP[53]. Strong positive association was observed between TFC and FRAP in Cuban honey[8]. A strong negative association has been established between iron chelating activity assay and TPC, with its activity being inversely proportional to the TPC. No significant association has been observed between TFC and iron chelating activity of the honeys studied. HO<sup>•</sup> scavenging assay had a negative, strong correlation with TPC while TFC and the antioxidant assay were moderately correlated.

A study conducted by Serem and Bester on various Southern African honey observed a strong positive significant correlation between TPC and DPPH as well as TFC and DPPH[41]. Similar correlation between total phenolic and DPPH scavenging had been stated by Saxena *et al.*[47]. Krpan *et al.* also observed a very strong positive correlation between TPC and DPPH demonstrating the role of phenolic compounds in the antioxidant potential of the acacia honey[54]. Additionally, Silici *et al.* observed a strong positive linear correlation between TPC and DPPH in *Rhododendron* honeys[12]. However, strong negative correlation has also been noted between TPC and DPPH:EC<sub>50</sub> as well as moderate negative association between TFC and DPPH:EC<sub>50</sub>[53]. These results are in line to those obtained from the current study where a strong inverse relationship has been attributed to TPC and DPPH activity. Thus, it can be suggested that other compounds were responsible for this antiradical activity.

A moderate negative association was noted between

TFC and DPPH. Thus, as the level of polyphenols in honey increases, the DPPH action decreases. However, this negative association could have resulted from the extremely low flavonoid content of the samples analysed. A strong negative correlation has been observed between TFC and HOCl scavenging activity while a strong positive relationship between TFC and NO<sup>•</sup> scavenging activity of honey. Thus, it can be concluded that NO scavenging activity was directly linked to the levels of flavonoids present in the local honeys. In the case of HOCl scavenging activity, it was noted that as the levels of TFC increased, the antiradical activity observed was lowered. Therefore, other compounds present in the honey samples could have interfered with antioxidant assay. However, no significant associations were noted between HOCl and NO scavenging activities of honey and TPC.

Similarly, no strong correlations were observed between ABTS radical scavenging and TPC and TFC in the present study. However, significant correlation was observed between ABTS<sup>•+</sup> assay and TPC in Argentinean honeys[51]. Moreover, Isla *et al.* concluded that the quality of honey polyphenols is the primary factor affecting its antioxidant potential[51]. The lack of correlation between polyphenols and antioxidant capacity could be explained by the different compounds namely organic acids, enzymes (glucose oxidase, catalase) and small levels of antioxidants (ascorbic acid) naturally present in honey which can further influence their antioxidant activity. Heat treatment during processing along with extensive storage periods can significantly alter the composition of honey where the Maillard reaction products can contribute to the antioxidant activity of honey[55]. Moreover, evidence showed that some bioelements namely copper, iron, magnesium and zinc correlated to the antioxidant activity, TFC and TPC of honey while manganese had been linked to the antioxidant activity only. Other factors such as colour, floral source and climatic and environmental conditions also affect the antioxidant potential of the honeys.

Phenolics content is also strongly correlated with colour of honey[43]. Islam *et al.* established a significant positive association between TPC and colour intensity in addition to colour and DPPH and colour and FRAP[43]. Colouring agents present in honey may therefore alter the antioxidant activity of honey, where darker honeys typically increase its antioxidant potential. Additionally, proline, a natural constituent of honey, was also moderately correlated with TPC, TFC, FRAP and DPPH in Bangladeshi, Indian and Cuban honeys[43]. Thus, the antioxidant potential of honey could be slightly altered by the presence of proline.

Moreover, different methods used for sample preparation might account for any difference observed between this study and previous studies. In the current study, the honey

samples were freshly prepared in sterile distilled water prior to conducting each assay. This particular approach has been selected for the *in vitro* assays since honey is typically used in its crude form. However, several methods describing the extraction of phenolic compounds for antioxidant and antimicrobial assays have been widely used in previous studies[56,57].

Honey preparation following sampling usually consists of thorough mixing, purification process and concentrating the sample before conducting assays[58]. In numerous studies, Amberlite XAD-2 was used during the extraction as it was observed to retain honey polyphenols, recovering 80%–90% of the compounds while sugars present in honey are eliminated along with other compounds which may affect the various assays[58,59].

Indeed, Ferreres *et al.* observed that extraction using Amberlite XAD-2 enabled the removal of sugars from honeys which can adversely affect flavonoid determination of honey using high performance liquid chromatography method[60]. Indeed, Silva *et al.* analysed TPC content of honey samples prepared from pure honey, methanol extract and ethyl acetate extract[48]. The highest TPC was found in ethanolic acid fraction followed by methanolic extract while pure honey had the lowest TPC content.

Overall, it was observed that both processed and raw honeys had approximately the same antioxidant power, with variation occurring which could be possibly attributed to the different food matrix, susceptibility to type of reagents used in the assay as well as mechanism of action of the oxidation process. These parameters may affect the outcome of the assays, therefore they need to perform different standard antioxidant assays. Thus, it can be suggested that the regular consumption of commercial or unprocessed unifloral honey could have potential health benefits in reducing oxidative stress in the body. Moreover, the assayed honeys possessed better antibacterial activity than antifungal capacity, which can explain the importance of honey in wound healing in traditional medicine and apitherapy. The antimicrobial activities of raw honeys were observed to be superior to that of commercially available honeys, with longan honey being the most active.

### Conflict of interest statement

We declare that we have no conflict of interest.

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