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Original article

# Physico-chemical, antioxidant and anti-microbial properties of some Ethiopian mono-floral honeys

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## ABSTRACT

Honey has been widely used to treat several human pathogens. However, all honeys may not have equal potencies against different human pathogens. The purpose of the current work was to investigate the physico-chemical and antimicrobial qualities of some mono-floral honeys from Ethiopia against some human pathogen bacteria and fungi. In the study, seven different botanical origin honeys were used of which some were from plants known for their medicinal properties. The samples were tested for their major physico-chemical properties (sugar profiles, total free acids, pH, color, electric conductivity and total soluble substances) and their medicinal values as total antioxidant capacity, total phenolic content and antimicrobial properties as minimum inhibitory concentration against some human pathogens, following standard protocols. Generally, the average values of the physico-chemical properties of the samples were within the acceptable ranges of world honey quality values. The average total antioxidant value of the samples was  $320.3 \pm 15.1$  with range of  $225.4 \pm 12.8$ – $465.7 \pm 21.8$   $\mu\text{M Fe(II)}/100\text{g}$ . Relatively higher values  $421.5 \pm 23.4$  and  $465.7 \pm 21.8$   $\mu\text{M Fe(II)}/100\text{g}$  recorded for *Croton macrostachyus* and *Vernonia amygdalina* honeys respectively. The average phenolic contents of the samples varied from  $233.3 \pm 24.0$  to  $693.3 \pm 26.8$   $\text{mgGAE}/\text{kg}$  and relatively higher values recorded for *C. macrostachyus* and *V. amygdalina* honeys. The significant proportion of the tested samples showed strong antimicrobial qualities inhibiting the growth of tested pathogens at concentration of 10.5%–28.6% of MIC (% v/v). Honeys from medicinal plants (*C. macrostachyus* and *V. amygdalina*) relatively showed more antimicrobial properties which could be due to the presence of plant specific phytochemicals which require further investigations.

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## 1. Introduction

Despite the general similarities in major physico-chemical properties of different honeys; there are no two identical honeys, unless both harvested from the same hive at the same time which indicates the very diverse nature of honeys in their composition

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and properties. In this regard, honey has been extensively analyzed in terms of its physico-chemical properties, geographical and botanical origins and reported to have significant variations among different honeys (Wang et al., 2009; Alvarez-Suarez et al., 2009; Gomes et al., 2010; Ansari et al., 2018; Kumar et al., 2018; Al-Ghamdi et al., 2019). The harvesting season and associated climatic conditions and postharvest handling techniques contribute for its variations (Alvarez-Suarez et al., 2010b).

Besides its dietary importance, honey has been taken as an essential ingredient of folk medicine and utilized against microbial infections, various diseases and ailments dates back to ancient times (Molan, 1992a, 1992b, 2006). Moreover, honey has been assessed for its antimicrobial action (wound healing, and infected surgical wounds), anti-inflammatory property (burns, skin grafting, etc.), anti-oxidant properties and surgical debridement and

has been reported for its high therapeutic potentials (Al-Wali, 2004; Vander berg et al., 2008; Noori et al., 2012; Ansari, et al., 2013, Noori, et al. 2013). Moreover, several studies supported the medicinal values of different honeys and the presence of significant variations in their medicinal properties (Molan, 1992a, b; Lusby et al., 2005; Saxena et al., 2010).

The variations in the antimicrobial potencies among the different honeys reported to be more than 100-folds, which is attributed to their botanical and geographical origins (Molan and Cooper, 2000). Such variations in the properties of honeys could be responsible for the broadly varying capabilities of honeys to serve as antioxidants (Lusby et al., 2005; French et al., 2005; Mullai & Menon 2007). The antimicrobial property of honey is mainly attributed to osmolality (sugar), pH, hydrogen peroxide levels and the methylglyoxal (Mavric et al., 2008; Alvarez-Suarez et al., 2010a). Moreover, the honey's non-peroxide aspects such as: lysozyme, some phytochemicals, phenolic acids and flavonoids are reported to play important role in its antimicrobial properties (Al et al., 2009). Particularly, the presence of phenolic compounds in honey, their anti-oxidant properties and their positive health role have been well documented (Gheldof and Engeseth, 2002; Nicholls and Miraglio, 2003). Moreover, the presence of differences in the amount of phenolic compounds based on its botanical and geographical origins have been also reported (Özkök et al. 2010).

Honeys composition is variable as they vary not only in their physico-chemical properties but also in their biological activities (Khalil et al., 2001). The difference in degrees of antimicrobial properties among different honeys is directly related to their floral source and geographical origins of the floral sources (Mavric et al., 2008; Molan, 1992a, 1992b). Moreover, the quality of honey is also utmost importance in determining the antimicrobial potential of honey because honey that has been mishandled (overheated or adulterated) may lose its medicinal properties. In addition, some of the physical properties (acidity, phenolic content and antioxidant level) of honey have direct relationships with its antimicrobial properties which are important to assess and correlate the physical properties and medicinal values of honeys.

In the very past and present days' folk medicines, selection of specific plant origin honeys for specific therapeutic purposes are widely used (Molan, 1992), indicating all honeys may not have equal therapeutic values. In this regard, Iirish et al. (2011) reported that out of 477 honey samples tested only 57% of them have antimicrobial activities, and exceptionally strong antimicrobial properties observed only in honeys derived from three bee plants. The composition of active ingredients in plants depends on different factors, mainly plant bio and chemo-type and environmental situations (Alvarez-Suarez et al., 2010b).

Today the occurrence of antibiotic-resistant pathogens poses a severe risk to public health, thus a high demand for alternative to antibiotics and conventional therapies are increasing more than ever (AL-Waili, et al., 2013). This generally shows the importance of screening of different floral origin honeys and identify the one with more antimicrobial potencies. The purpose of this study was to examine the physico-chemical and antimicrobial properties of some Ethiopian mono-floral honeys against selected drug resistant human pathogen bacteria and fungi isolates using broth microdilution assay. Some of the tested mono-floral honeys were from plants those have been reported for their medicinal properties.

## 2. Materials and methods

### 2.1. Honey samples and their classification

Honey samples were collected from farm gates targeting the harvesting periods of the specific mono-floral honeys from various

apiaries in the different regions of Ethiopia. In the study, six mono- and one poly floral honey samples were collected. For each honey type, six independent samples were collected. The samples were kept in sterile screw capped glass jars and stored in refrigerator below 5 °C until analysis.

### 2.2. *Melissopalynological analysis*

The botanical origins of the honey samples were authenticated based on the analysis of the honey pollen grains following the methodology of Louveaux et al. (1978). The identification of the pollen grains was made based on the pollen reference slides of honey plants of the target areas and also related publication (Atlas of pollen grains of major honey source plants) (Nuru, 2007). In addition to the pollen analysis, sensorial and physico-chemical properties were applied to differentiate the honey types. Moreover, the honey samples were collected from the farm gates considering the harvesting season and geographical regions of the targeted honey samples.

### 2.3. *Physico-chemical analysis*

The major compositions of honey such as: sugar profiles, total minerals (ash) content, acidity (total free acid) as meq.acid/kg of honey, pH and electric conductivity were determined following the International Honey Commission (2009) protocols. Every sample was tested in triplicate for every parameter and their average values were taken.

#### 2.3.1. *Sugar profile analysis*

The major honey sugars (fructose, glucose and sucrose) contents were investigated using HPLC following International Honey Commission, (2009) protocols. Standard sugars (fructose, glucose, sucrose) were obtained from Sigma chemicals (Germany). Before the analysis, the appropriateness of the methods, validation parameters (average recover values, limits of detection, quantification and the linearity response index) were confirmed.

#### 2.3.2. *Total free acid*

The total free acid in the honeys was determined based on the following protocols: 10 g of honey sample was dissolved in 75 ml of carbon dioxide-free water in a 250 ml beaker. The solution was stirred with the magnetic stirrer. The honey solution was titrated with 0.1 M NaOH to pH 8.30, using Hanna® pH measuring device. The result was expressed as free acidity, (Milliequivalents of acid/kg honey = ml of 0.1 M NaOH × 10).

#### 2.3.3. *pH determination*

The honey pH was determined by dissolving the samples in Milli-Q water (10% w/v) and measuring its pH using 3510 pH meter (Jenway® Instrument UK).

#### 2.3.4. *Electrical conductivity (EC)*

For EC test, a 20% (w/v) honey water solution was prepared using Milli-Q water. EC was determined by a conductivity meter (HI98311 Hanna Instruments, Mauritius). The result was presented as mS/cm.

#### 2.3.5. *Color*

The honey samples colour was determined using digital honey colorimeter (Portable Photometer, Hanna®, Instrument USA). Before the analysis the debris and air bubbles were removed. The results were expressed in mm P-fund (0–150 mm).

### 2.3.6. Total soluble substance (TSS)

The TSS as °Brix was determined through measuring, the total soluble substances in the honeys using a refractometer (Atago, No. 3840, Japan). During testing some honey drops were smeared on the prism of the refractometer and a reading was made directly from the scale. For the values above or below the standard temperature of 20 °C were corrected through adding or subtracting 0.00023/°C as correction factor. The results were expressed in °Brix as total soluble substance (%) = 100 – % moisture content).

### 2.3.7. Total antioxidant capacity (TAC) determination

Total antioxidant capacity of the honey samples was investigated chemically applying FRAP (Ferric Reducing Antioxidant Power) assay following Benzie and Strain (1996) and Alvarez-Suarez et al. (2010b) protocols.

### 2.3.8. Total phenolic content

The total phenolic contents of the honey samples were investigated based on Folin-Ciocalteu reactive, following the Singleton et al. (1999) and Alvarez-Suarez et al. (2010a,b) protocols. For each sample 5 g of liquid honey was dissolved in 50 ml of distilled water and the solution was filtered using Whatman No. 1 filter paper (0.5 ml). Then the honey solution was added to 2.5 ml of 0.2 N Folin-Ciocalteu reagent and mixed for 5 min then 2 ml 0.7 M Sodium carbonate ( $\text{Na}_2\text{CO}_3$ ) was added. The solution was incubated in dark at 25 °C for 2 h. The absorbance of the reaction mixture was determined at 760 nm using Agilent Cary 60 UV–Vis (© Agilent Technologies, Inc). The calibration curve was determined using standard Gallic acid following Özkök et al. (2010) protocol. Results were expressed as mg Gallic Acid Equivalents/Kg of honey (mgGAE/Kg of honey).

## 2.4. Antimicrobial assays

### 2.4.1. Pathogenic isolates

Cultures of various human pathogenic strains (isolated from human specimens) which are reported to be resistance to common antibiotics were obtained from Department of Microbiology, College of Pharmacy, King Saud University, Riyadh. The pathogen strains include: *Escherichia coli*, *Staphylococcus aureus*, *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Klebsiella pneumonia*, *Salmonella typhimurum*, *Micrococcus luteus*, *Staphylococcus epidermidis*, *Bacillus cereus*, *Aspergillus nidulans*, and *Enterobacter aerogene*. The isolates were identified by the standard bacteriological techniques.

### 2.4.2. Determination of minimum inhibitory concentration (MIC)

The antimicrobial activities and Minimum Inhibitory Concentration (MIC) of the honey samples were determined against some gram negative bacteria (*Escherichia coli*, *Salmonella typhimurum*, *Pseudomonas aeruginosa*, *Klebsiella pneumonia*, *Enterobacter aerogenes*), gram positive bacteria (*Bacillus subtilis*, *Staphylococcus aureus*, *Staphylococcus epidermis*, *Bacillus cereus*, *Micrococcus luteus*) and some common human fungal pathogens (*Candida albicans*, *Candida tropicalis* and *Aspergillus Nodules*). The antimicrobial activities and minimum inhibitory concentration were determined following Franch et al. (2005) & Alvarez-Suarez et al. (2010b) protocols with some modifications. A dilution series with honey concentrations that ranges from 5 to 50% v/v with an increment of 5% were added aseptically into sterile test tubes. The required honey concentration with nutrient broth solutions were mixed by stirring with small vortexing. Specimen of each microorganism was taken from pure culture grown in 10 ml nutrient broth. These specimens were cultured in broth containing different concentrations of individual honey by using a standard loop (10 µl). Control plates of nutrient without honey were included in each susceptibility assay to check the viability and density of the bacterial cells.

The cultures were incubated at 37 °C for 24 h. Then a loopful (10 µl) of the cultures of each of the specimens of microorganisms was streaked onto agar plates. The streaked plates were incubated aerobically at 37 °C and inspected after 24 h to measure MIC. Then the growth of the bacterial cells was assessed as partial or total inhibition and recorded for each strain. The minimum inhibitory concentration was considered to be the lowest honey concentration at which bacterial growth was totally inhibited. The testing was repeated three times for each strain at each concentration level. The average value for the minimum inhibitory concentration was taken from the three replicates for each strain for each concentration level.

## 3. Results and discussion

### 3.1. Pollen analysis

In general, all the six honey samples were classified as mono-floral honeys based on consisting of a greater proportion of pollen grains counts from the respective species (Table 1) and also supported with sensorial and physico-chemical properties and conformity in its harvesting season and geographical origins.

**Table 1**  
Melissopalynological analysis of the honey samples.

Floral origin	Quantitative melissopalynological result of the honey samples
<i>Schefflera abyssinica</i>	<i>S. abyssinica</i> honey samples were characterized by the low pollen representation, that range between 25 and 30% with the presence of other species pollen also. But the honey samples were authenticated based on sensorial and physico-chemical analysis and its distinctive harvesting season and regions.
<i>Eucalyptus globules</i>	<i>Eucalyptus globules</i> honey samples were characterized by relatively high pollen content accounting for $\geq 45\%$ of the typical pollen grains of the respective species.
<i>Guizotia scabra</i>	<i>G. scabra</i> honey samples were characterized by predominant $\geq 45\%$ content of the typical <i>G. scabra</i> pollen grains.
<i>Vernonia amygdalina</i>	<i>V. amygdalina</i> honey samples were characterized by low pollen representation, that ranges between 25 and 30% of the <i>V. amygdalina</i> pollen grains with the presence of other species pollen.
<i>Croton macrostachyus</i>	<i>C. macrostachyus</i> honey samples were characterized by low pollen representation between 25 and 30% with the presence of other species pollen, but the honey samples were authenticated based on sensorial and physicochemical analysis,
<i>Becium grandiflorum</i>	<i>B. grandiflorum</i> honey samples were characterized by under representation ( $\leq 25\%$ ) of its pollen, because of the flower morphology (long filament that keeps the dehiscence of anther faraway from its nectar location, which limits the direct contamination of the nectar by its pollen. The honey samples were further confirmed based on sensorial and physico-chemical analysis and its distinctive harvesting season and geographical origins.
Multi-floral honey	The multi-floral honeys were characterized by the presence of very diverse pollen grains from various cultivated oil crops pulses and annual weeds.

\*The mono-floral honeys considered in this study, besides their pollen analysis, distinctiveness in geographical location and harvesting seasons, it has been easily differentiated by experts familiar with the honeys, by assessing their peculiar tastes, aroma, color, structure of their crystals.

### 3.2. Physico-chemical analysis

The mean and standard deviations of the physico-chemical properties of the honey samples are shown in Table 2. The average major sugars: sucrose, glucose and fructose contents of the honey samples were  $3.7 \pm 0.9$ ,  $30.3 \pm 2.5$  &  $37.1 \pm 2.6$  respectively and the results are closer to the reports of Ouchemoukh et al. (2010) and Nuru et al. (2017). The mean of EC, acidity, mineral (ash) content and pH values of the honey samples were  $0.5 \pm 0.14$ ,  $27.0 \pm 3.07$ ,  $0.4 \pm 0.15$  &  $3.8 \pm 0.25$  respectively and the results are within the values reported by Saxena et al. (2010) and Feás et al. (2010).

The average colors of the honey samples were varying from  $6.1 \pm 1.3$  mm to  $115.5 \pm 7.2$  mm P-fund scale. Relatively, darker color with high P-fund value of  $115.5 \pm 7.2$  mm and  $93.3 \pm 5.3$  mm recorded for *Vernonia amygdalina* and *Croton macrostachys* honeys respectively while very light color with P-fund value of  $6.1 \pm 1.3$  mm and  $13.5 \pm 2.1$  mm recorded for *Becium grandiflorum* and *Schefflera abyssinica* honey samples respectively (Table 2). The significant variations in physico-chemical properties the different floral origin honeys (Table 2) could be due to the variations in their botanical and geographical origins of the samples. Generally, the mean values of the physico-chemical properties of the honey samples were within the acceptable ranges of honey quality standards set by Codex, (2001) and EU (2001).

### 3.3. Antioxidant

The average total antioxidant capacities of the honey samples as Ferric Reducing Antioxidant Power (FRAP) were varying  $225.4 \pm 12.8$ – $465.7 \pm 21.8$  FRAP ( $\mu\text{M Fe(II)}/100$  g with a mean of  $320.3 \pm 15.1$   $\mu\text{M Fe(II)}/100$  g (Table 2). Relatively lower FRAP values of  $225.4 \pm 12.8$  &  $237.1 \pm 11.7$   $\mu\text{M Fe(II)}/100$  g recorded for *Guizotia scabra* and multi-floral honeys while relatively higher FRAP values of  $421.5 \pm 23.4$  and  $465.7 \pm 21.8$   $\mu\text{M Fe(II)}/100$  g recorded for *C. macrostachys* and *V. amygdalina* honeys respectively. Generally, all the honey samples have possessed strong antioxidant properties and the values were closer or within the values reported by Alvarez-Suarez et al. (2010b) and Bundit et al. (2016) for Cuban and Thai honeys respectively. The antioxidant capacities of the *V. amygdalina* and *C. macrostachys* honeys were within the ranges of the antioxidant values reported for Manuka honey (Bundit et al., 2016). The differences in antioxidant properties of the honey samples could be due to the variations in phytochemicals of the respective plants and their geographical origins. The presence of variations in antioxidant properties of different honeys as a result of their floral sources, seasonal and environmental factors are well reported (Estevinho et al., 2008; Jantakee and Tragoolpua, 2015).

### 3.4. Phenolic contents

In general, the total phenolic contents of the honey samples obtained in this study were high and varied from  $233.3 \pm 24.0$  mg

GAE/kg to  $693.3 \pm 26.8$  mg GAE/kg (Fig. 1). Relatively higher phenolic contents of  $574.2 \pm 40.8$  mg GAE/kg and  $693.3 \pm 26.8$  mg GAE/kg were recorded for *C. macrostachys* and *V. amygdalina* honeys respectively. The phenolic content obtained in this study were closer or within the reported ranges of phenolics (325.9–1147.5 mg GAE/kg of honey) for Burkina Fasan's honeys (Meda et al. 2005); 213.9–595.8 mg GAE/kg of Cuban honeys (Alvarez-Suarez et al., 2010a,b) and 250–548 mg GAE/kg for some Brazilian honeys (Potis et al., 2014). The darker honeys from *V. amygdalina* and *C. macrostachys* have higher phenolic contents than lighter color honeys. It was also obtained strong correlation ( $r^2 = 0.925$ ,  $P < 0.0001$ ) between phenolic contents and the color values of the honey samples. Similarly, the presence of higher phenolic contents of darker color honeys than lighter honeys and their strong correlations are well documented Alvarez-Suarez et al. (2010a,b) for Cuban honeys and Isla et al. (2011) for Argentina honeys.

### 3.5. Microbial assay

Most of the studied honey samples showed strong antimicrobial properties in inhibiting the growth of tested human pathogens at relatively lower concentration which averagely range from  $10.5 \pm 0.5$ – $28.6 \pm 0.9$  of MIC (% v/v) (Table 3). In regarded to the gram negative bacteria, the average MIC (% v/v) of the studied honeys samples averagely varied from  $14.0 \pm 0.7$ – $25.5 \pm 1.1$  % v/v (Table 3).

However, specific honeys from *V. amygdalina*, *C. macrostachys*, *E. globules* and *B. grandiflorum* have showed relatively stronger antimicrobial properties in inhibiting the growth of the tested gram negative bacteria at an averagely of  $14.0 \pm 0.7$ ,  $16.3 \pm 0.68$ ,  $20.0 \pm 0.7$  and  $21.1 \pm 0.9$  MIC (% v/v) respectively. Among the gram negative bacteria, *Salmonella typhimurum* and *Klebsiella pneumonia* become more susceptible to *C. macrostachys* and *V. amygdalina* honeys at  $10.5 \pm 0.5$  and  $10.5 \pm 0.6$  MIC (% v/v) respectively.

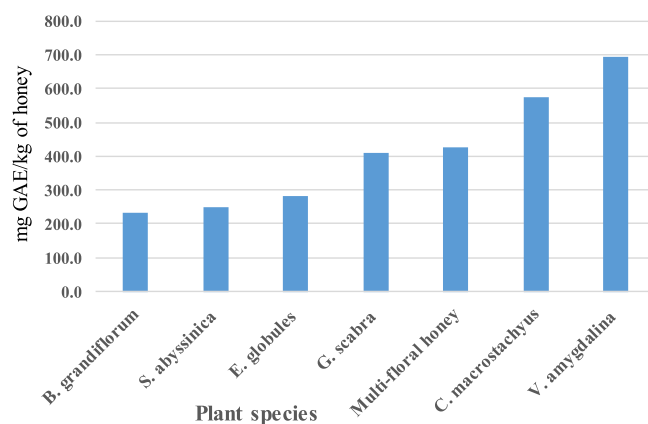


Fig. 1. The average phenolic contents (in mg GAE/kg) of different botanical origin honeys.

Table 2

The mean and standard deviation of physicochemical and antioxidant properties of the tested honey samples (n = 6).

Botanical sources of the samples	Sucrose %	Glucose %	Fructose %	TSS	EC (mS/cm)	Acidity meq/kg	ash %	PH	Color mm	FRAP ( $\mu\text{M Fe(II)}/100$ g of honey)
<i>Schefflera abyssinica</i>	$2.0 \pm 0.5a$	$29.5 \pm 2.2a$	$36.5 \pm 3.2b$	$79.5 \pm 3.3a$	$0.3 \pm 0.1a$	$19.9 \pm 2.3$	$0.2 \pm 0.1a$	$3.7 \pm 0.3b$	$13.5 \pm 2.1b$	$285.6 \pm 10.2b$
<i>Eucalyptus globules</i>	$3.1 \pm 1.1b$	$30.5 \pm 1.9b$	$35.2 \pm 3.5a$	$82.2 \pm 2.5b$	$0.4 \pm 0.1a$	$20.2 \pm 2.5$	$0.4 \pm 0.2b$	$3.4 \pm 0.2a$	$20.1 \pm 2.4c$	$310.9 \pm 17.5b$
<i>Guizotia scabra</i>	$4.5 \pm 0.8c$	$28.6 \pm 3.4a$	$37.0 \pm 2.6b$	$81.9 \pm 1.8b$	$0.6 \pm 0.2b$	$28.9 \pm 3.6$	$0.5 \pm 0.2b$	$4.2 \pm 0.3c$	$50.8 \pm 3.5d$	$225.4 \pm 12.8a$
<i>Vernonia amygdalina</i>	$4.4 \pm 0.5c$	$29.0 \pm 2.5a$	$39.5 \pm 1.1c$	$77.1 \pm 4.2a$	$0.8 \pm 0.2b$	$39.8 \pm 4.2$	$0.3 \pm 0.1a$	$4.1 \pm 0.1c$	$115.5 \pm 7.2f$	$465.7 \pm 21.8c$
<i>Croton macrostachys</i>	$2.3 \pm 1.5a$	$31.4 \pm 1.6b$	$38.8 \pm 2.4c$	$78.2 \pm 2.9a$	$0.5 \pm 0.1b$	$37.0 \pm 4.1$	$0.3 \pm 0.1a$	$3.5 \pm 0.2a$	$93.3 \pm 5.3e$	$421.5 \pm 23.4c$
<i>Becium grandiflorum</i>	$4.4 \pm 1.3c$	$33.6 \pm 2.9b$	$37.0 \pm 2.3b$	$84.0 \pm 3.1b$	$0.3 \pm 0.1a$	$12.4 \pm 1.3$	$0.4 \pm 0.2b$	$3.7 \pm 0.3b$	$6.1 \pm 1.3a$	$295.7 \pm 8.6b$
Multi-floral honey	$4.2 \pm 0.7c$	$29.4 \pm 3.1a$	$35.6 \pm 3.4a$	$81.7 \pm 2.4b$	$0.6 \pm 0.2b$	$31.0 \pm 3.5$	$0.5 \pm 0.2b$	$4.1 \pm 0.4c$	$52.6 \pm 4.0d$	$237.1 \pm 11.7a$
Mean	$3.7 \pm 0.9$	$30.3 \pm 2.5$	$37.1 \pm 2.6$	$80.7 \pm 2.9$	$0.5 \pm 0.14$	$27.0 \pm 3.1$	$0.4 \pm 0.15$	$3.8 \pm 0.25$	$50.3 \pm 3.7$	$320.3 \pm 15.1$
P	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.001	< 0.01	< 0.01	< 0.0001	< 0.0001

**Table 3**  
The antimicrobial property of different mono-floral honeys as Minimum Inhibitory Concentration (MIC) that necessary to inhibit 100% of the microbial growth in vitro expressed as mean and standard deviation of v/v % solution (n = 7).

Pathogenic strains	<i>Schefflera Abyssinica</i>	<i>Eucalyptus globulus</i>	<i>Guizotia scabra</i>	<i>Vernonia amygdalina</i>	<i>Croton macrostachyus</i>	<i>Becium grandiflorum</i>	Multi- floral
<b>Gram (–) bacteria</b>							
<i>Escherichia coli</i>	25.5 ± 0.5	20.5 ± 0.9	25.2 ± 0.8	15.5 ± 0.3	17.5 ± 0.8	21.5 ± 0.9	25.2 ± 0.3
<i>Salmonella typhimurum</i>	24.6 ± 0.5	18.5 ± 0.5	23.4 ± 0.5	15.2 ± 0.2	10.5 ± 0.5	22.5 ± 1.0	24.4 ± 0.5
<i>Pseudomonas aeruginosa</i>	24.5 ± 1.0	18.5 ± 0.6	26.2 ± 0.6	14.5 ± 1.0	18.5 ± 0.5	20.5 ± 1.0	23.5 ± 1.0
<i>Klebsiella pneumonia</i>	24.5 ± 1.0	20.5 ± 1.0	24.5 ± 0.7	10.5 ± 0.6	17.5 ± 0.6	20.5 ± 1.0	25.5 ± 1.1
<i>Enterobacter aerogenes</i>	23.5 ± 0.6	22.2 ± 0.6	23.2 ± 1.3	14.5 ± 1.3	17.5 ± 1.0	20.5 ± 0.8	24.4 ± 1.1
Group mean	24.5 ± 0.72	20.0 ± 0.7	24.5 ± 0.8	14.0 ± 0.7	16.3 ± 0.68	21.1 ± 0.9	24.6 ± 0.8
<b>Gram (+) bacteria</b>							
<i>Bacillus subtilis</i>	26.5 ± 1.3	20.5 ± 1.0	27.5 ± 1.0	16.5 ± 0.5	18.5 ± 0.9	24.5 ± 0.9	25.5 ± 0.7
<i>Staphylococcus aureus</i>	25.5 ± 0.9	22.5 ± 0.7	25.5 ± 1.0	15.5 ± 1.0	10.5 ± 0.9	23.5 ± 0.6	26.4 ± 0.4
<i>Staphylococcus epidermis</i>	25.5 ± 0.7	20.5 ± 1.1	24.5 ± 0.9	10.5 ± 0.5	18.5 ± 0.5	20.5 ± 1.2	25.6 ± 0.9
<i>Bacillus cereus</i>	27.5 ± 0.7	24.5 ± 1.0	26.5 ± 0.3	16.5 ± 0.6	17.5 ± 1.0	22.5 ± 0.6	26.5 ± 0.7
<i>Micrococcus luteus</i>	25.5 ± 1.0	24.5 ± 0.7	25.5 ± 1.0	15.5 ± 1.0	18.5 ± 1.0	21.5 ± 0.5	24.4 ± 1.2
Group mean	26.1 ± 0.92	22.5 ± 0.9	25.9 ± 0.84	14.9 ± 0.72	16.7 ± 0.86	22.5 ± 0.76	25.7 ± 0.78
<b>Fungal pathogens</b>							
<i>Candida albicans</i>	28.6 ± 0.9	25.5 ± 0.1	26.5 ± 1.1	18.5 ± 0.9	20.0 ± 0.4	25.5 ± 0.5	26.5 ± 1.0
<i>Candida tropicalis</i>	27.5 ± 1.0	24.0 ± 0.6	25.5 ± 0.2	16.5 ± 0.1	20.5 ± 1.0	26.5 ± 0.3	27.5 ± 0.1
<i>Aspergillus Nodules</i>	27.5 ± 0.3	22.5 ± 0.1	26.5 ± 0.1	15.5 ± 0.1	20.0 ± 0.5	23.5 ± 0.7	26.5 ± 1.1
Group mean	27.9 ± 0.73	24.0 ± 0.27	26.2 ± 0.47	16.8 ± 0.37	20.2 ± 0.63	25.2 ± 0.5	26.8 ± 0.73

Moreover, the same *V. amygdalina*, *C. macrostachyus*, *Eucalyptus globules* and *B. grandiflorum* honeys have showed relatively strong antimicrobial properties in inhibiting the growth of the tested gram positive bacteria at an average of 14.9 ± 0.72, 16.7 ± 0.86, 22.5 ± 0.9 & 22.5 ± 0.76 MIC (% v/v) respectively. Among the gram positive bacteria, *Staphylococcus aureus* and *Staphylococcus epidermis* become more susceptible to *C. macrostachyus* and *V. amygdalina* honeys at 10.5 ± 0.9 and 10.5 ± 0.5 MIC (% v/v) respectively. The MIC results of the current study were closer to the findings of Mullai and Menon (2007) who reported MIC of 11–20% v/v for isolates of *Pseudomonas aeruginosa*. Moreover, most of the current study results were within the MIC range of 10–25% v/v reported for the antimicrobial properties of Argentina honeys against different gram positive and gram negative bacteria [(Isla et al., 2011)]. However, the MIC of the current study results were relatively higher from the findings of Alvarez-Suarez et al. (2010b) who reported MIC 2.5–10.4%v/v for gram positive bacteria and 7.2–16% v/v for gram negative bacteria for Cuban mono-floral honeys. Moreover, the MIC values of the current results were higher than the report of Andargachew et al. (2004) who recorded MIC (6.25% and 7.5% v/v) activities of honeys against some human pathogens: *P. aeruginosa*, *E. coli* and *S. aureus* respectively. Moreover, Sherlock et al. (2010) reported MIC of 6.3% and 12.5% v/v for Ulmo (Chile) and Manuka honeys respectively. The variations among results could be due to the qualities, freshness and the botanical and geographical sources of the honeys. The existences of large variation in the antimicrobial properties of natural honeys due to their spatial and temporal variations in sources of nectar is well documented (Saxena et al., 2010; Alvarez-Suarez et al., 2010a,b; Mandal & Mandal, 2011; Isla et al., 2011). On other hand relatively higher MIC of 32 ± 8.3% to 58 ± 13% v/v of honey was recorded against drug resistant bacteria and pathogenic fungi (AL-Waili et al., 2013).

Regarding the antifungal pathogens, *V. amygdalina*, *C. macrostachyus* and *E. globules* honeys showed relatively stronger potency in inhibiting the growth of the tested human fungal pathogens at an average MIC (v/v%) of 16.8 ± 0.37, 20.2 ± 0.63 and 24.0 ± 0.27 respectively. The study indicated that fungi were less susceptible than bacteria. The less susceptibility of fungi to different honey samples than bacteria were also reported (AL-Waili et al., 2013).

Generally, honeys from *V. amygdalina*, *C. macrostachyus*, *E. globules* and *B. grandiflorum* showed relatively stronger antimicrobial

potencies compared to others. These could be due to the medicinal properties of the plants and the presence of some floral specific bioactive substances in their honeys. In this regard, besides the widely utilization of the different parts of the above plants in traditional medicines, the strong antimicrobial properties of the different parts of the plants have been well documented. For instance: the medicinal potentials of *C. macrostachyus* leaves, barks, stem and fruits for treatment of wider range of human and animal diseases and ailments (Maroyi, 2017), the strong antimicrobial properties of *C. macrostachyus* extracts against wide range of human pathogens (2010; Mesfin et al. 2010); the antimicrobial and the various medicinal properties of *V. amygdalina* leaves extracts have been well documented (Ogbulie et al., 2007; Ijeh, & Ejike, 2011).

Moreover, the antimicrobial properties of different eucalyptus oils against different pathogenic micro-organisms (Gilles et al., 2010) and the significantly more effectiveness of *Eucalyptus marginata* honey against *Candida* spp are reported (Irish et al., 2006). Moreover, the wound healing and antimicrobial properties of leaf extract of *B. grandiflorum* are well documented (Khalid, 2017). These generally may indicate that honey obtained from medicinal plants may have more medicinal values.

The presence of significant variations in medicinal properties among the different botanical origin honeys are well established (Lusby et al., 2005; Molan, 1992; Saxena et al., 2010). In this regard the variations in antimicrobial potencies among the different honeys reported to be more than 100-folds, which is attributed to their botanical and geographical origins (Molan and Cooper, 2000). The difference in levels of antimicrobial activities and the functional properties in human health promotion among the different honeys have been associated to their floral source, geographical location, climatic conditions and the plant bio and chemo-types (Alvarez-Suarez et al., 2010a, 2010b; Mavric et al., 2008; Molan, 1992a, 1992b). Moreover, the more antimicrobial properties of some of the honeys could be due to their higher phenolic content and antioxidant properties. The strong relations of antimicrobial properties of honeys with their antioxidant properties and phenolic contents well discussed (Isla et al., 2011). Particularly phenolic compounds are believed to defend against diseases like cancer, cardiovascular disorders and neurodegenerative diseases by cleaning up potentially damaging free radicals occurring in our body (Özkök et al., 2010).

In the very past and present days' folk medicines, selection of specific plant origin honeys for specific therapeutic purposes are widely used (Molan, 1992), indicating that all honeys may not have equal therapeutic values. In this regard, Irish, et al. (2011) reported that exceptionally strong antimicrobial properties observed only in honeys derived from three out of 477 bee plants.

#### 4. Conclusion

Based on the current research findings and the available literature supports, honeys derived from plants those have medicinal properties may have more medicinal values. Hence, more research would be important to focus on honeys from medicinal plants and to establish the possible relations between the bioactive substances in plant parts and their nectars.

#### Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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