Article

Quality Evaluation of Saudi (*Moringa Alfileria*) Honey: Anti-microbial, Antioxidant Activities, Physiochemical Properties and Melissopalynological Analyses

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Featured Application: This study was conducted with *Moringa alfileria* **honey through the anti-microbial, antioxidant activities, physicochemical, melissopalynological analysis,total phenolic and flavonoid contents. For this purpose** *Moringa alfileria* **honey samples** were **collected from four localities in Saudi Arabia were analyzed based on results of melissopalynological analyses.**

Abstract:

Objective: The aim of the study was to characterize varieties of ***Moringa alfileria*** honey (unfloral and polyfloral) from Saudi Arabia based on antibacterial, antioxidant activities, physicochemical, melissopalynological analysis, total phenolic and flavonoid contents.

Material and Methods: The fresh 376 honey samples (3 kg of each) were kindly provided by Alnahal aljwal Company, 2021 flowering season. The honey samples collected in sterile universal glass containers and kept at 2– 8°C until tested. Antibacterial, antioxidant activities and physiochemical analysis were done. Determination of sediment content, total grains, moisture content, water-soluble solids, acidity, electrical conductivity, total sugars content, inverted sugars, glucose (g/100 g), fructose (g/100 g), total glucose + fructose, fructose/ glucose ratio, sucrose (g/100 g), diastase enzyme activity and HMF were calculated. As well as total phenolic and flavonoid contents **Results:** Antibacterial activity and physiochemical analysis of honey samples w varied. All parameters studied were significantly different ($P < 0.05$) among all honey varieties. The results of the physiochemical analysis were compared with Saudi National Standard, Codex standard, as well as published data in the literature. **Conclusion:** It was obvious that the honey quality was varied based on the botanical origins

Keywords: Antioxidant activity; Antibacterial activity; Melissopalynological and Physiochemical analysis; ***Moringa alfileria*** honey

1. Introduction

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All honey types vary in their colors from nearly colorless to dark brown, the type of nectar gathered by the bees from various floral sources is influenced directly and its flavor varies from delectably mild to distinctively bold the flavor. Each honey is unique on the basis of a combination of the various chemical components that attributes to its quality (1). In apiculture, the control and characterization of quality are of great importance and interest. *Moringa alfileria* honey is one of the potential local materials that are rich in micronutrients and widely available but not fully utilized by the community. it is well known to have potent antimicrobial properties (2)and antioxidant activity that can prevent oxidative stress, DNA damage, and repair hematologic status. (3). Drugs misused to control bacterial infection led to the rapid emergence of antibiotic resistance and encourage scientists to use a novel natural product such as honey (4)

Natural foods that are widely used today are known to be *Moringa oleifera* and honey which improve the nutritional status of the community, they contain various nutrients and active ingredients that can overcome the problem of malnutrition (5). The Moringa tree has been widely used in various parts of the world and is currently known as the miracle tree (6).

Moringa oleifera has large polyphenols and flavonoids that have biological activities (7). Their effects are involved in stimulating pancreatic Beta cells and subsequently increasing insulin secretion (8).

An indicator of the quality and origin of honey is its physicochemical properties. The characteristics of honey depend on many factors such as the flowers nectar, regional, beekeeping practices, and environmental climatic variations. Moisture content (9) , sucrose content and reducing sugars content (10), pH value, electrical conductivity, ash content, free acidity (9), diastase activity (10) and hydroxymethyl furfural (HMF) content (11) are the major quality criteria of honey (12).

There are no studies that have tried to compare variations of active ingredients in *Moringa alfileria* honey from 4 regions in Saudi Arabia. This investigation was conducted to assess differences in antimicrobial, antioxidant activities, physiochemical properties, and melissopalynological analyses of four *Moringa alfileria* honey from different geographic areas in Saudi Arabia.

2. Materials and Methods

Materials

All reagents and chemicals used in this investigation were of analytical grade and purchased from Sigma (St. Louis, MO, USA).

Honey samples

A total of 376 fresh Moringa alfileria honey samples (3 kg each) were collected (95 each) from four (Al Taef, Al Kaseem, Al Madinah and Al Alla) localities in Saudi Arabia during 2021. Honey samples were kept at 2-8°C in sterile universal glass container until tested.

Bacterial strains:

In this investigation six antibiotic-resistant bacterial strains (Gram-positive and Gram-negative) were used to determine the antibacterial activities of honey against *Staphylococcus aureus* (ATCC 25923), *Streptococcus pyogenes* (ATCC 19615), *Proteus vulgaris* (ATCC 13315), *Pseudomonas aeruginosa* (ATCC 27853) *Klebsiella pneumoniae* (ATCC 27736) and *Escherichia coli* (ATCC 35218). These bacterial strain were kindly provided and maintained in the Department of Zoonotic Diseases, National Research Centre, Egypt. VITEK 2 compact system (bioMérieux, Inc.) was performed for identification and susceptibility patterns of all clinical isolates (13). Each bacterial strains were subculture on Muller Hinton broth (Sigma Aldrich company). The suspension was standardized to provide 0.5 McFarland = 1 − 2xCFU/mL using calibrated VITEK 2 DENSICHEK (14)

Disc diffusion method:

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The bacterial growth inhibitions were measured by using the disc diffusion method after mixing with honey. Disc diffusion method was performed by using prepare discs approximately 6 mm in diameter Whatman filter paper no. 1. Then were sterilized in a hot air oven according to the Clinical and Laboratory Standards Institute (CLSI) guideline(15) and spotted with 0.2 mg ***Moringa alfileria*** honey samples then placed on a Mueller Hinton agar (MHA) plate. The plates were incubated at 37°C for 24 h and the diameter of the inhibition zones of growth produced by each spot was measured in millimeters(16). The mean values of inhibition were calculated from triple reading in each tested bacterial strains to evaluate the antibacterial activity of different honey according to (17).

Minimum Inhibitory Concentration (MIC):

Moringa alfileria honey was serially diluted Mueller Hinton broth (100 μL containing the bacteria 0.5 McFarland) by transferring 100 μL to the next well to produce final concentrations of 0.32, 0.75, 1.5, 3, 6, 12, 25, and 50 mg/mLin microdilution plates (18). The microdilution plates were incubated at 37°C overnight. The MIC was determined by selecting the lowest concentration of ***Moringa alfileria*** honey that completely inhibited the growth of the organism and compared with the growth control (19). Wells with no visible growth in MIC were subculture using 10 μL of the selected wells and placed on Muller Hinton agar plates. The MBC was determined by taking 10 μL of the selected column and placing it on the Mueller Hinton agar plates as well. All plates were incubated for 24 h at 37°C and the colony forming units (CFUs) were counted. MIC was determined by selecting the lowest concentration of *Tamarix gallica* honey that completely inhibited the visible growth of a microorganism after overnight incubation in the well (20).

Total Phenolic Content (TPC):

Total phenolic compounds from of *Moringa alfileria* honey were detected by a modified spectrophotometric Folin-Ciocalteu method (21). The total flavonoid concentration of each honey sample was determined according to the colorimetric assay developed by (22) . UV-Visible spectrophotometer (Perkin-Elmer Lambda 25, Waltham, MA, USA) used tomeasure the absorbance at 765 nm after incubation using. Gallic acid (0–1000 mg/L) was used as a standard for the calibration curve preparation. The mean value of triplicate assays of TPC was reported and expressed as milligrams of gallic acid equivalent (GAE) per gram of honey (23).

Total Flavonoid Content (TFC):

For the determination of total flavonoid content (TFC) using a volume of 5 ml diluted honey with 0.1 g/ml concentration. This solution was mixed with 5 ml of 2% aluminum chloride (AlCl3). The mixture was then incubated for 10 min at 25ºC. Using a UV-Visible spectrophotometer at 415 nm was measured the absorbance of the formed complex. The standard chemical for the calibration curve preparation was rutin with a concentration 0–100 mg/L. The mean value of triplicate assays of TFC was reported and expressed as milligram of rutin equivalent (RE) per gram of honey (24)((25).

Antioxidant capacity (DPPH) Scavenging Activity:

The DPPH scavenging activity of the different honey samples was determinethe antioxidant activity. This test is based on the change in the absorbance by reducing the purple DPPH radical using an oxidizing antioxidant. The scavenging effect of vitamin C, caffeic acid and honey samples were corresponded to the quenching intensity of 1,1-diphenyl-2-picrylhydrazyl (DPPH) as carried out. The degree of absorbance by reducing the purple DPPH radical by an oxidizing antioxidant was measured at 520 nm (26).

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Melissopalynological and physicochemical analysis:

Melissopalynological and physicochemical analyses were determined (27). The pollen content was identified by the sedimentation technique(28,29). Water content (30), water-soluble solids (31), pH, acidity, and electrical conductivity (32). According to Official Methods sugars content, glucose, fructose, fructose/ glucose ratio, fructose plus glucose %, and sucrose were performed by HPLC-DAD (33) were determined. hydroxymethylfurfural (HMF) (34)and diastase enzyme activity (35) were analyzed.

Minerals

Minerals in *Moringa alfileria* honey were detected and identifed of twelve minerals; four elements (Ca: calcium; K: potassium; Mg: magnesium; Na: sodium) and eight of heavy metals (Cd: cadmium; Cr: chromium; Cu: copper ; Fe: iron; Mn: manganese; Ni: nickel ; Pb: lead; Zn: zinc). An atomic absorption spectrophotometer (Model 3300, MS-DOS, detection limit is 3 s, lg/L, PerkinElmer Inc., USA) was used according to the method described by(36).

Statistical Analysis:

The statistical analysis was subjected on triplicate results were applied SPSS Ver. 21 (IBM, New York, US) software. One-way ANOVA applied to compare between and within the tested groups. All data and the P value less than 0.05 was taken as significant to the mean ± standard error (SE).

3. Results

A total of 376 *Moringa oleifera* honey samples were collected from four regions in Saudi Arabia. The antibacterial activities of *Moringa oleifera* honey were evaluated according to the zone of inhibition. The antibacterial potency of honey four regions was determined against various pathogenic bacteria (Gram-negative and Gram-positive). all honey types tested showed growth suppression of pathogenic bacterial strains (Table 1). Al Madeniah *Moringa oleifera* honey showed the highest zones of inhibition against all tested pathogen. The inhibition was 43.22 ± 0.18 mm (*Staphylococcus aureus*) 38..11± 0.15 mm *(Streptococcus pyogenes)*, 35.15 ± 0.26 mm (*Klebsiella pneumoniae)*, 35.10 ± 0.10 mm (*Proteus vulgaris*) and 39.01± 0.25 mm (*Pseudomonas aeruginosa*) respectively. While *Moringa oleifera* honey showed the zones of inhibition ranged from 23.10 ± 0.38 mm to 43.22 ± 0.18 mm against *Staphylococcus aureus, while* 26.43 ± 0.18 - 38..11 ± 0.15 mm against *Streptococcus pyogenes* , 25.05 ± 0.46 - 35.15 ± 0.26 mm against *Klebsiella pneumoniae*, 13.39 ± 0.33 - 23.19 ± 0.13 mm against *Escherichia coli,* 21.01± 0.14 - 35.10 ± 0.10 mm against *Proteus vulgaris* and 23.16 ± 0.53- 39.01 ± 0.25 mm against *Pseudomonas aeruginosa* respectively. The antibiotic Tetracyclin, Oxacillin and Clindamycin showed varible inbition (Figure 1).

The MIC test was performed of the different examined *Moringa alfileria* honey against the pathogenic bacteria strains as shown in Table 2. The results showed significant differences in MIC values against tested antibiotic-resistant strains. The strongest antibacterial potential against all tested bacteria tested toward *Moringa alfileria* honey against *Staphylococcus aureus*, *Streptococcus pyogenes* , *Escherichia coli*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* (MIC=0.4 g/ml). *Escherichia coli,* showed the lowest antibacterial potential against all bacteria. The therapeutic antibiotics Tetracyclin, Oxacillin and Clindamycin showed highest MIC activity against all tested bacteria (Table 2).

The highest level of total phenolic, flavonoid and DPPH of *Moringa alfileria* honey was observed in honey in Table 3 and Figure 2. The highest level of total phenolic of *Moringa alfileria* honey was observed in Al Madeniah honey 245.29 ± 16.32 (mg GAE/100g honey), with the lowest level observed in Al Taef honey 179.89 ± 12.66 (mg GAE/100g honey). The highest level of total flavonoid 183.1 ± 19.33 mg RE/100g honey) was detected in Al Taef honey, where the lowest level was in Al allia 163.86± 15.57 mg RE/100g honey. Antioxidant activitiesin of the *Moringa alfileria* honey were evaluated with the 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay. The highest DPPH value was detected in Al Madeniah *Moringa alfileria* honey with value of

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237.80 ±15.51 mg AAE/100g honey and the lowest value was observed in Al allia honey (77.07 ± 09.51 mg AAE/100g honey (Table 3 and Figure 2).

Table 1. The inhibition zone of *Moringa alfileria* honey against various pathogenic microorganisms by well diffusion method. Each test was run in triplicate.

Antibacterial activity	Gram-positive		Gram-negative			
Honey origin	*Staphylococcus aureus*	*Streptococcus pyogenes*	*Klebsiella pneumoniae*	*Escherichia coli*	*Proteus vulgaris*	*Pseudomonas aeruginosa*
Al Taef	33.23 ± 0.88	28..86 ± 0.18	25.05 ± 0.46	13.39 ± 0.33	25.10 ± 0.20	29.01 ±1.15
Al Kaseeem	29.01 ± 0.85	26.43 ± 0.18	21.06 ± 0.42	18.12 ±0.08	22.01 ± 0.11	23.16 ± 0.53
Al Madeniah	43.22 ± 0.18	38..11 ± 0.15	35.15 ± 0.26	23.19 ± 0.13	35.10 ± 0.10	39.01 ± 0.25
Al allia	23.10 ± 0.38	29.33 ± 0.14	24.02 ± 0.54	19.16 ± 0.16	21.01 ± 0.14	25.05 ± 0.08
Tetracyclin	41.02 ± 0.24	39.11 ± 0.33	36.15 ± 0.18	28.15 ±0.22	46.24 ± 0.42	42.25 ± 0.36
Oxacillin	46. 21 ± 0.13	36.12 ± 0.16	50.61 ± 0.13	36.19 ±0.13	35.42 ± 0.59	37.33 ± 0.36
Clindamycin	52. 05 ± 0.12	35.01 ± 0.28	41.66 ± 0.26	31.16 ±0.31	42.24 ± 0.38	40.67 ± 0.39

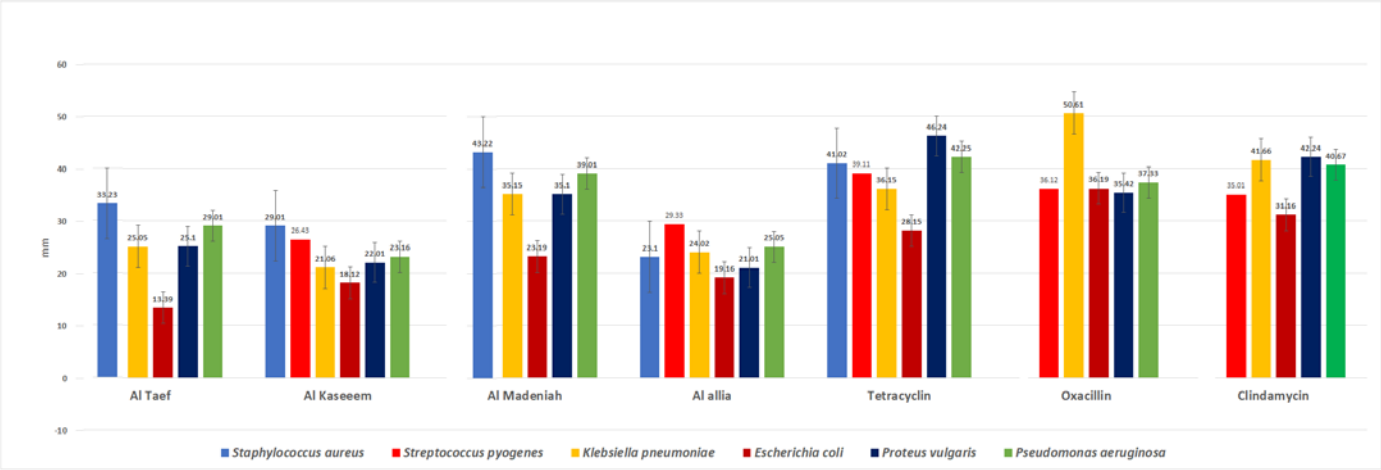

Figure 1. The inhibition zone of *Moringa alfileria* honey honey against various pathogenic microorganisms by well diffusion method.

Table 2. Minimal inhibitory concentration (MIC) of *Moringa alfileria* honey against the antibiotic resistant bacterial strains.

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MIC	Minimal inhibitory concentration (MIC) of bacterial strain (g/ml)*					
	Gram-positive		Gram-negative			
Honey origin	*Staphylococcus aureus*	*Streptococcus pyogenes*	*Klebsiella pneumoniae*	*Escherichia coli*	*Proteus vulgaris*	*Pseudomonas aeruginosa*
Al Taef	0.4	0.4	0.2	0.1	0.2	0.4
Al Kaseeem	0.4	0.4	0.4	0.2	0.4	0.1
Al Madeniah	0.4	0.4	0.4	0.4	0.1	0.4
Al allia	0.4	0.1	0.4	0.1	0.1	0.4
	Drugs for positive control for growth inhibition					
Tetracyclin	0.000004	0.0000016	0.0000064	0.0000016	0.000064	0.0000016
Oxacillin	0.00000016	0.00000032	0.000000128	0.00000016	0.0000016	0.00000016
Clindamycin	0.00000016	0.00000128	0.000000064	0.0000008	0.0000016	0.00000016

*MIC, concentration required for 99% bacteriostatic effect

Table 3: The content of total phenolic, flavonoids and the antioxidant capacity of the ***Moringa alfileria*** honey from the re four rgions of Saudi Arbia.

Honey	Samples (n)	Total phenolic* X SD	Total flavonoid** X SD	Antioxidant capacity (DPPH)*** X SD
Al Taef	94	179.89 ± 12.66	183.1 ± 19.33	153.30 ± 12.18
Al Kaseeem	94	194.11 ± 18.30	173.6 ± 14.13	161.00 ± 18.82
Al Madeniah	94	245.29 ± 16.32	201.0 ± 12.47	237.80 ±15.51
Al allia	94	187.29 ± 13.32	163.86± 15.57	77.07 ± 09.51

All results are expressed as means of triplicate standard deviation (SD). *mg GAE/100g honey ** mg RE/100g honey ***mg AAE/100g honey)

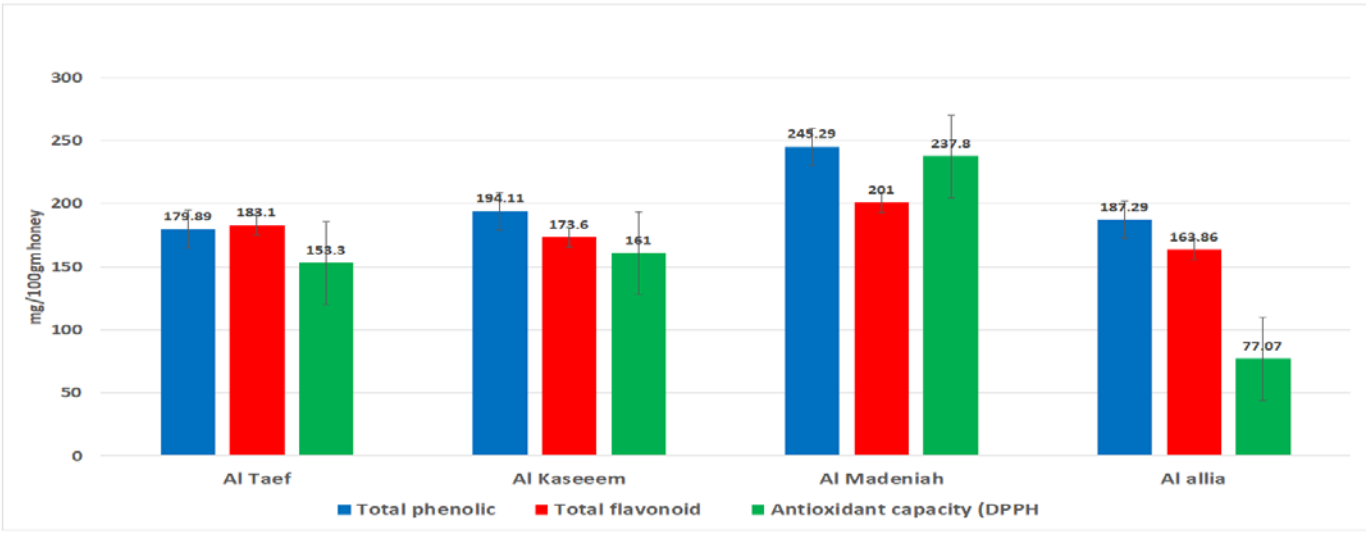

Figure 2: The content of total phenolic, flavonoids and the antioxidant capacity of the *Moringa alfileria* honey from the re four rgions of Saudi Arbia.

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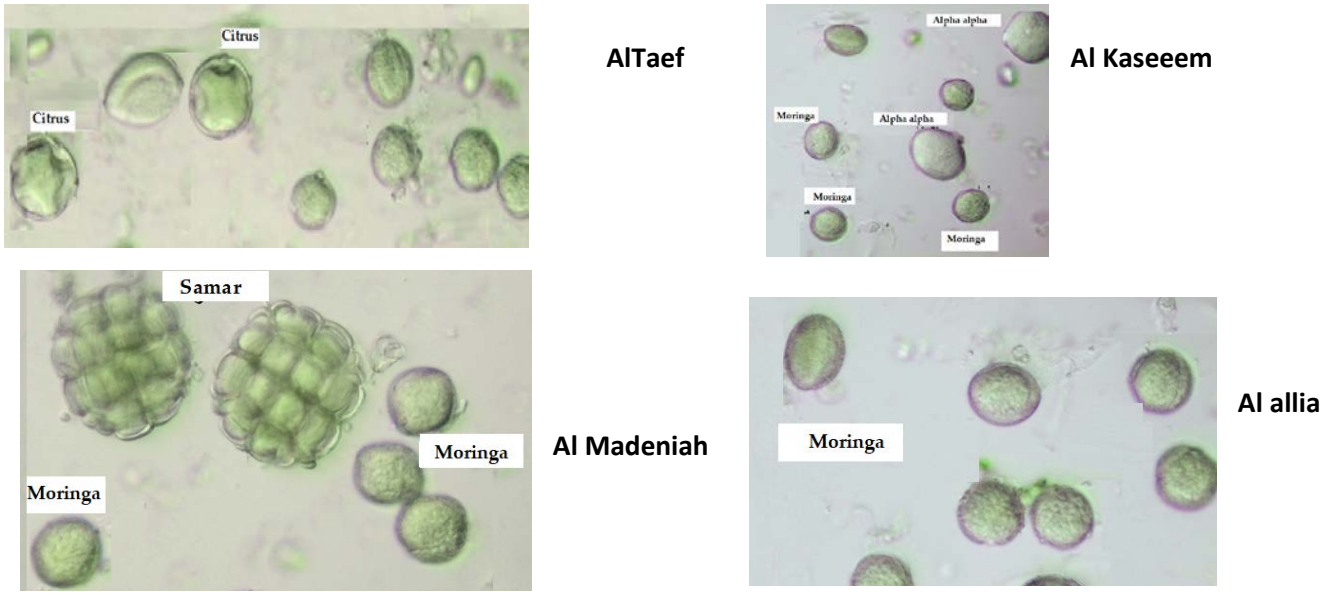

Figure 3: Images pollen grains from microscopic preparations of ***Moringa alfileria*** honey from the re four rgions of Saudi Arbia.

Melissopalynological analysis of *Moringa alfileria* honey from four origins indicated that not only the expected pollen type based on the specific source of nectar but also pollen from some other sources were present depending of the origin (Fig.3).

The results of the physicochemical properties as shown in Table 4 and Fig. 4 revealed that *Moringa alfileria* honey samples were comparable in water content which ranged from 14.11±0.25 % (Al Kaseeem) to 16.35±0.12% (Al allia). The pH ranged from 4.18±0.13 (Al allia) to 4.24±0.22 (Al Kaseeem). The acidity also varied from 10±0.11 (Al Taef) to 31.00±0.13 meq/l (Al Madeniah). Electrical conductivity was 0.26±0.004 (Al Taef) to 0.69±0.002mS/cm (Al Madeniah). The insoluble solids ranged from 0.055±0.004 (Al allia) to 0.079±0.007% (Al Madeniah).

The results of glucose, fructose, sucrose, diastase activity and levels in different *Moringa alfileria* honey samples were illustrated in Table 4. Glucose was detected at the level 26.52±0.14% in Al Taef honey, where the Al Kaseeem honey (23.81±0.12%), Al Madeniah honey (27.43±0.16%), and Al allia honey (32.97±0.18%) were observed.

The fructose level ranged between 34.54±0.23% (Al allia honey) and 39.33±0.48% (Al Kaseeem). Sucrose levels ranged from 1.00±0.30 % (Al Taef honey) to 3.60±0.22 % (Al Kaseeem honey). Diastase activity had a range of 10.25±0.14 D.U. (Al Kaseeem honey) to 20.70±0.13 D.U. (Al Madeniah honey). The lowest HMF (mg/kg) was observed in Al Taef and Al Taef honey (0.00±0.00) where the highest level was detected in Al Kaseeem honey (7.50±0.10).

Identification and determination of twelve minerals; four elements (Ca: calcium; K: potassium; Mg: magnesium; Na: sodium) and eight heavy metals (Cd: cadmium; Cr: chromium; Cu: copper; Fe: iron; Mn: manganese; Ni: nickel; Pb: lead; Zn: zinc) in *Moringa alfileria* honey samples were analyzed (Table 5, Figure 5 and 6).

Table 5 Figures 4 and 5 illustrated the 12 minerals identified in the examined Moringa alfileria honey samples. The highest potassium (K) content (85.70 ± 0.0002ppm) was found in Madenia Moringa alfileria, and the lowest (74.36 ±0.0002 ppm) was found in Al Kaseeem. The most prevalent mineral was Mg, with significantly different ($P < 0.05$) values ranging between 54.28 ± 0.0001 ppm in (Madenia) and 35.17 ± 0.0002 ppm in Al allia. The Calcium (Ca) was ranged from 32.52 ± 0.002 ppm (Madenia) to 82.36 ±0.002 ppm (Al allia) where Sodium (Na) was 7.59 ± 0.0001ppm (Al allia) to 13.02 ± 0.0001ppm (Madenia). The Iron (Fe) content was ranging from 7.218 ± 0.0002 ppm in (Al allia) to 8.45 ± 0.0002 ppm in (Madenia). Copper (Cu), values range between 4.505 ±0.001ppm (Al allia) and 6.98 ±0.001ppm

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Table 4:Physicochemical parameters of *Moringa alfileria* honey from the re four rgions of Saudi Arbia.

Physicochemical parameters	Honey origin			
	Al Taef	Al Kaseeem	Al Madeniah	Al allia
Moisture	15.04±0.18	14.11±0.25	14.8±0.13	16.35±0.12
Fructose	35.85±0.22	39.33±0.48	35.31±0.43	34.54±0.23
Glucose	26.52±0.14	23.81±0.12	27.43±0.16	32.97±0.18
Fructose/ glucose ratio	1.14	1.11		1.11
Fructose + Glucose	62.37±0.85	57.92±2.16	62.74±0.12	67.51±3.11
Sucrose	1.00±0.30	3.60±0.22	2.50±0.14	1.4.11±0.32
HMF	0.00±0.00	0.00±0.00	7.50±0.10	2.8±0.15
Acidity	10±0.11	14.4±0.18	31.00±0.13	17±0.16
Diastase	16±0.18	10.25±0.14	10.70±0.13	11.80±0.27
conductivity	0.26±0.004	0.64±0.003	0.69±0.002	0.42±0.001
Water Insoluble Solids Content	0.066±0.006	0.075±0.003	0.075±0.007	0.055±0.004
PH	4.19±0.17	4.24±0.22	4.22±0.27	4.18±0.13

Table 5: Minerial levels of *Moringa alfileria* honey from the re four rgions of Saudi Arbia.

Mineral content	Honey origin			
	Al allia	Al Madeniah	Al Kaseeem	Al Taef
Potassium (K)	81.33 ± 0.0002	85.70 ± 0.0002	74.36 ±0.0002	78.07± 0.0002
Magnesium (Mg)	35.17 ± 0.0002	54.28 ± 0.0001	39.84 ± 0.0001	38.09 ± 0.0001
Calcium (Ca)	32.52 ± 0.002	82.36 ±0.002	65.08 ± 0.002	64.97 ± 0.002
Sodium (Na)	12.51 ± 0.0001	13.02 ± 0.0001	8.20 ± 0.0001	7.59 ± 0.0001
Iron (Fe)	7.218 ± 0.0002	8.45 ± 0.0002	6.23 ± 0.0002	7.28 ± 0.0002
Copper (Cu)	4.505 ±0.001	6.98 ±0.001	5.03 ±0.001	5.05 ± 0.001
Zinc (Zn)	3.881 ± 0.0001	4.55 ± 0.0001	4.35 ± 0.0001	3.13 0.0001
Manganese (Mn)	1.345 ± 0.003	4.99 ± 0.0001	1.12 ± 0.003	2.90 ± 0.003
Nickel (Ni)	0.199 ± 0.001	0.24 ± 0.001	0.21± 0.001	0.20 ±0.002
Chromium (Cr)	0.10 ±0.001	0.18 ± 0.001	0.11 ± 0.001	0.11 ± 0.001
Cadmium (Cd)	0.099 ± 0.001	0.13 ±0.001	0.12 ±0.001	0.06 ±0.001
Lead (Pb)	0.163 ± 0.010	0.131 ± 0.010	0.149 ±0.011	0.118 ± 0.010

All results are expressed as means of triplicate standard deviation (SD).

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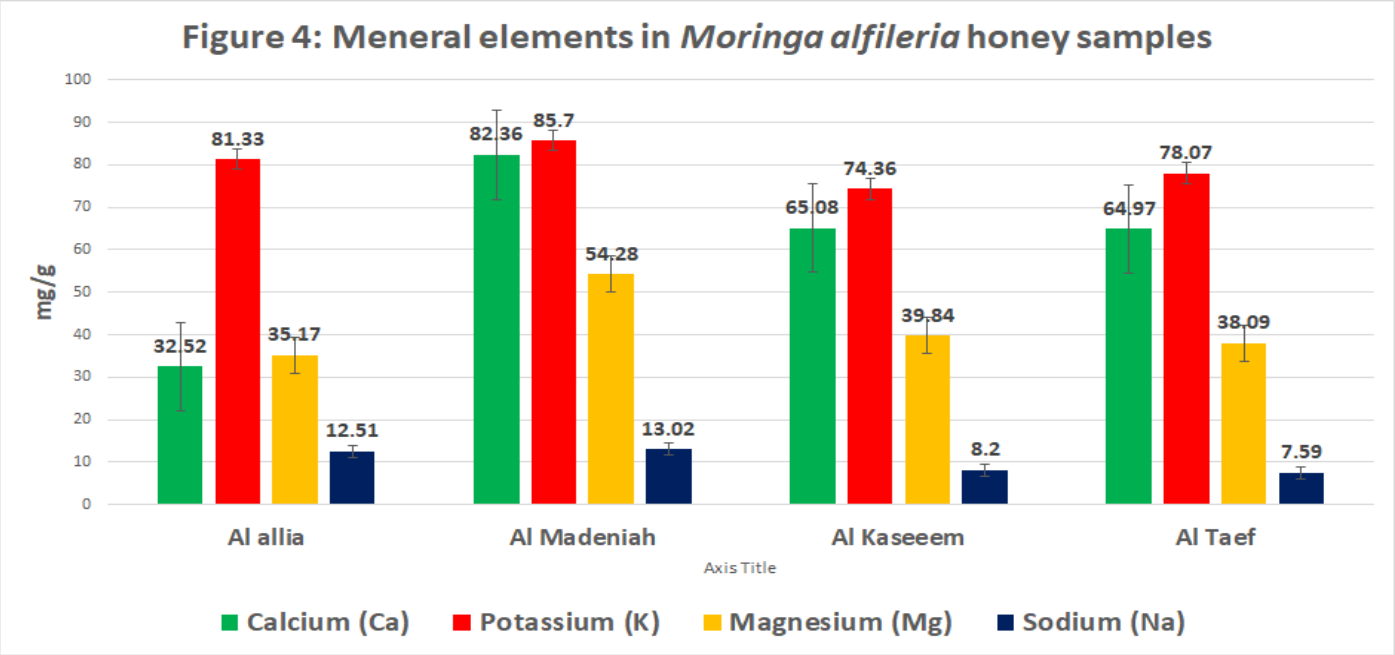
in (Madenia)., with a significant difference ($P < 0.05$). The Zinc (Zn) content was ranged between 3.13 0.0001ppm in Al Taef and 4.55 ± 0.0001ppm in Madenia, with a significant difference ($P < 0.05$). Contrary the, Manganese (Mn) exhibited the content (1.12 ± 0.003ppm) in Al Kaseeem, whereas Madenia had the highest (4.99 ± 0.0001ppm), with a significant difference ($P < 0.05$). The Nickel (Ni) showed lowest content in the Al allia tested honeys 0.199 ± 0.001ppm while in Madenia honey showed 0.24 ± 0.001ppm. The data showed that Al allia honey had the lowest Chromium (Cr) value 0.10 ±0.001 ppm. The Cadmium (Cd) detected in honey samples was 0.06 ±0.001 ppm where the highest level was detected 0.13 ±0.001 ppm.in Madenia listed in Table 5. The values for Lead (Pb) ranged between 0.131 ± 0.010 ppm in Madenia and 0.163 ± 0.010 ppm in Al allia, with a significant difference ($P < 0.05$) between the two values.



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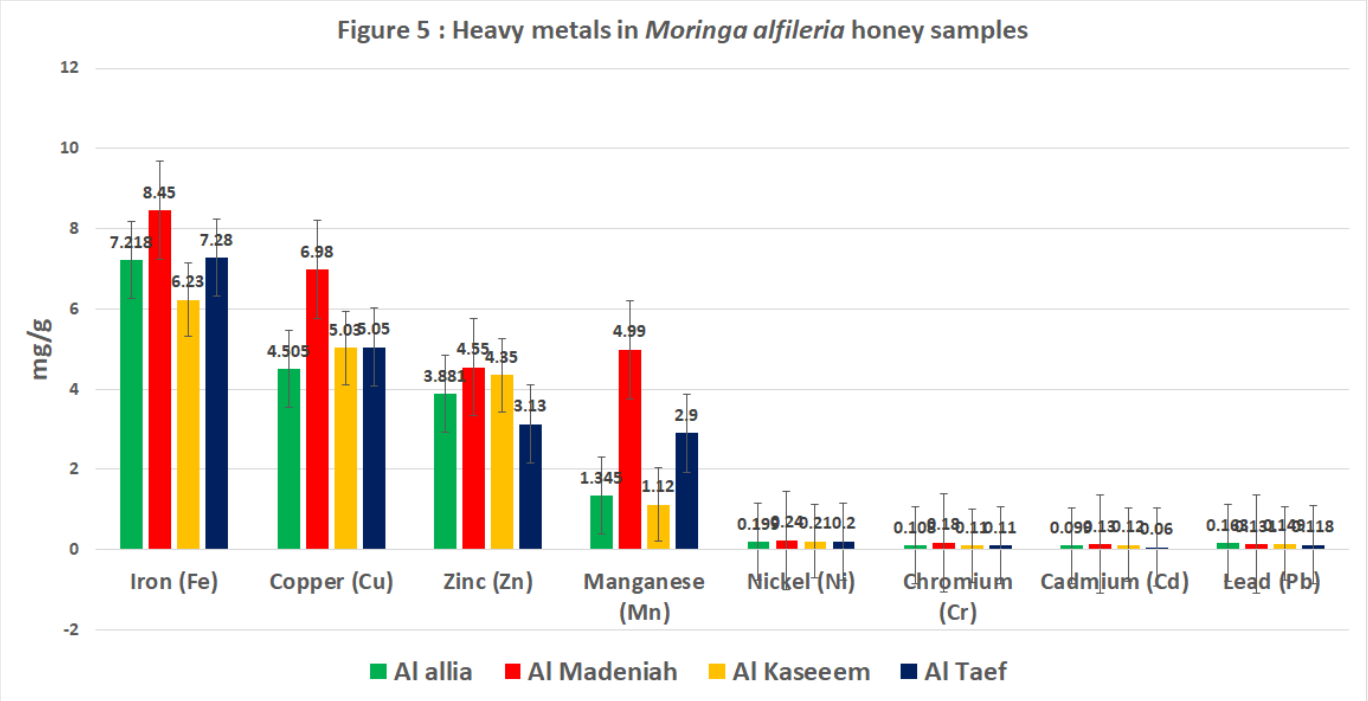

4. Discussion

Bee workers collect honey from nectar from many plants and processed honey with variable composition depending on to the differences in plant types, environmental conditions, climate and contribution of the bee keeper (37,38). One of the bee products, honey is most appreciated widely used (39). The natural authentic honey used in the prophylaxis and therapeutic of many diseases (40) The identification of novel antimicrobial compounds well documented natural authentic honey (2). Some bacterial strains have antibiotics resistance incrage the micorobolist to improve research on natural honey as antimicrobial agents (4).

In the present study, the antimicrobial activities of *Moringa alfileria* honey was determined. The antibacterial potency of *Moringa alfileria* honey from four regoins was investigated against various Gram-negative and Gram-positive pathogenic bacteria. The using *Moringa alfileria* honey revealed a suppression in growth of different pathogens tested. All honey tested showed growth suppression of pathogenic bacterial strains. Al Madeniah *Moringa oleifera* honey showed the highest zones of inhibition against all tested pathogen. The results showed significant differences in MIC values against tested antibiotic-resistant strains. The therapeutic antibiotics Tetracyclin, Oxacillin and Clindamycin showed highest MIC activity against all tested bacteria (Table 2).

Significant differences in MIC values of tested antibiotic-resistant strains. The strongest antibacterial potential against all tested bacteria tested toward *Moringa alfileria* honey from 4 regions gainst *Staphylococcus aureus, Streptococcus mutans, Escherichia coli, Klebsiella pneumoniae* and *Pseudomonas aeruginosa* (MIC=0.4 g/ml). *Moringa oleifera* honey showed the strongest antibacterial potential against all bacteria (MIC=0.4 g/ml). On the contrary, the lowest antibacterial activity was shown , *Escherichia coli* (MIC=0.2 g/ml). The therapeutic antibiotics Penicillin, Oxacillin and Clindamycin showed highest MIC activity against all tested bacteria. Similar results were observed (2,4,41,42).

In our investigation, the antibacterial activities of all honeys were comparable. This may be attributed to the narrow ranges of their total phenolic and total flavonoid. Where there is a positive correlation between TPC and the antibacterial activity of

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honey (43), and inhibition in the virulence factors of the pathogen (44). While, Nayaka et al., 2020) found a weak correlation between the antioxidant activity and antimicrobial activity of some polish commercial honey.

Many factors such as phenolic compounds, low pH, high osmolarity, and hydrogen peroxide-produced glucose oxidase enzyme lead to the strong antibacterial activity (17,46–49)(50) presence of lysozyme, methylglyoxal, bee peptides and honey high sugar contents (51). Our results clearly show significant variations among honey samples for allevaluated parameters.

The investigated *Moringa alfileria* honey in this investigation revealed different inhibition activities against the tested bacteria based on its origin. Significant differences in MIC values of *Moringa alfileria* honey against tested antibiotic-resistant strains were observed. Also, the therapeutic antibiotics showed highest MIC activity against all tested bacteria. Our results were in accordance with the research of (51), which also confirmed that Gram-positive bacteria were more sensitive to the honey samples than Gram-negative ones (52) The uses of honey for treatement of microbial infections has been attributed to a high sugar osmolarity as and presence of other biologically active compounds (53–55) (56), directly related to the botanical origin (57) and phenolic compounds, flavonoids, and phenolic acids (51) (58). Moreover, honey phenolics possess antimicrobial capacity against great numbers of pathogenic strains (59) (60).
The total phenolic content was ranged from 187.29 ± 13.32 to 245.29 ± 16.32 mg GAE/100 g honey which is higher than the results found in honey from India (61) (47–98 mg GAE/100 g honey), Poland (62) (71.7 to 202.6 µg/g honey), Argentina (23) from (18.730– 107.213 mg GAE/100 g honey), Burkina Faso (63)from (32.59–114.75 mg GAE/100 g honey), Portugal (53)from (30.87 to 87.27 mg GAE/100 g) and Romania (64)from (2–125 mg GAE/100 g honey).

The variability total phenolic contents was associated with the floral origin of the honey (65)and multi-floral honey was found to have higher phenolic contents than monofloral honey (66). The phenolic compounds, especially flavonoids in honey, are worth reporting antiviral, antimicrobial, antifungal, antioxidant and anti-inflammatory activities (67).

Contents total phenolic and flavonoid in honey were depend mainly on the geographical and botanical origins (68). Our results on *Moringa alfileria* honey showed dark honey contained the highest concentration ($p < 0.05$) of TFC. Total flavonoid (mg RE/100g honey) ranged from 163.86 ± 15.57 to 183.1 ± 19.33. Similar results were observed by analysis of three types of monofloral honey from Portugal which showed that dark honey was richer in phenolics content (69).

DPPH radical scavenging method used to detemine the antioxidant activity of the *Moringa alfileria* honey samples. The *Moringa alfileria* honey antioxidant activity ranged from 77.07 ± 09.51 mg AAE/100g honey (Al allia) to 237.80 ±15.51 mg AAE/100g honey (Al Madeniah). These results are in accordance with the results reported by others (70). There is a positive relationship between phenolic concentration, antioxidant capacity, and the color of honey (71). was demonstrated.

Our study revealed that monofloral honey produced from the *Moringa alfileria* plant nectar of different localities reached more than 70 % in particular of specific pollen. Water content is the most important physicochemical parameter of honey (72).
In our investigation, the honey samples had a moisture content between 14.11±0.25% (Al Kaseeem) and 16.35±0.12 % (Al allia). (Table 4), they were concordant with the funding observed in the literature (73,74) with small differences between the recorded values(42). The differences may be attributed to the geographical region, climate changes, the maturation temperature and the humidity during the harvest period (75,76). High moisture of honey promotes the process of fermentation during storage, while low water content elongated honey shelf life. Our study revealed that water content of investigated *Moringa alfileria* honey samples was within the accepted range. The relative humidity and temperature affect the water content during honey production by bees

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(77). Honey moisture content provides information in its degree of maturation (78). It is depending on the degree of honey maturation, botanical origin, processing technique and storage conditions (79–81) Fechner et al., 2016). According to the codex alimentary honey humidity should not be higher than 20% (Council Directive 2001/110/EC Relating to Honey. | FAOLEX; Devi & Jangir, 2018). Moisture content affects some characteristics of honey such as maturation, crystallization and viscosity (84,85). Moisture content of honey causes fermentation which affecting honey quality(86).

Sugar content is one of parameters used to assess authenticity and the overall quality of honey (87). Analysis of honey sugar is a good indicator of whether the honeybees were naturally fed with flower nectar or they were fed sugar solution (88), or when honey glucose content is seen much higher than its fructose content (9).

The results of our study revealed that the measures the sum of glucose and fructose content (reducing sugars) in *Moringa alfileria* was within the accepted range to prove the standardization and authenticity of honey as(89) and (90). The most dominant sugar in honey is fructose (9) The ratio of fructose to glucose (F/G) indicated the natural feeding of honeybees (91). Content of sucrose in all *Moringa alfileria* honey samples in our study did not exceed 5% which is the accepted level to prove the authenticity of honey as observed by (92). Hydroxymethylfurfural content and diastase activity are important parameters used to prove the freshness of honey (93) (94). The *Codex Alimentarius* of the World Health Organization and the European Union and the Gulf Technical Regulation on honey (GSO 147:2008 - Standards Store - GCC Standardization Organization EOSC, 2005) recommended that the maximum level for content in honey does not exceed 40 mg/kg but in countries with tropical temperatures, the HMF content should not exceed of honey 80 mg/kg. (46,94,95).

Many factors affected Diastase activity as age of bees, nectar collection period, quantity of nectar & its sugar content and physiological period of the colony (96). In this study, all examined *Moringa alfileria* honey samples were within the accepted range for HMF content and diastase number. These finding indicates the authenticity and freshness of honey as well as these honeys not exposde to heat and short storage time before testing in our experiment. All *Moringa alfileria* honey samples were found to be within the accepted range of acidity. The honey acidity is due to the presence of organic acids, in particular gluconic acid, which was found to affect honey flavor, texture, shelf life, and stability (97). Physical characteristics of all *Moringa alfileria* honey samples tested were found to be in the normal range, comparing with other studies (37).

5. Conclusions

It was concluded that *Moringa alfileria* honey from all four regoins in Saudi Arabia has the capacity to suppress pathogenic bacterial growth as well as significant free radical scavenging activities. Also, these findings suggest that *Moringa alfileria* honey may be considered as an interesting source of antimicrobial and antioxidants for therapeutic or for food manufactures or nutraceutical industries.

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Ethical issues Including plagiarism, informed consent, misconduct, data fabrication and/or falsification, double publication and/or submission, redundancy, etc.) have been completely observed by the authors.

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References

1. Grembecka M, Szefer P. Evaluation of honeys and bee products quality based on their mineral composition using multivariate techniques. Environmental Monitoring and Assessment [Internet]. 2013 May [cited 2022 Aug 5];185(5):4033. Available from: /pmc/articles/PMC3613575/
2. Santos-Beneit F, Ceniceros A, Nikolaou A, Salas JA, Gutierrez-Merino J. Identification of Antimicrobial Compounds in Two Streptomyces sp. Strains Isolated From Beehives. Frontiers in Microbiology. 2022 Feb 3;13:125.
3. Khuzaimah A, Hadju V, As S, Abdullah N, Bahar B, Riu DS, et al. Effect of Honey and Moringa Oleifera Leaf Extracts Supplementation for Preventing DNA Damage in Passive Smoking Pregnancy. International Journal of Sciences: Basic and Applied Research (IJSBAR) International Journal of Sciences: Basic and Applied Research [Internet]. 2015 [cited 2022 Aug 5];24(1):138–45. Available from: http://gssrr.org/index.php?journal=JournalOfBasicAndApplied
4. Adinortey CA, Wilson M, Kwofie SK. Honey as a Natural Product Worthy of Re-Consideration in Treating MRSA Wound Infections. The Global Antimicrobial Resistance Epidemic – Innovative Approaches and Cutting-Edge Solutions [Working Title] [Internet]. 2022 May 4 [cited 2022 Aug 15]; Available from: https://www.intechopen.com/online-first/81351
5. Hadju V, Dassir M, Putranto A, Sadapotto A. Chemical composition of Moringa oleifera and Honey from three different Areas in South Sulawesi, Indonesia. Gaceta Sanitaria. 2021 Jan 1;35:S396–9.
6. Saini RK, Sivanesan I, Keum YS. Phytochemicals of Moringa oleifera: a review of their nutritional, therapeutic and industrial significance. 3 Biotech [Internet]. 2016 Dec 1 [cited 2022 Aug 6];6(2):1–14. Available from: https://link.springer.com/article/10.1007/s13205-016-0526-3
7. Sreelatha S, Padma PR. Antioxidant activity and total phenolic content of Moringa oleifera leaves in two stages of maturity. Plant Foods for Human Nutrition [Internet]. 2009 Nov 11 [cited 2022 Aug 6];64(4):303–11. Available from: https://link.springer.com/article/10.1007/s11130-009-0141-0
8. Onyagbodor OA, Aprioku JS. Moringa oleifera leaf extract inhibits diabetogenic effect of alloxan in rats. IOSR Journal Of Pharmacywww.iosrphr.org [Internet]. 2017 [cited 2022 Aug 6];7(10):7–12. Available from: www.iosrphr.org
9. el Sohaimy SA, Masry SHD, Shehata MG. Physicochemical characteristics of honey from different origins. Annals of Agricultural Sciences. 2015 Dec 1;60(2):279–87.
10. Tigistu T, Worku Z, Mohammed A. Evaluation of the physicochemical properties of honey produced in Doyogena and Kachabira Districts of Kembata Tambaro zone, Southern Ethiopia. Heliyon [Internet]. 2021 Apr 1 [cited 2022 Aug 6];7(4). Available from: /pmc/articles/PMC8060584/
11. Shapla UM, Solayman M, Alam N, Khalil MI, Gan SH. 5-Hydroxymethylfurfural (HMF) levels in honey and other food products: effects on bees and human health. Chemistry Central Journal 2018 12:1 [Internet]. 2018 Apr

4 [cited 2022 Aug 6];12(1):1–18. Available from: https://bmcchem.biomedcentral.com/articles/10.1186/s13065-018-0408-3

12. Yayinie M, Atlabachew M, Tesfaye A, Hilluf W, Reta C. Quality authentication and geographical origin classification of honey of Amhara region, Ethiopia based on physicochemical parameters. Arabian Journal of Chemistry. 2021 Mar 1;14(3):102987.
13. Joyanes P, Conejo MDC, Martínez-Martínez L, Perea EJ. Evaluation of the VITEK 2 System for the Identification and Susceptibility Testing of Three Species of Nonfermenting Gram-Negative Rods Frequently Isolated from Clinical Samples. Journal of Clinical Microbiology [Internet]. 2001 [cited 2022 Jul 12];39(9):3247. Available from: /pmc/articles/PMC88326/
14. Idelevich EA, Schüle I, Grünastel B, Wüllenweber J, Peters G, Becker K. Acceleration of Antimicrobial Susceptibility Testing of Positive Blood Cultures by Inoculation of Vitek 2 Cards with Briefly Incubated Solid Medium Cultures. Journal of Clinical Microbiology [Internet]. 2014 Nov 1 [cited 2022 Jul 12];52(11):4058. Available from: /pmc/articles/PMC4313230/
15. Matuschek E, Brown DFJ, Kahlmeter G. Development of the EUCAST disk diffusion antimicrobial susceptibility testing method and its implementation in routine microbiology laboratories. Clinical Microbiology and Infection. 2014 Apr 1;20(4):O255–66.
16. Dahiya P, Purkayastha S. Phytochemical Screening and Antimicrobial Activity of Some Medicinal Plants Against Multi-drug Resistant Bacteria from Clinical Isolates. Indian Journal of Pharmaceutical Sciences [Internet]. 2012 Sep [cited 2022 Jul 12];74(5):443. Available from: /pmc/articles/PMC3660871/
17. Hegazi AG, al Guthami FM, al Gethami AFM, Abd Allah FM, Saleh AA, Fouad EA. Potential antibacterial activity of some Saudi Arabia honey. Veterinary World [Internet]. 2017 Feb 21 [cited 2022 Feb 7];10(2):233. Available from: /pmc/articles/PMC5352850/
18. Patton T, Barrett J, Brennan J, Moran N. Use of a spectrophotometric bioassay for determination of microbial sensitivity to manuka honey. Journal of Microbiological Methods. 2006 Jan 1;64(1):84–95.
19. Wu JC, Cheng J, Shi XL. Preparation of ace inhibitory peptides from mytilus coruscus hydrolysate using uniform design. BioMed Research International. 2013;2013.
20. Salissou MTM, Mahaman YAR, Zhu F, Huang F, Wang Y, Xu Z, et al. Methanolic extract of Tamarix Gallica attenuates hyperhomocysteinemia induced AD-like pathology and cognitive impairments in rats. Aging (Albany NY) [Internet]. 2018 Nov 1 [cited 2022 Jul 12];10(11):3229. Available from: /pmc/articles/PMC6286848/
21. Singleton VL, Orthofer R, Lamuela-Raventós RM. [14] Analysis of total phenols and other oxidation substrates and antioxidants by means of folin-ciocalteu reagent. Methods in Enzymology. 1999 Jan 1;299:152–78.
22. Zhishen J, Mengcheng T, Jianming W. The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. Food Chemistry. 1999 Mar 1;64(4):555–9.
23. Isla MI, Craig A, Ordoñez R, Zampini C, Sayago J, Bedascarrasbure E, et al. Physico chemical and bioactive properties of honeys from Northwestern Argentina. LWT - Food Science and Technology. 2011 Nov 1;44(9):1922–30.
24. Chua LS, Rahaman NLA, Adnan NA, Eddie Tan TT. Antioxidant activity of three honey samples in relation with their biochemical components. Journal of Analytical Methods in Chemistry. 2013;2013.
25. Isla MI, Craig A, Ordoñez R, Zampini C, Sayago J, Bedascarrasbure E, et al. Physico chemical and bioactive properties of honeys from Northwestern Argentina. LWT - Food Science and Technology. 2011 Nov;44(9):1922–30.

 doi:10.20944/preprints202211.0146.v1

26. Ruiz-Ciau D, Cuevas-Glory L, Quijano L, Sauri-Duch E. Chemical Composition and Antioxidant DPPH Activity of the Floral and Leaves Essential Oils of cMontanoa speciosa</i> DC. American Journal of Plant Sciences. 2017;08(04):745–53.
27. Kirs E, Pall R, Martverk K, Laos K. Physicochemical and melissopalynological characterization of Estonian summer honeys. Procedia Food Science. 2011 Jan 1;1:616–24.
28. Chukwuemeka R, Simeonov V. Evaluation of Pollen and Chemical Composition of Honey Samples Sourced from Open Markets in Anambra State, Nigeria to Ascertain their Authenticity. Journal of Applied Life Sciences International [Internet]. 2019 Dec 12 [cited 2022 Jan 8];22(3):1–12. Available from: https://www.journaljalsi.com/index.php/JALSI/article/view/30128
29. Mijanur Rahman M, Gan SH, Khalil MI. Neurological effects of honey: Current and future prospects. Vol. 2014, Evidence-based Complementary and Alternative Medicine. Oxford University Press; 2014.
30. Chataway HD. THE DETERMINATION OF MOISTURE IN HONEY. https://doi.org/101139/cjr32-041 [Internet]. 2011 May 1 [cited 2022 Jan 8];6(5):532–47. Available from: https://cdnsciencepub.com/doi/abs/10.1139/cjr32-041
31. Nyau V, Mwanza E, Moonga H. Physico- chemical qualities of honey harvested from different beehive types in Zambia. African Journal of Food, Agriculture, Nutrition and Development [Internet]. 2013 Apr 18 [cited 2022 Jan 8];13(2). Available from: https://www.ajol.info/index.php/ajfand/article/view/87467
32. Yadata D. Detection of the Electrical Conductivity and Acidity of Honey from Different Areas of Tepi. Food Science and Technology [Internet]. 2014 [cited 2022 Jan 8];2(5):59–63. Available from: http://www.hrpub.org
33. Zhou J, Yao L, Li Y, Chen L, Wu L, Zhao J. Floral classification of honey using liquid chromatography–diode array detection–tandem mass spectrometry and chemometric analysis. Food Chemistry. 2014 Feb 15;145:941–9.
34. Shapla UM, Solayman M, Alam N, Khalil MI, Gan SH. 5-Hydroxymethylfurfural (HMF) levels in honey and other food products: effects on bees and human health. Chemistry Central Journal 2018 12:1 [Internet]. 2018 Apr 4 [cited 2022 Jan 8];12(1):1–18. Available from: https://bmcchem.biomedcentral.com/articles/10.1186/s13065-018-0408-3
35. Miłek M, Bocian A, Kleczyńska E, Sowa P, Dżugan M. The Comparison of Physicochemical Parameters, Antioxidant Activity and Proteins for the Raw Local Polish Honeys and Imported Honey Blends. Molecules [Internet]. 2021 May 1 [cited 2022 Jan 8];26(9). Available from: /pmc/articles/PMC8122680/
36. Bilandžić N, Dokić M, Sedak M, Kolanović BS, Varenina I, Končurat A, et al. Determination of trace elements in Croatian floral honey originating from different regions. Food Chemistry. 2011 Oct 15;128(4):1160–4.
37. Habib HM, al Meqbali FT, Kamal H, Souka UD, Ibrahim WH. Physicochemical and biochemical properties of honeys from arid regions. Food Chemistry. 2014 Jun 15;153:35–43.
38. Habib HM, al Meqbali FT, Kamal H, Souka UD, Ibrahim WH. Bioactive components, antioxidant and DNA damage inhibitory activities of honeys from arid regions. Food Chemistry. 2014 Jun 15;153:28–34.
39. Cornara L, Biagi M, Xiao J, Burlando B. Therapeutic properties of bioactive compounds from different honeybee products. Frontiers in Pharmacology. 2017;8(JUN):412.
40. Eteraf-Oskouei T, Najafi M. Traditional and Modern Uses of Natural Honey in Human Diseases: A Review. Iranian Journal of Basic Medical Sciences [Internet]. 2013 Jun [cited 2022 Aug 15];16(6):731. Available from: /pmc/articles/PMC3758027/
41. Boulaaba M, Snoussi M, Saada M, Mkadmini K, Smaoui A, Abdelly C, et al. Antimicrobial activities and phytochemical analysis of Tamarix gallica extracts. Industrial Crops and Products. 2015 Dec 15;76:1114–22.

 doi:10.20944/preprints202211.0146.v1

42. Pătruică S, Alexa E, Obiștioiu D, Cocan I, Radulov I, Berbecea A, et al. Chemical Composition, Antioxidant and Antimicrobial Activity of Some Types of Honey from Banat Region, Romania. Molecules [Internet]. 2022 Jun 29 [cited 2022 Aug 7];27(13):4179. Available from: /pmc/articles/PMC9268046/
43. Nayaka NMDMW, Fidrianny I, Sukrasno, Hartati R, Singgih M. Antioxidant and antibacterial activities of multiflora honey extracts from the Indonesian Apis cerana bee. J Taibah Univ Med Sci. 2020 Jun 1;15(3):211–7.
44. Stagos D, Soulitsiotis N, Tsadila C, Papaeconomou S, Arvanitis C, Ntontos A, et al. Antibacterial and antioxidant activity of different types of honey derived from Mount Olympus in Greece. International Journal of Molecular Medicine [Internet]. 2018 [cited 2022 Feb 17];42(2):726. Available from: /pmc/articles/PMC6034916/
45. Makarewicz M, Kowalski S, Lukasiewicz M, Małysa-Paśko M. Antimicrobial and Antioxidant Properties of Some Commercial Honeys Available on the Polish Market. Czech J Food Sci. 35(5):0.
46. Hegazi AG, Guthami FM al, al Gethami AFM, Fouad EA, Abdou & AM, Hegazi S, et al. ANTIBACTERIAL ACTIVITY AND CHARACTERISATION OF SOME EGYPTIAN HONEY OF DIFFERENT FLORAL ORIGIN. Bulgarian Journal of Veterinary Medicine. 2021;24(2):278–90.
47. Hegazi AG. Antimicrobial activity of different Egyptian honeys as comparison of Saudi Arabia honey. Research Journal of Microbiology. 2011;
48. Hegazi AG, Abd Allah FM. Antimicrobial Activity of Different Saudi Arabia Honeys. Global Veterinaria. 2012;9(1):53–9.
49. Hegazi AG, Guthami FMA, Gethami AFMA, Fouad EA. Antibacterial and Antioxidant Activities of Some Saudi Arabia Honey Products. Iranian Journal of Medical Microbiology [Internet]. 2020 Sep 26 [cited 2022 Feb 21];14(5):490–500. Available from: http://ijmm.ir/article-1-1167-en.html
50. Almasaudi S. The antibacterial activities of honey. Saudi Journal of Biological Sciences. 2021 Apr 1;28(4):2188–96.
51. Mandal MD, Mandal S. Honey: its medicinal property and antibacterial activity. Asian Pacific Journal of Tropical Biomedicine [Internet]. 2011 [cited 2022 Jul 14];1(2):154. Available from: /pmc/articles/PMC3609166/
52. Matzen RD, Leth-Espensen JZ, Jansson T, Nielsen DS, Lund MN, Matzen S. The Antibacterial Effect in Vitro of Honey Derived from Various Danish Flora. Dermatology Research and Practice. 2018 Jun 19;2018.
53. Gonçalves J, Ribeiro I, Marçalo J, Rijo P, Faustino C, Pinheiro L. Physicochemical, Antioxidant and Antimicrobial Properties of selected Portuguese Commercial Monofloral Honeys. Journal of Food and Nutrition Research [Internet]. 2018 [cited 2022 Feb 16];6(10):645–54. Available from: http://pubs.sciepub.com/jfnr/6/10/
54. Kwakman PHS, te Velde AA, de Boer L, Vandenbroucke-Grauls CMJE, Zaat SAJ. Two major medicinal honeys have different mechanisms of bactericidal activity. PLoS ONE. 2011;6(3).
55. Anthimidou E, Mossialos D. Antibacterial activity of greek and cypriot honeys against staphylococcus aureus and pseudomonas aeruginosa in comparison to manuka honey. Journal of Medicinal Food. 2013 Jan 1;16(1):42–7.
56. Leyva-Jimenez FJ, Lozano-Sanchez J, Borras-Linares I, Cadiz-Gurrea M de la L, Mahmoodi-Khaledi E. Potential antimicrobial activity of honey phenolic compounds against Gram positive and Gram negative bacteria. LWT. 2019 Mar 1;101:236–45.
57. Pontis JA, da Costa LAMA, da Silva SJR, Flach A. Color, phenolic and flavonoid content, and antioxidant activity of honey from Roraima, Brazil. Food Science and Technology. 2014;34(1):69–73.
58. Bhatt P, Rathi K, Hazra S, Sharma A, Shete V. Prevalence of multidrug resistant Pseudomonas aeruginosa infection in burn patients at a tertiary care centre. Indian Journal of Burns. 2015;23(1):56.

59. Rodríguez Vaquero MJ, Tomassini Serravalle LR, Manca de Nadra MC, Strasser de Saad AM. Antioxidant capacity and antibacterial activity of phenolic compounds from argentinean herbs infusions. Food Control. 2010 May 1;21(5):779–85.
60. Eldeen IMS, Seow EM, Abdullah R, Sulaiman SF. In vitro antibacterial, antioxidant, total phenolic contents and anti-HIV-1 reverse transcriptase activities of extracts of seven Phyllanthus sp. South African Journal of Botany. 2011 Jan 1;77(1):75–9.
61. Saxena S, Gautam S, Sharma A. Physical, biochemical and antioxidant properties of some Indian honeys. Food Chemistry. 2010 Jan 15;118(2):391–7.
62. Kaškonienė V, Maruska A, Kornysova O, Ligor M, Kaškonienė V, Maruška A, et al. Quantitative and qualitative determination of phenolic compounds in honey Plantarum project View project Impact of clear cuttings on transformation of biodiversity in forest ecosystems View project Quantitative and qualitative determination of phenolic compounds in honey. 2009 [cited 2022 Feb 16]; Available from: https://www.researchgate.net/publication/228494420
63. Meda A, Lamien CE, Romito M, Millogo J, Nacoulma OG. Determination of the total phenolic, flavonoid and proline contents in Burkina Fasan honey, as well as their radical scavenging activity. Food Chemistry. 2005 Jul 1;91(3):571–7.
64. Al M, Daniel D, Moise A, Bobis O, Chemistry LLF, 2009 undefined. Physico-chemical and bioactive properties of different floral origin honeys from Romania. Elsevier [Internet]. [cited 2021 Apr 21]; Available from: https://www.sciencedirect.com/science/article/pii/S0308814608007681?casa_token=3ZmpsjzgpboAAAAA:LnGJAfWuyUedUEdudzKS4iUcjfgHlNYAfGJ7-O-Y49-v0N3CzIt3KQws26HaBDwLzpteyjbBrEyH
65. Roby MHH, Abdelaliem YF, Esmail AHM, Mohdaly AAA, Ramadan MF. Evaluation of Egyptian honeys and their floral origins: phenolic compounds, antioxidant activities, and antimicrobial characteristics. Environmental Science and Pollution Research. 2020 Jun 1;27(17):20748–56.
66. Perna A, Intaglietta I, Simonetti A, Gambacorta E. A comparative study on phenolic profile, vitamin C content and antioxidant activity of Italian honeys of different botanical origin. International Journal of Food Science and Technology. 2013 Sep;48(9):1899–908.
67. Srećković NZ, Mihailović VB, Katanić Stanković JS. PHYSICO-CHEMICAL, ANTIOXIDANT AND ANTIMICROBIAL PROPERTIES OF THREE DIFFERENT TYPES OF HONEY FROM CENTRAL SERBIA. Kragujevac J Sci. 2019;41:53–68.
68. Silici S, Sagdic O, Ekici L. Total phenolic content, antiradical, antioxidant and antimicrobial activities of Rhododendron honeys. Food Chemistry. 2010 Jul 1;121(1):238–43.
69. Ferreira ICFR, Aires E, Barreira JCM, Estevinho LM. Antioxidant activity of Portuguese honey samples: Different contributions of the entire honey and phenolic extract. Food Chemistry. 2009 Jun 15;114(4):1438–43.
70. van den Berg AJ, van den Worm E, van Ufford HCQ, Halkes SB, Hoekstra MJ, Beukelman CJ. An in vitro examination of the antioxidant and anti-inflammatory properties of buckwheat honey. J Wound Care. 2008;17(4).
71. Pontis JA, da Costa LAMA, da Silva SJR, Flach A. Color, phenolic and flavonoid content, and antioxidant activity of honey from Roraima, Brazil. Food Science and Technology. 2014;34(1):69–73.
72. Manyi-Loh CE, Ndip RN, Clarke AM. Volatile Compounds in Honey: A Review on Their Involvement in Aroma, Botanical Origin Determination and Potential Biomedical Activities. International Journal of Molecular Sciences [Internet]. 2011 Dec [cited 2022 Jul 14];12(12):9514. Available from: /pmc/articles/PMC3257144/
73. Sakač MB, Jovanov PT, Marić AZ, Pezo LL, Kevrešan ŽS, Novaković AR, et al. Physicochemical properties and mineral content of honey samples from Vojvodina (Republic of Serbia). Food Chemistry. 2019 Mar 15;276:15–21.

74. Lazarević KB, Andrić F, Trifković J, Tešić Ž, Milojković-Opsenica D. Characterisation of Serbian unifloral honeys according to their physicochemical parameters. Food Chemistry. 2012 Jun 15;132(4):2060–4.
75. Pătruică S, Peț I, Simiz E. BEEKEEPING IN THE CONTEXT OF CLIMATE CHANGE.
76. Đogo Mračević S, Krstić M, Lolić A, Ražić S. Comparative study of the chemical composition and biological potential of honey from different regions of Serbia. Microchemical Journal. 2020 Jan 1;152:104420.
77. Abou-Shaara HF, Owayss AA, Ibrahim YY, Basuny NK. A review of impacts of temperature and relative humidity on various activities of honey bees. Insectes Sociaux 2017 64:4 [Internet]. 2017 Jul 6 [cited 2022 Aug 18];64(4):455–63. Available from: https://link.springer.com/article/10.1007/s00040-017-0573-8
78. Gallina A, Stocco N, Mutinelli F. Karl Fischer Titration to determine moisture in honey: A new simplified approach. Food Control. 2010 Jun 1;21(6):942–4.
79. Pouliarekou E, Badeka A, Tasioula-Margari M, Kontakos S, Longobardi F, Kontominas MG. Characterization and classification of Western Greek olive oils according to cultivar and geographical origin based on volatile compounds. Journal of Chromatography A. 2011 Oct 21;1218(42):7534–42.
80. Karabagias IK, Badeka A v., Kontakos S, Karabournioti S, Kontominas MG. Botanical discrimination of Greek unifloral honeys with physico-chemical and chemometric analyses. Food Chemistry. 2014 Dec 15;165:181–90.
81. Karabagias IK, Badeka A, Kontakos S, Karabournioti S, Kontominas MG. Characterisation and classification of Greek pine honeys according to their geographical origin based on volatiles, physicochemical parameters and chemometrics. Food Chem [Internet]. 2014 [cited 2022 Aug 7];146:548–57. Available from: https://pubmed.ncbi.nlm.nih.gov/24176380/
82. Fechner DC, Moresi AL, Ruiz Díaz JD, Pellerano RG, Vazquez FA. Multivariate classification of honeys from Corrientes (Argentina) according to geographical origin based on physicochemical properties. Food Bioscience. 2016 Sep 1;15:49–54.
83. Devi A, Jangir J. Chemical characterization complemented with chemometrics for the botanical origin identification of unifloral and multifloral honeys from India. 2018 [cited 2022 Feb 13]; Available from: https://doi.org/10.1016/j.foodres.2018.02.017
84. Machado De-Melo AA, Almeida-Muradian LB de, Sancho MT, Pascual-Maté A. Composition and properties of Apis mellifera honey: A review. https://doi.org/101080/0021883920171338444 [Internet]. 2017 Jan 1 [cited 2022 Aug 7];57(1):5–37. Available from: https://www.tandfonline.com/doi/abs/10.1080/00218839.2017.1338444
85. Bogdanov S, Jurendic T, Sieber R, Gallmann P. Honey for nutrition and health: A review. J Am Coll Nutr. 2008 Dec 1;27(6):677–89.
86. Bogdanov S, Jurendic T, Sieber R, Gallmann P. Honey for Nutrition and Health: A Review. https://doi.org/101080/07315724200810719745 [Internet]. 2013 Dec 1 [cited 2022 Aug 7];27(6):677–89. Available from: https://www.tandfonline.com/doi/abs/10.1080/07315724.2008.10719745
87. Geană EI, Ciucure CT, Costinel D, Ionete RE. Evaluation of honey in terms of quality and authenticity based on the general physicochemical pattern, major sugar composition and δ13C signature. Food Control. 2020 Mar 1;109:106919.
88. Moumeh B, Dolores Garrido M, Diaz P, Peñaranda I, Linares MB. Chemical analysis and sensory evaluation of honey produced by honeybee colonies fed with different sugar pastes. Food Science & Nutrition [Internet]. 2020 Nov 1 [cited 2022 Feb 16];8(11):5823–31. Available from: https://onlinelibrary.wiley.com/doi/full/10.1002/fsn3.1843
89. Council Directive 2001/110/EC relating to honey. | FAOLEX [Internet]. [cited 2022 Aug 7]. Available from: https://www.fao.org/faolex/results/details/ru/c/LEX-FAOC037441/

 doi:10.20944/preprints202211.0146.v1

90. Aljohar HI, Maher HM, Albaqami J, Al-Mehaizie M, Orfali R, Orfali R, et al. Physical and chemical screening of honey samples available in the Saudi market: An important aspect in the authentication process and quality assessment. Saudi Pharmaceutical Journal 2018, Vol 26, Pages 932-942. 2018 Apr 25;26(7):932–42.
91. Szczesna T, Waś E, Semkiw P, Skubida P, Jaśkiewicz K, Witek M. Changes in the Physicochemical Properties of Starch Syrups after Processing by Honeybees. Agriculture 2021, Vol 11, Page 335 [Internet]. 2021 Apr 8 [cited 2022 Feb 16];11(4):335. Available from: https://www.mdpi.com/2077-0472/11/4/335/htm
92. Kazeminia M, Mahmoudi R, Aali E, Ghajarbygi P. Evaluation of Authenticity in Honey Samples from Qazvin, Iran. Journal of Chemical Health Risks. 2021;0.
93. Bentabol Manzanares A, Hernández García Z, Rodríguez Galdón B, Rodríguez Rodríguez E, Díaz Romero C. Physicochemical characteristics of minor monofloral honeys from Tenerife, Spain. LWT - Food Science and Technology. 2014;
94. Pasias IN, Kiriakou IK, Proestos C. HMF and diastase activity in honeys: A fully validated approach and a chemometric analysis for identification of honey freshness and adulteration. Food Chemistry [Internet]. 2017 Feb 22 [cited 2022 Feb 16];229:425–31. Available from: https://europepmc.org/article/MED/28372195
95. Bentabol Manzanares A, Hernández García Z, Rodríguez Galdón B, Rodríguez-Rodríguez EM, Díaz Romero C. Physicochemical characteristics and pollen spectrum of monofloral honeys from Tenerife, Spain. Food Chem [Internet]. 2017 Aug 1 [cited 2022 Feb 16];228:441–6. Available from: https://pubmed.ncbi.nlm.nih.gov/28317747/
96. Belay A, Haki GD, Birringer M, Borck H, Lee YC, Kim KT, et al. Enzyme activity, amino acid profiles and hydroxymethylfurfural content in Ethiopian monofloral honey. Journal of Food Science and Technology [Internet]. 2017 Aug 1 [cited 2022 Feb 16];54(9):2769. Available from: /pmc/articles/PMC5583106/
97. Warui MW, Hansted L, Gikungu M, Mburu J, Kironchi G, Bosselmann AS. Characterization of Kenyan Honeys Based on Their Physicochemical Properties, Botanical and Geographical Origin. International Journal of Food Science. 2019;2019.