

Screening invitro antibacterial properties of *Apis mellifera* L. monofloral honey in Ethiopia

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Research Article

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Abstract

Background

Honey is a natural sweetener and viscous food which is synthesized from the nectar of flowering plants by bees. As a worldwide, it has been traditionally used for incurring different diseases. However, In recent times, the emergence of multi and extended drug-resistant human pathogens called for an urgent search for more effective sources of natural products to treat infectious diseases. The biological activity of honey primarily relies on its botanical and entomological origin. Hence, the objective of this study was to screen the in vitro antibacterial activity of honey based on botanical origin produced in Ethiopia.

Methods

Botanical origin was identified by the Melisopalynological analysis method. The inhibition zone was determined by agar well diffusion assay. Minimum inhibitory concentration was done by broth diffusion.

Result

Six different monofloral honey namely: *Guizotia* (75.3% pollen grain counted), *Coffee arabica* (68.6%), *Vernonia* (90.5%), *Schefflera abyssinica* (100%), *Croton macrostachyus* (64.4%) and *Eucalyptus* (100%) honey was harvested through December, February, February, April, May, and June respectively from the study area. All the monofloral honey inhibited the growth of all the bacterial strains even though the diameter of the inhibition zone varied. *C. macrostachyus*, *Vernonia*, *Guizotia*, and *C. arabica* were comparatively more whilst *S. abyssinica* was the least effective against all the tested bacterial strains. The grand mean inhibition zone ranged from 17.0 ± 1 mm by *C. macrostachyus* to 10.5 ± 1 mm by *S. abyssinica* against all the bacterial strains. Besides, the MIC of botanical honey type ranged from $11.7 \pm 3.3\%$ w/v by *C. macrostachyus* against *S. aureus* to $37.5 \pm 1\%$ w/v by *S. abyssinica* against *P. aeruginosa*.

Conclusion

The current result indicated that there is a disparity in antibacterial properties between monofloral honey. The more effective honey against the tested bacterial strain were those produced from the nectar of Ethiopian medicinal plants; based on literatures. Therefore, further in-vivo and in-vitro research would be expected from the concerned researchers on the association between the biological compounds present in each part of medicinal plants and honey samples.

Background

Honey is a natural sweetener and viscous food which is synthesized from the nectar of flowering plants through regurgitation, enzymatic activity, and water evaporation by bees [1]. Honey has been used as an antimicrobial agent to treat ulcers, bed sores, skin infections, wounds, and respiratory tract infections [2], which depends on the peroxide and non-peroxide factors found in the product. The peroxide (hydrogen peroxide) which is produced by the oxidation of glucose by Glucose oxidase plays a key role in the antimicrobial activity of honey [3]. As hydrogen peroxide decomposes, it generates highly reactive free radicals that react and kill the bacteria. The non-peroxide factors are high osmotic effect; low water activity, acidic environment; low pH, phenolic acids, flavonoids, low protein content, high carbon-to-nitrogen ratio, and low redox potential; high content of reducing sugars [4].

Infectious disease is one of the major public health problems leading to morbidity and mortality [5] and worldwide, more than 50 million people died each year in society and healthcare settings [6]. In Ethiopia, WHO reported that lower respiratory tract infections caused by human pathogens are one of the top three causes of disability-adjusted life years [7]. To solve this problem drugs have been introduced in the field of pharmacology as they intensely reduced human illness and mortality. Besides, WHO (2008) estimated, 70% – 80% of the world's population depends on traditional medicines as the main health care, alternative, and balancing medicines. However, through time, pathogenic microbes evolve multidrug and extended resistance mechanisms against commonly available drugs due to indiscriminate usage of it, and on the other hand, consuming a drug has its own side effect on human health [8]. Furthermore, the new generations of drugs are less available and are expensive for resource-poor societies [9]. Thus, the resistance or slow killing of antibiotics calls for an urgent search for more effective sources of natural products to tackle problems due to drugs' failure to treat infectious diseases.

Honey is one of the natural products which has been widely used as a traditional medicine for the treatment of gastrointestinal infection and human respiratory ailments starting from the origin of mankind [10]. It has a very diverse nature in its composition and properties, and there are no two identical kinds of honey unless both are uniformly harvested from the same hive at the same time [11]. Its characteristics primarily rely on botanical, entomological, and geographical origins, storage conditions, environmental factors, and processing methods [12]. The difference in degrees of bactericidal and bacteriostatic activities between different kinds of honey is directly related to the botanical and geographical origins of the floral sources which create differences in peroxide and non-peroxide factors [13].

Ethiopia has a great potential for the production of quantity and quality of honey since it is endowed with a huge diversity of bee flora; over 8000 bee flora, and a high population density of bees; over 10 million bee colonies [14]. This makes the country, the leading honey producer in Africa and the 10th in the world [15]. The sources of natural honey in the country vary from region to region depending on the agroecology, soil types, bee forages, and bee species. But there are insufficient data that identifies the antibacterial potency based on the nectar type from which honey is produced. The objective of the current study is therefore to screen the antibacterial potential between floral sources and geographical origin from different parts of Ethiopia.

Materials And Methods

Study site and honey sample collection

The study was conducted in different parts of the Oromia region, Ethiopia. Honey samples were collected based on the availability of monofloral honey (plant abundance and dominance). For this from Kellem and West Wollega Zone (*Guizotia*, *C. arabica*, and *C. macrostachyus* honey), West Shoa (*Vernonia* honey), Holota (*Eucalyptus* spp honey) and Guji (*S. abyssinica* honey) was collected from a beekeeper apiary site and assigned as a source of honey type. A total of 10kg (sample size) of honey samples for each source of honey was collected separately from 10 different farmer beekeepers (about 1kg per farmer) in the study area. The collected honey samples were brought to Oromia Agricultural Research Institute; Food Science and Nutrition, using sterile glass cup honey containers for analysis. During harvesting, visual observation of the apiary environment was carried out in addition to personal communication with experienced beekeepers of each study area to get the floral source of the honey samples and bee forages growing in the area. Then pollen grain and antibacterial screening were carried out at Holota Apiculture Research Center (Bee Botany) and Oromia Agricultural Research Institute (Food Microbiology) Laboratory respectively from 2020 to 2021.

Determining botanical origin

The floral source of each honey type was first authenticated based on the melissopalynological analysis of the honey following the standard methodology [16]. Then, pollen grains were identified based on the pollen reference slides of honey plants from Atlas of pollen grains of major honey source plants [14]. Besides the pollen analysis sensorial properties, harvesting season and geographical regions were considered to differentiate the honey types. Moreover, during harvesting, visual observation of the apiary environment was carried out in addition to personal communication with very experienced beekeepers of each study area to get the floral source of the honey samples and bee forages growing in the area.

Antibacterial assay

Human pathogens used

Cultures of already identified common human pathogens which were isolated from human specimens were obtained from the Ethiopian public health institute. The isolates were *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, and *Enterobacter cloacae*. Then they were further confirmed by the standard bacteriological techniques.

Inoculums preparation

Standard 0.5 McFarland was prepared following the procedure [17]. Then, the standard was distributed into a capped test tube of the same size and volume as those used to prepare the test inoculums. Then, inoculums were prepared by picking 2-3 colonies from a 24-hour-old culture that was grown on their selective media and suspended in 5 ml saline solution (0.85% saline). The suspended inoculums were vortexed for 15 seconds and their turbidity was adjusted by adding colony or saline solution into the microbial stock solution and compared visually with standardized 0.5 McFarland (turbidity adjusted spectrophotometrically to 0.08-0.12 at OD_{625nm}) against a white paper background and contrasting black lines.

Inhibition zone susceptibility test

Agar well diffusion tests were used to record an observable inhibition zone [18]. For this, Muller Hinton Agar plates were uniformly seeded using a sterile cotton swab from already an adjusted inoculated saline solution containing the bacterial pathogen. Then, using a sterile cork borer, 6 mm diameter and 4 mm deep wells were made in the seeded agar medium. Using a micropipette, 60µL of the honey sample with a concentration of 75 % (3g of honey in 1mL sterile distilled water) each sample was added into the wells in the plates. Positive controls (chloramphenicol (30µg/60µL), as well as sterile distilled water negative control, were equally filled in the well. The plates were incubated at 37°C for 24 h. The diameters of inhibition zones including the wells were measured using a caliper. All the assays were replicated three times.

Minimum inhibitory concentration (MIC)

The broth dilution method was used [19]. Stock honey solution (75%) which was prepared by diluting 3g/ml of Muller Hinton Broth that shows high inhibition zone against the test microbes was used to determine the lowest concentration which inhibits bacterial growth. Then, a two-fold serial dilution was made from the stock solution. An 18-24h bacterial culture was used and standardized by comparing its turbidity with McFarland 0.5 standard. Then, 4ml (75%), 2ml (37.5%), 1ml (18.75%), 0.5ml (9.375%), 0.25ml (4.68%), 0.125ml (2.35%) and 0.0625ml (1.17%) was pipetted from stock honey solution (75%) within a series of seven tubes. Then to each test tube, 1ml of the standardized inoculum suspensions was pipetted while the negative control tube (8th test tube) contained only broth and the positive control tube (9th test tube) contained one milliliter tested bacteria plus broth for comparison. The tubes were vortexed and incubated for 24h at 37°C. Finally, by comparing with the control tube which showed clear tubes were used as the least honey concentration that inhibits bacterial growth.

Data analysis

SAS version 9.1.3 computer package was used. Means and standard deviations were calculated using SAS Software. Determination of the significant differences between each monofloral honey type was done using one-way ANOVA. The inhibition zone and MIC of the tested honey types were used for mean separation. Multiple pairwise comparisons between the mean values were done using the Least Significant Difference (LSD).

Results

Melissopalynological (Botanical origin) analysis

Pollen grain morphology was counted and categorized based on pollen grain frequency (Table 1). All honey types had a predominant pollen source since their pollen grain count was > 45%. These were *Guizotia* spp from the Nedjo honey sample, *Vernonia* spp from the Gedo honey sample, *C. arabica* from the Haro Sebu honey sample, *C. macrostachyus* from the Haro Sebu honey sample, *S. abyssinica* from the Bore honey sample and *Eucalyptus* spp from Holota honey sample which accounts 75.3, 90.5, 68.6, 64.4, 100 and 100% respectively (Fig. 1). No secondary pollen source plant was observed from all the honey samples whilst important minor pollen and minor pollen were recorded from each honey type. Besides, pollen grain morphology of each monofloral honey type were listed in Fig. 2.

Table 1
The characteristics of identified honey plants with their pollen frequency class from honey

Zone	District	Altitude	Agroecology	Month	Predominant pollen source(> 45%)	Secondary pollen source(16–45%)	Important minor pollen source (3–15%)	Minor pollen source (< 3%)
West Wollega	Nedjo	1892	Midland	December	<i>Guizotia</i> spp (Monofloral honey type)	-	<i>Bidens</i> spp, <i>Trifolium</i> spp & Grass type	<i>Plantago lanceolate</i> , <i>Sesamum indicum</i> , <i>Allophylus abyssinicus</i> , <i>Lipidium sativum</i> , <i>Brassica carinata</i> & <i>Satureja paradoxa</i>
West Shoa	Gedo		Highland	February	<i>Vernonia</i> spp (Monofloral honey type)	-	<i>Guizotia abyssinica</i>	<i>Eucalyptus camaldulensis</i> , <i>Brassica carinata</i> , <i>Carolus ayasanyu</i> , <i>Plantago lanceolate</i>
Kellem Wollega	Haro Sebu	1461	Lowland	February	<i>Coffee arabica</i> (Monofloral honey type)	-	<i>Vernonia</i> , <i>Terminalia</i> & <i>Guizotia</i> spp.	<i>Apodytes dimidiata</i> , <i>Bidens</i> spp, <i>Eucalyptus</i> spp, <i>Hypoestes trifolia</i> , <i>Galiniera saxifrage</i> , <i>Justicia schimperiana</i> , <i>Bersama abyssinica</i> , <i>Rumex nervosus</i> , <i>Euphorbia ampliphylla</i> & <i>Dobiya torida</i>
Guji	Bore	2800	Highland	April	<i>Schefflera abyssinica</i> (Monofloral honey type)	-		
Kellem Wollega	Haro Sebu	1524	Midland	May	<i>Croton macrostachyus</i> (Monofloral honey type)	-	<i>Syzium guineense</i> , <i>Eucalyptus</i> & <i>Acacia</i> spp	<i>Justicia schimperiana</i> , <i>Rumex nervosus</i> , <i>Coffee arabica</i> , <i>Guizotia</i> spp & <i>Bidens</i> spp
West Shoa	Holota	2700	Highland	June	<i>Eucalyptus</i> spp (Monofloral honey type)	-		

Inhibition zone

The mean diameter inhibition zone (mm) of botanical origin-based honey types against the human pathogenic strain was listed in Table 2. In addition, the produced zones of inhibition by the tested monofloral honey against *K. pneumonia* was indicated in Fig. 3 whereas against *P. aeruginosa* was listed in Fig. 4. Accordingly, the antibacterial activity ranged from 9.3 ± 0.6 mm by the *S. abyssinica* honey against the *P. aeruginosa* strain to 19.3 ± 0.6 mm by the *C. macrostachyus* against the *S. aureus* strain. The *S. abyssinica* honey types were statistically ($p < 0.0001$) less effective against all the tested bacterial pathogenic strains compared to other monofloral honey types. Of all the tested pathogenic strains, *S. aureus* was the most susceptible to all the monofloral honey types ranging from 12 ± 1 mm by *S. abyssinica* to 19.3 ± 0.6 mm by *C. macrostachyus* honey. In contrary *P. aeruginosa* was relatively the least susceptible bacterial strain ranging from 9.3 ± 0.6 mm by *S. abyssinica* to 14.6 ± 1.5 mm by *C. macrostachyus* honey. The grand mean inhibition zone of the tested strain was varied from 17.0 ± 0.9 mm by *C. macrostachyus* to 10.5 ± 0.9 mm by *S. abyssinica*.

Table 2
Inhibition zone of different honey types at 75% (w/v) honey concentration against human bacterial pathogenic strain by agar well diffusion

Pathogenic strains	Inhibition zone of honey type (Mean $\pm$ standard deviation)						chloramphenicol	Least significant difference	P-Value
	<i>C. macrostachyus</i>	<i>Guizotia</i> spp	<i>Vernonia</i> spp	<i>C. arabica</i>	<i>Eucalyptus</i> spp	<i>S. abyssinica</i>			
<i>S. aureus</i>	19.3 $\pm$ 1 ^b	18 $\pm$ 1 ^b	18 $\pm$ 1 ^b	17 $\pm$ 3 ^b	14 $\pm$ 1 ^c	12 $\pm$ 1 ^d	26.3 $\pm$ 1 ^a	2.4	< .0001
<i>E. coli</i>	15.3 $\pm$ 1 ^b	14.3 $\pm$ 1 ^b	14 $\pm$ 0 ^b	12.3 $\pm$ 1 ^c	11 $\pm$ 1 ^{cd}	9.6 $\pm$ 1 ^d	22 $\pm$ 1 ^a	1.4	< .0001
<i>P. aeruginosa</i>	14.6 $\pm$ 2 ^b	13.6 $\pm$ 2 ^{bc}	14 $\pm$ 1 ^{bc}	12.3 $\pm$ 1 ^c	12 $\pm$ 1 ^c	9.3 $\pm$ 1 ^d	23.3 $\pm$ 0 ^a	2.1	< .0001
<i>E. cloacae</i>	17.6 $\pm$ 2 ^b	16.3 $\pm$ 1 ^b	16 $\pm$ 1 ^b	15.3 $\pm$ 2 ^b	11.3 $\pm$ 2 ^c	11 $\pm$ 1 ^c	24.6 $\pm$ 1 ^a	2.4	< .0001
<i>K. pneumonia</i>	16.3 $\pm$ 1 ^{bc}	17 $\pm$ 1 ^b	15 $\pm$ 1 ^{bc}	15.4 $\pm$ 2 ^{bc}	11 $\pm$ 1 ^d	10 $\pm$ 1 ^d	23.6 $\pm$ 1 ^a	1.6	< .0001
<i>A. baumannii</i>	18.6 $\pm$ 1 ^b	16 $\pm$ 1 ^c	18 $\pm$ 0 ^{bc}	17 $\pm$ 2 ^{bc}	13 $\pm$ 1 ^d	11 $\pm$ 1 ^e	25.3 $\pm$ 1 ^a	1.7	< .0001
Grand mean	17.0 $\pm$ 1	15.9 $\pm$ 1	15.8 $\pm$ 1	14.9 $\pm$ 2	12.05 $\pm$ 1	10.5 $\pm$ 1	24.2 $\pm$ 1		

Minimum inhibitory concentration (MIC)

The least concentration of honey type which inhibits bacterial pathogenic growth was indicated in Fig. 5. The MIC of botanical honey type ranged from 11.7 $\pm$ 3.3% w/v by *C. macrostachyus* against *S. aureus* to 37.5 $\pm$ 1% w/v by *S. abyssinica* against *P. aeruginosa*. However, *Guizotia*, *Vernonia*, and *C. arabica* honey had similar MIC against *S. aureus*, *E. coli*, *E. cloacae*, and *K. pneumonia*. From the test bacterial pathogenic strains, *S. aureus* was the most sensitive whilst *P. aeruginosa* was the least sensitive to all the honey types. The highest and lowest grand mean MIC was recorded by *C. macrostachyus* (15.8 $\pm$ 2.9% w/v) and *S. abyssinica* (31.3 $\pm$ 2.4% w/v) honey type respectively against all the test pathogens.

Discussion

The present study identified the most commonly used monofloral honey based on the honey harvesting calendar and geographical origin from Ethiopia. each honey type was characterized into pollen grain frequency class based on Melissopalynological (botanical) analysis, sensorial, color, its distinctive harvesting season, and regions. Hence a monofloral honey namely: *Guizotia* from Nedjo, *C. arabica* from Haro Sebu, *Vernonia* from Gedo, *S. abyssinica* from Bore, *C. macrostachyus* from Haro Sebu and *Eucalyptus* honey from Holota was harvested through December, February, February, April, May, and June respectively.

This finding is inconvenient to different investigators. For instance, a study by Bareke and Addi [20], have indicated that honey from *S. abyssinica* is harvested in April-May and is whitish in color with an aroma and very pleasant characteristics, whereas *Vernonia* honey is harvested in February with a dark to a black color and has a very strong flavor and bitter test [21]. *Guizotia* honey is mostly harvested from November through December and its honey is very thick with a yellow color. The predominant pollen source in honey samples is due to their abundance and nectar potentiality of that area [22].

The current study screened the antibacterial activities of honey samples from different botanical origins. The study indicated that Chloramphenicol (used as a control) had shown statistically ($p < 0.0001$) more diameter of inhibition zone than all the honey types against tested pathogenic strain. However, *C. macrostachyus*, *Vernonia*, *Guizotia*, and *C. arabica* honey had statistically more effective and comparable antibacterial activity ($p > 0.05$) against all the tested pathogenic strains whilst *S. abyssinica* had shown statistically ($p < 0.0001$) less effective against all the tested strain compared to other honey types. The grand mean inhibition zone ranged from 17.0 $\pm$ 0.9 mm by *C. macrostachyus* to 10.5 $\pm$ 0.9 mm by *S. abyssinica*.

The overall mean diameter of the inhibition zone of this study was higher than that by Ofijan et al [23] who recorded 10.80 $\pm$ 2.63 mm from *A. mellifera* honey purchased from the market to 14.83 $\pm$ 2.45 mm from *A. mellifera* honey directly harvested from the hive at 75% w/v honey concentration. However, a study by Yalemwork et al [24] reported a higher mean diameter of inhibition zone (18.07 to 21 mm) at

50% v/v honey concentration by *A. mellifera* yellow and *A. mellifera* white honey respectively with the same bee species but different plant species and honey concentration. Moreover, higher than the present result was obtained from Cameroonian *A. mellifera* honey from the market which exhibited 29.3 ± 0.6 mm against *E. coli* to 37.0 ± 0.0 mm against *P. aeruginosa* at 75% (w/v) concentration by using agar well diffusion method [19].

Regarding MIC, the current studied honey types had inhibited the growth of the tested pathogenic strains which averagely varied from 11.7 ± 3.3 – $37.5 \pm 1\%$ w/v. The overall mean MIC of honey samples against all the examined bacterial pathogens ranged from 15.8 ± 2.9 (*C. macrostachyus*) to $31.3 \pm 2.4\%$ w/v (*S. abyssinica*). Gram-positive bacteria were less susceptible and inhibited at low honey concentrations relatively by all the examined honey types than gram-negative bacteria.

The average MIC result of this study is closer or within the values reported by Adgaba et al [11] who obtained an average range from 10.5 ± 0.5 – 28.6 ± 0.9 of MIC (% v/v), and they also indicated *C. macrostachyus* and *V. amygdalina* as a strong inhibitor at low honey concentration against the tested pathogens than other monofloral honey which is comparable with this finding. However, AL-Waili et al [25] recorded relatively higher MIC ($32 \pm 8.3\%$ to $58 \pm 13\%$ v/v) against drug-resistant bacteria than the current result.

The MIC of the current study results was relatively higher than the findings of Alvarez-Suarez et al [26] who reported MIC 2.5–10.4%v/v from Cuban monofloral honey. Less MIC than the current result was reported [27] who obtained 11–20% v/v for *P. aeruginosa* whilst very closer was recorded [11] which is 10.5 ± 0.9 and 15.5 ± 1.0 by *C. macrostachyus* and *V. amygdalina* respectively against *S. aureus*.

The variation in results could be due to the methods and materials used. For instance, the turbidity of the inoculum suspension, incubation time and temperature, depth of poured medium, the medium used for susceptibility test, the diameter of well formation, and measurement of inhibition zone diameter [28]. Besides, honey qualities and freshness, botanical and entomological origin, geographical sources, and the plant bio and chemo-types of the honey bring a disparity in its antimicrobial and biological activities [29].

In general, the present finding indicated relatively strong antibacterial activities of *C. macrostachyus*, *V. amygdalina*, *G. scabra*, and *C. arabica* against all the tested pathogenic strains which could be probably due to their medicinal properties. In Ethiopia, different plant part extracts of these plants have been traditionally used in curing different diseases. For example, the bark part of *C. macrostachyus* is used for treating splenomegaly and gonorrhea [30], whereas its leaves, stem, and fruits extracts are for the treatment of a wide range of human and animal diseases [31]; *V. amygdalina* leave extract for preventing headache and intestinal worm, and for treating tumor/cancer in general [32], the leaves of *G. scabra* used as wound treatment [33], seeds of *C. arabica* for curing diarrhea by pounding and mixing with honey [33]. In Ethiopia, the leaves of *V. amygdalina* is used to wash materials that are used in preparation of homemade beverage such as “Tella and Tej” and food processing, and preparation.

The susceptibility of *S. aureus* from the present study to all monofloral honey types compared to all the gram -ve bacteria was comparison with the finding by Adgaba et al [11] and Ofijan et al [23]. The difference in cell wall structure between gram + ve and gram-ve bacteria might be made *S. aureus* to be inhibited at less honey concentration. The complex cell wall with an additional outer membrane protects the Gram -ve bacteria from external influence by excluding toxic molecules and providing an additional stabilizing layer around the cell [34].

Conclusion

A monofloral honey namely: *Guzotia* from Nedjo, *C. arabica* from Haro Sebu, *Vernonia* from Gedo, *S. abyssinica* from Bore, *C. macrostachyus* from Haro Sebu and *Eucalyptus* honey from Holota was harvested through December, February, February, April, May, and June respectively. All the monofloral honey types were not the same in inhibiting the growth of human bacterial strains. Relatively honey produced from *C. macrostachyus*, *Vernonia*, *Guzotia*, and *C. arabica* plants were more effective whilst *S. abyssinica* honey was the least effective against all tested bacteria. Gram + ve bacteria were more susceptible to all honey types than gram –ve bacteria. The current result indicated that honey which is produced from medicinal plants has more antibacterial properties. Therefore, further in vivo and invitro research would be expected from the concerned researchers on the association between the biological compounds present in each part of medicinal plants and honey samples.

Declarations

Ethics approval and consent to participate

Not applicable

Consent for publication

Not applicable

Availability of data and materials

The data sets used and/or analyzed during the current study are available from the corresponding author upon reasonable request.

Competing interest

The authors declare that they have no competing interests

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Authors' contribution

Ofijan Tesfaye did all activities starting from conceptual framework to manuscript preparation and edition.

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References

1. Alvarez-Suarez JM, Tulipani S, Romandini S, Vidal A, Battino M: Methodological Aspects about determination of Phenolic Compounds and invitro evaluation of antioxidant capacity in the honey: a review. *Curr Anal Chem* 2009, 5 (4):293-302.
2. Abdullah FE, Aftab K, Khanum R, Khan SS: Antibacterial activity of honey against bacteria isolated from respiratory tract infections. *J Dow Uni Health Sci* 2012, 6: 95-99.
3. Kačániová M, Vukovic N, Bobková A, Fikselová M, Rovná K, Haščík P, Čuboň J, Hleba L, Bobko M: Antimicrobial and antiradical activity of Slovakian Honeydew honey samples. *J Microbiol Biotechnol Food Sci* 2011, 1 (3): 354-36.
4. Ramos OY, Salomon V, Libonatti C, Cepeda R, Maldonado L, Basualdo M: Effect of botanical and physicochemical composition of Argentinean honey on the inhibitory action against food pathogens. *Food Sci Tech* 2018, 87: 457- 463.
5. WHO. World Health Report; Geneva: Switzerland, 2002.
6. Andersson DI, Hughes D: Persistence of Antibiotic Resistance in Bacterial Populations. *FEMS Microbiol Rev* 2011, 35(5), 901–911. DOI: 10.1111/j.1574-6976.2011.00289.x
7. Laxminarayan R, Duse A, Wattal C, Zaidi AK, Wertheim HF, Sumpradit N, Vlieghe E, Hara GL, Gould IM, Goossens H: Antibiotic Resistance—the Need for Global Solutions. *Lancet Infect Dis* 2013, 13(12), 1057–1098.
8. Feleke M, Mengistu E, Andargachew M, Belay T, Yeshambel B, Yitayal S, Kahsay H, Chandrashekhar U, Afework K: The growing challenges of antibacterial drug resistance in Ethiopia. *Journal of Global Antimicrobial Resistance* 2014, 2: 148-154
9. Yitbarek H, Tadesse E, Alehegne W, Takele S: In vitro antimicrobial activity of selected Ethiopian medicinal plants against some bacteria of veterinary importance. *Afr J Microbiol Res* 2010, 4 (12): 1230-1234.
10. Molan PC: The antibacterial activity of honey: 1. the nature of the antibacterial activity. *Bee World*, 1992, 73: 5-28.
11. Adgaba N, Al-Ghamdi A, Sharma D, Tadessa Y, Alghanem SA, Khan KA, Ansari MJ, Mohamed GKA: Physico-chemical, antioxidant and anti-microbial properties of some Ethiopian mono-floral honey. *Saudi Journal of Biological Sciences* 2020, 27: 2366–2372. <https://doi.org/10.1016/j.sjbs.2020.05.031>
12. Silva TMS, dos Santos FP, Evangelista-Rodrigues A, da Silva EMS, da Silva GS, de Novais JS, Camara CA: Phenolic compounds, melissopalynological, physicochemical analysis and antioxidant activity of jandaíra (*Melipona subnitida*) honey. *J Food Compos Anal* 2013, 29:10–18.
13. Saranraj P, Sivasakthi S, Dire GF: Pharmacology of Honey: A Review. *Advances in Biological Research*. 2016, 10 (4): 271-289.

14. Adgaba N: Atlas of Pollen Grains of Major Honey Bee Flora of Ethiopia, Vol. 152, *Holeta Bee Research Centre, Commercial Printing Enterprise*, Addis Ababa, Ethiopia, 2007.
15. Estevinho L, Pereira AP, Moreira L, Dias LG, and Pereira E: Antioxidant and Antimicrobial Effects of Phenolic Compounds Extracts of Northeast Portugal Honey. *Food Chem Toxicol* 2008, 46(12): 3774–3779. DOI: 10.1016/j.fct.2008.09.062
16. Louveaux J, Maurizio A, Vorwohl G: Methods of melissopalynology. *Bee World*. 1978, 59 (4):139-157. <https://doi.org/10.1080/0005772X.1978.11097714>
17. Andrews J: BSAC Standardized Disc Susceptibility Testing Method (Version 5). *J Antimicrob Chemother* 2006, 58(3):511–529. DOI: 10.1093/jac/dkl277.
18. Moussa A, Saad A, Djebli ND, Meslem A, Benhalima AEK: Antifungal Activity of Four Honeys of Different Types from Algeria against Pathogenic Yeast: *Candida Albicans* and *Rhodotorula Spp.* *Int J Microbiol Res* 2011, 2(3): 276–279.
19. Boateng J, Diunase KN: Comparing the Antibacterial and Functional Properties of Cameroonian and Manuka Honeys for Potential Wound Healing—Have We Come Full Cycle in Dealing with Antibiotic Resistance? *Molecules* 2015, 20(9): 16068–16084. DOI: 10.3390/molecules200916068
20. Tura B, Admassu A: POLLEN ANALYSIS OF HONEY FROM BORANA ZONE OF SOUTHERN ETHIOPIA. *J APIC SCI* 2019, 63: 2.
21. Admassu A, Kibebew W, Amssalu A, Ensermu K: Honeybee forages of Ethiopia. United Printers, Addis Ababa 2014b.
22. Tura B, Admassu A: Honeybee flora resources of Guji Zone, Ethiopia. *J Biol Agric Healthcare* 2018, (21): 1-9.
23. Ofijan T, Diriba M, Asnake D: In vitro antimicrobial properties of *apis mellifera* L. and *Meliponulla beccarii* L. honeys from Kellem and West Wollega Zones, Western Ethiopia. *International Journal of Food Properties* 2022, 25:1, 65-75, DOI: 10.1080/10942912.2021.2019761
24. Yalemwork E, Wossenseged L, Nega B: Antibacterial Effects of *Apis Mellifera* and Stingless Bees Honeys on Susceptible and Resistant Strains of *Escherichia Coli*, *Staphylococcus Aureus* and *Klebsiella Pneumoniae* in Gondar, Northwest Ethiopia. *BMC Complement Alternat Med* 2013, 13(1):269.
25. Al-Waili, Noori S: Investigating the antimicrobial activity of natural honey and its effects on the pathogenic bacterial infections of surgical wounds and conjunctiva. *J Med Food* 2004, 7 (2): 210–222. <https://doi.org/10.1089/1096620041224139>.
26. Alvarez-Suarez JM, Tulipani S, Romandini S, Bertoli E, Battino M: Contribution of honey in nutrition and human health: a review. *Mediterr J Nutr Metab* 2010b, 3 (1):15–23. <https://doi.org/10.1007/s12349-009-0051-6>
27. Mullai V, Menon T: Bactericidal activity of different types of honey against clinical and environmental isolates of *Pseudomonas aeruginosa*. *J Alternative Complementary Med* 2007, 13 (4): 439–441. <https://doi.org/10.1089/acm.2007.6366>
28. Coyle MB: Manual of Antimicrobial Susceptibility Testing; Departments of Laboratory Medicine and Microbiology. University of Washington: Seattle, 2005.
29. Alvarez-Suarez JM, Tulipani S, Díaz D, Estevez Y, Romandini S, Giampieri S, Damiani E, Astolfi P, Bompadre S, Battino M: Antioxidant and antimicrobial capacity of several mono-floral Cuban honey and their correlation with color, polyphenol content and other chemical compounds. *Food Chem Toxicol* 2010a, 48 (8–9): 2490–2499. <https://doi.org/10.1016/j.fct.2010.06.021>
30. Teklehaymanot T: Ethnobotanical study of knowledge and medicinal plants use by the people in Dek Island in Ethiopia. *Journal of Ethnopharmacology* 2009, 124(1):69-78.
31. Balemie K, Kelbessa E, Asfaw Z. Indigenous medicinal plant utilization, management and threats in Fentalle area, Eastern Shewa, Ethiopia. *Ethiopian J of Bio Sci.* 2004; 3:37–58.
32. Abera B: Medicinal plants used in traditional medicine by Oromo people, Ghimbi District, Southwest Ethiopia. *Journal of Ethnobiology and Ethnomedicine* 2014, 10(40):1-15. <http://www.ethnobiomed.com/content/10/1/40>
33. Tolossa T, Megersa M: Ethnobotanical Study of Medicinal Plants Used to Treat Human Diseases in Berbere District, Bale Zone of Oromia Regional State, South East Ethiopia. London: Hindawi 2018. <https://doi.org/10.1155/2018/8602945>
34. Silhavy TJ, Kahne D, Walker S: The bacterial cell envelope. *Cold Spring Harb Perspect Biol* 2010, 2: a000414.

Figures

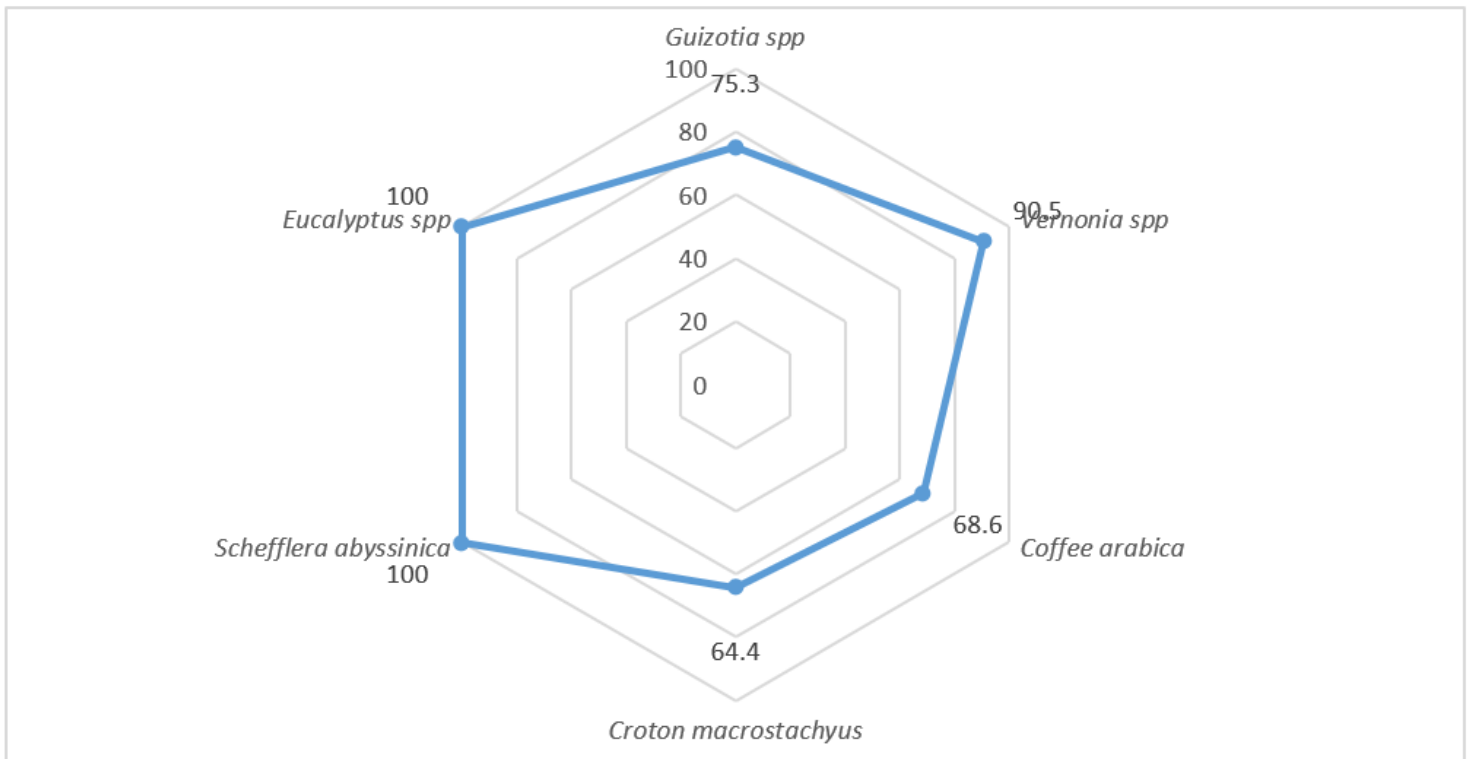


Figure 1

Major bee forage plant that provides monofloral honey in different part of Ethiopia

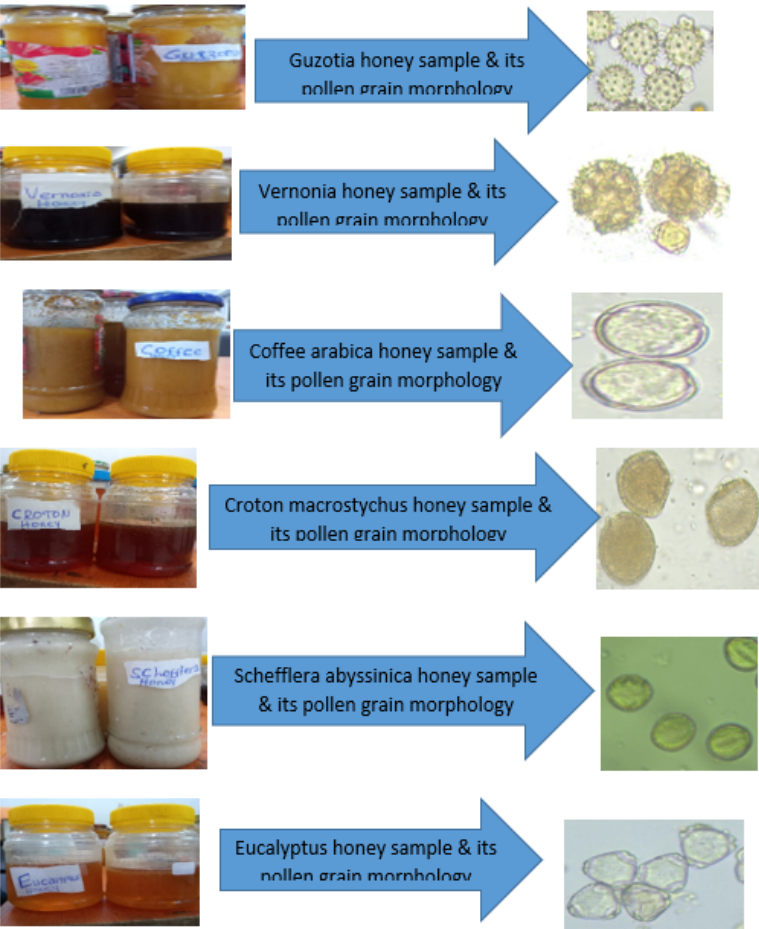


Figure 2

Honey sample color with its pollen grain morphology of each monofloral honey from Ethiopia

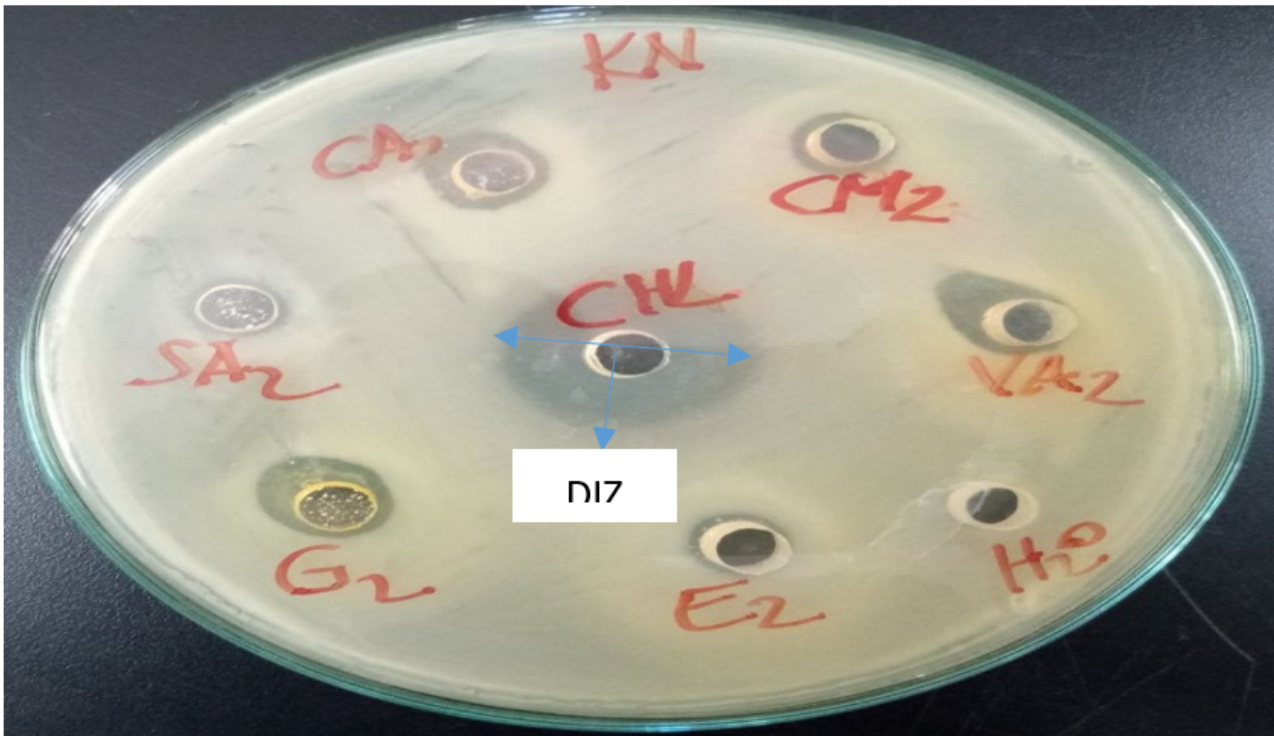


Figure 3

Agar plate showing inhibition zone generated by 75% w/v solution of the monofloral honey types. Where: KN; K. pneumonia, CHL; Chloramphenicol, DI7; Diameter of inhibition zone, CA2; C. arabica sample number 2, CM2; C. macrostachyus sample number 2, VA2; Vernonia sample number 2, H2O; Water, E2; Eucalyptus sample number 2, G2; Guizotia sample number 2, and SA2; S. abyssinica sample number 2.

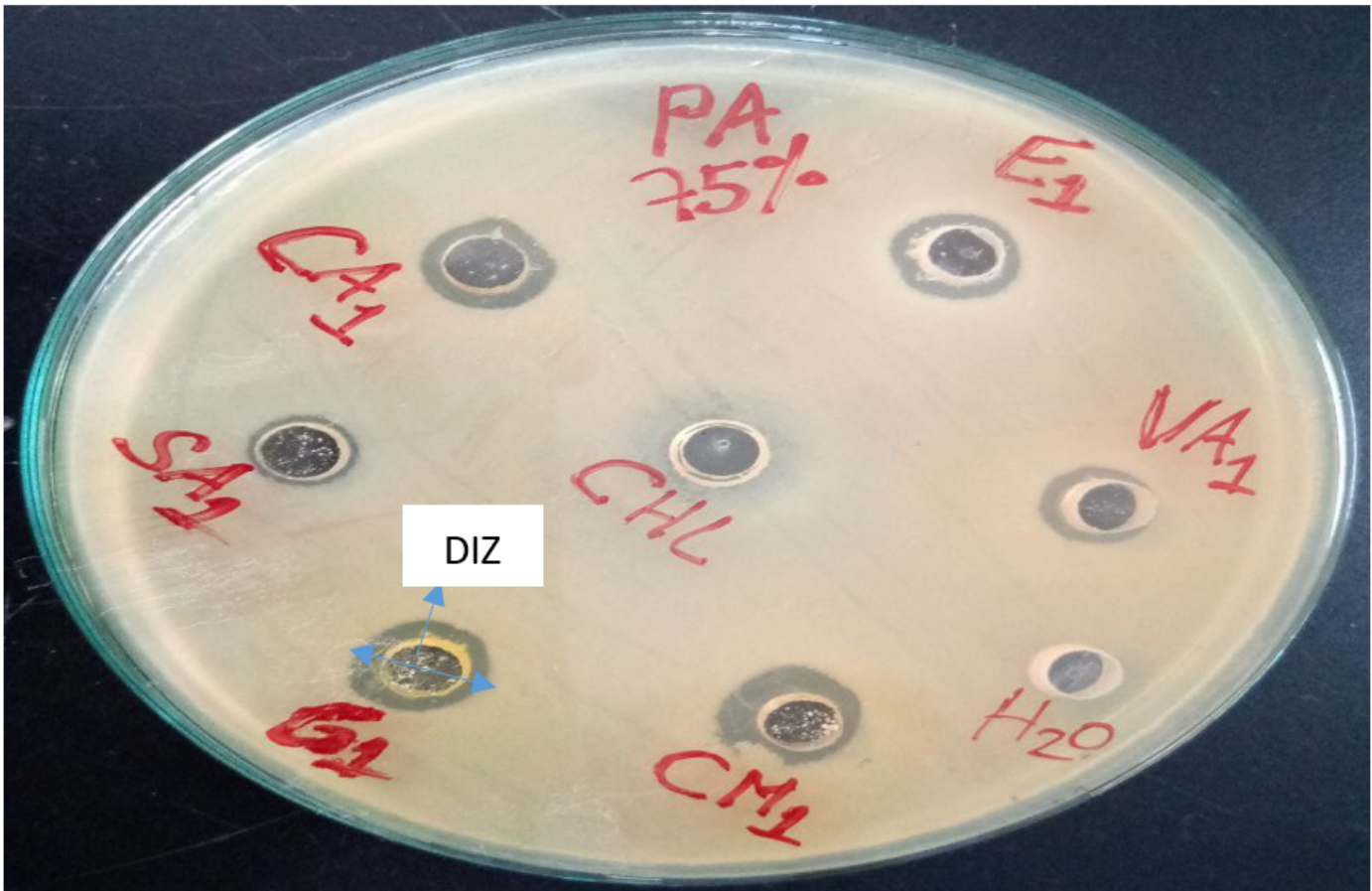


Figure 4

Agar plates showing inhibition zone generated by 75% w/v solution of the monofloral honey types. Where: PA; *P. aeruginosa*, CHL; Chloramphenicol, CA1; *C. arabica* sample number 1, CM1; *C. macrostachyus* sample number 1, VA1; *Vernonia* sample number 1, H2O; Water, E1; *Eucalyptus* sample number 1, G1; *Guizotia* sample number 1, and SA1; *S. abyssinica* sample number 1.

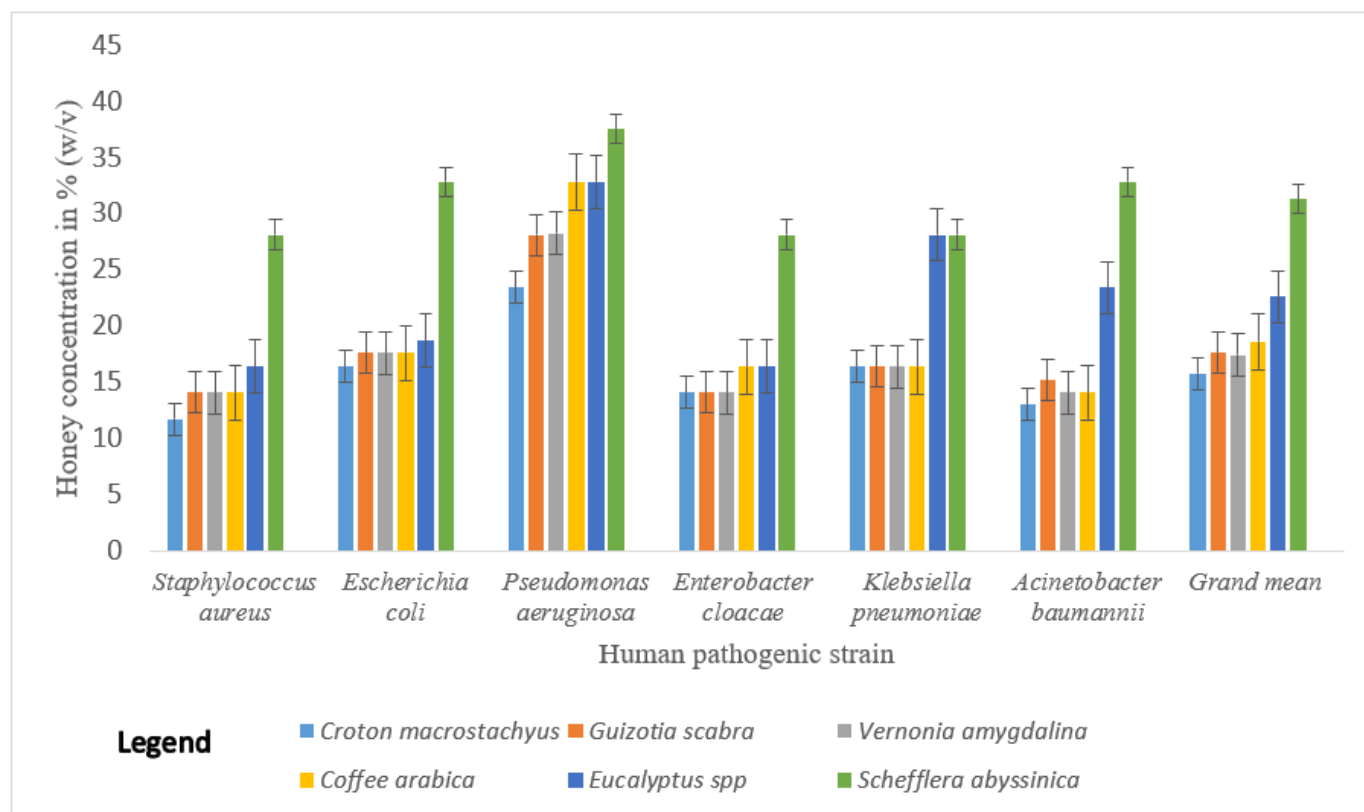


Figure 5

The Minimum inhibitory concentration (% w/v) of monofloral honey types against the tested bacterial strains