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Original Research

Physicochemical and Antibacterial Activity of Honey Sold in Nekemte, East Wollega Zone, Western Ethiopia

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Abstract

This study investigated the physicochemical properties and antibacterial activities of honey samples from Nekemte, East Wollega Zone, Ethiopia. A total of 21 honey samples were collected from seven locations: Arjo Guddatu, Mettu (I/Bora), Bedelle (B/Bedele), Gidda Ayena, Limu (Gelila), Jemoa, and Ebantu (Qello), with three replicates per area. The samples were analyzed for pH, electrical conductivity (EC), total sugar content, and ash content using a randomized design. pH of the samples ranged from 3.5 to 3.96, with Jemoa honey being the lowest pH (3.5) and Ebantu (Qello) honey being the highest pH (3.96). EC ranged from 0.48 to 1.17 mS/cm, with Jemoa honey being the highest EC (1.17 mS/cm). Sugar content ranged from 65 to 83 g/100 g, and ash content ranged from 0.28 to 0.75 g/100 g. Antibacterial analyses showed that the honey samples inhibited five bacterial pathogens (Staphylococcus aureus, Escherichia coli, Salmonella Typhimurium, Shigella flexneri, and Pseudomonas aeruginosa) by 100%. Jemoa honey had the maximum number of inhibition zones, whereas Ebantu (Qello) honey was broad-spectrum at low concentrations. The findings demonstrate the local honey's antimicrobial potency, particularly that of Ebantu (Qello).

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INTRODUCTION

Honey, a natural sweet substance derived from nectar or plant secretions, has been used as a human food since ancient times due to its high nutritional content, including carbohydrates, organic acids, proteins, polyphenolic compounds, free amino acids, minerals, and vitamins (Moore et al., 2001; Mohammad et al., 2012). Honey, produced by *Apis mellifera*, is a traditional medicine used to treat various human ailments. Research shows its antibacterial activity against pathogenic, oral, and -food spoilage bacteria, with natural, unheated honey showing broad-spectrum effectiveness (Lusby et al., *2005*). Antimicrobial agents reduce infectious diseases globally, but resistant pathogens weaken their effectiveness, posing a serious threat to public health and all antibiotics, including major last-option drugs (Mandal et al., 2009; Mohammed et al., 2014). Honey's antimicrobial properties come from its high sugar content, low pH, and hydrogen peroxide. However, a high pH can reduce its palatability. Honey has been used in traditional medicine for its antimicrobial properties, including wound healing (Al-Waili, 2004). There haven't been many studies on natural honey from Ethiopia to assess its antibacterial properties. Honey produced by honeybees (*Apis mellifera*) exhibits both bacteriostatic and bactericidal action when tested in vitro. However, pharmacological standardization and clinical assessment of honey's impact are necessary before adopting honey as a preventive and therapeutic strategy for common diseases associated with the studied bacterial species (Andargachew et al., 2004).

Statement of the problem

This study examined various kinds of honey sold in Nekemte, collected from Ebantu, Gidda, Arjo Guddatu, Mettu, Bedelle, Gidda Ayena, Limu, and Haro Limu, for their antibacterial activity and effects on pathogenic microbes. Nekemte town, located in the East Wollega Zone, is surrounded by rural districts rich in natural resources and diverse flora, which have a high potential for honey production. However, the physicochemical properties and antimicrobial activity of honey sold in this town have not been studied. Therefore, this study was conducted to investigate the physicochemical characteristics and antimicrobial activity of honey available in Nekemte. Despite increased consumption of honey in Nekemte and the surrounding area, there is no scientific data on the quality, physicochemical properties, and antimicrobial activity of commercially harvested honey from the area. Lack of this prevents the determination of honey as an effective, safe, natural medicine or complementary therapy product.

Research questions

Public health practitioners and the public are unaware of the possible health effects and benefits of the lack of the above studies, without which this study is conducted to address the following research questions.

1. What are the physicochemical characteristics (ash, sugar level, pH, electrical conductivity) of honey found on the market in Nekemte, East Wollega Zone?
2. Is honey found on the market in Nekemte antimicrobial against most pathogenic bacteria?
3. Which honey samples have maximum antibacterial activity, and how does it vary with concentration?
4. Is there a correlation between the physicochemical properties of honey and antibacterial activity?

MATERIALS AND METHODS

Study area and design

The study was carried out in the East Wollega Zone, Nekemte town, and honey samples marketed in Nekemte town were obtained from several zones, including Buno Bedelle, Illuababora, and East Wollega districts. This laboratory investigation looked into the antibacterial activity of honey against numerous human pathogenic microorganisms. Honey's physicochemical properties were examined, as well as the minimal inhibitory concentration, inhibitory concentration, and bactericidal concentration. The study employed an experimental laboratory-based research design to investigate the physicochemical properties and antimicrobial activity of honey sold in Nekemte town. This design involved sample collection, physicochemical analysis, and antibacterial assays against selected pathogenic bacteria to assess the potential of honey as a natural antimicrobial agent.

Samples and sampling techniques

This study employed an experimental laboratory-based research design to investigate the physicochemical properties and antimicrobial activity of honey sold in Nekemte town. Honey samples were purchased from local vendors in the town, and a total of 7 honey samples were included in the study. The samples were obtained from different regions within the town, which included varieties from Ebantu (Qello), Gidda, Arjo Guddatu, Mettu (I/Bora), Bedelle (B/Bedele), Gidda Ayena, Limu (Gelila), and Haro Limu

(Haro). The Ethiopian Public Health Institute in Addis Ababa, Ethiopia, provided the pathogenic bacteria cultures. These include *S. aureus* (ATCC 25923), *S. flexneri* (ATCC 12022), *S. typhimurium* (ATCC 14028), *P. aeruginosa* (ATCC 27853), and *E. coli* (ATCC 25922).

Physicochemical analysis

This study employed an experimental laboratory-based research design to investigate the physicochemical properties and antimicrobial activity of honey sold in Nekemte town. A total of 7 honey samples were collected from different districts in the East Wollega Zone, including Ebantu (Qello), Gidda, Arjo Guddatu, Mettu (I/Bora), Bedelle (B/Bedele), Gidda Ayena, Limu (Gelila), and Haro Limu (Haro). Each site provided three replicates of honey samples to ensure consistency in results. Physicochemical analyses such as pH, electrical conductivity (EC), total sugar, and ash content, as well as antimicrobial activity tests, were performed on each of the samples with three replicates per site to assess variability and confirm the findings.

The physicochemical analysis of the honey samples included determining the acidic content using titration. Using phenolphthalein as an indicator, the honey samples were titrated against 0.1 N NaOH to measure the acidity. This method provided insights into the honey's pH and its potential antimicrobial properties, as higher acidity often correlates with stronger antimicrobial activity. To measure the amount of carbohydrates, a honey sample was weighed, mixed, and diluted with distilled water and chloric acid reagent. The extract was then diluted and pipetted into test tubes using various sugar standards. The tubes were then placed in a boiling water bath for 12 minutes, and after cooling, the absorbance at 630 nm was measured against blanks. The results were computed using the formula developed by Agbagwa et al. (2010).

Antimicrobial activity

Using the agar diffusion method, the study assessed honey's antibacterial efficacy against five bacterial strains. As a positive control, chloramphenicol was added to wells containing the honey sample. The inhibition zone surrounding each tested drug was evaluated to determine the antibacterial activity. The antibacterial properties (inhibitions) of various kinds of honey were compared using descriptive statistics and a one-way ANOVA. All statistical analyses were conducted using the Statistical Package for Social Science (SPSS) version 20.

RESULTS AND DISCUSSIONS

Results

Physicochemical Analysis

The physicochemical analysis of the honey samples revealed notable differences in pH, electrical conductivity, and sugar content. The pH values of the kinds of honey ranged from 3.32 in Gida Ayana honey to 3.96 in Ebantu (Qello) honey, with an average value of 3.9, indicating all samples were acidic. Electrical conductivity (EC) varied significantly among the different types of honey, with Limu honey recording the lowest value at 0.48 mS/cm and Qello honey showing the highest at 1.17 mS/cm. In terms of sugar composition, the total sugar content of honey sold in Nekemte town ranged from 50 g/100 g in Haro Limu to 83 g/100 g in Bedelle honey.

Some of the honey samples met the QSAE (2005) standard of a minimum of 65% total sugars, while others fell below this threshold. The mean moisture content of the honey samples examined in this study ranged from 11.05 g/100 g to 13.65 g/100 g. These values fall well within the permissible range for export-quality honey according to international honey standards. Specifically, the lowest moisture content was recorded in the honey from Bedelle (11.05 g/100 g), while the highest was found in the honey from Gida Ayana (13.65 g/100 g). The overall average moisture content across all samples was found to be 12.36 g/100 g. These results suggest that the honey samples collected from different districts are relatively low in moisture, which is generally favorable for longer shelf life and reduced risk of fermentation (Table 1).

Table 1

Physicochemical analysis of honey samples collected from different areas and sold at Nekemte town, East Wollega Zone.

Sites	Moisture (Mean ± SD)	pH (Mean ± SD)	EC (m/Scm) (Mean ± SD)	Ash (%) (Mean ± SD)	Titrable Acidity (%) Mean ± SD	Total sugar (g/100 g) (Mean ± SD)	Non-reducing sugar (g/100g) (Mean ± SD)	Reducing sugars (g/100g) (Mean ± SD)
Arjo Guddatu	13.65±0.05[a]	3.56±0.03[a]	0.78±0.06[a]	0.75±0.01[a]	0.66±0.16[ab]	79±1.0[a]	31.6±1.52[a]	74±0.57[ab]
Mettu(I/Bora)	12.6±0.04[ab]	3.76±0.05[a]	0.98±0.01[a]	0.62±0.02[a]	1.28±0.02[ab]	65.00±1 lb	27.6±1.11[a]	62±2[b]
Bedelle (B/Bedele)	12.61±0.02[ab]	3.7±0.1[a]	0.97±0.01[a]	0.55±.01[a]	1.15±0.01[ab]	67±0.5[b]	24±1[ab]	60±1 lb
Gidda Ayena	13.8±0.0[a]	3.32±0.02[a]	0.87±0.01[a]	0.64±0.0[a]	1.24±0.01[ab]	72±1[ab]	16±1[b]	67±0.5[b]
Limu (Gelila)	11.06±0.01[b]	3.86±0.05[a]	0.48±0.03[a]	0.44±0.01[a]	0.89±0.02[ab]	83±1.52[a]	15±1.52[b]	81±1.52[a]
Haro Limu(Haro)	11.23±0.00[b]	3.80±0.01[a]	0.60±0.01[a]	0.28±0.01[a]	1.11±0.00[ab]	50±0.57[c]	18±1.52[ab]	46±0.05
Ebantu(Qello)	13.64±0.02[a]	3.96±0.05[a]	1.17±0.02[a]	0.39±0.00[a]	2.8±1.72[a]	77±2.51[a]	24±1[ab]	72.±2.0[ab]
National standards	17.5 – 21	-	-	0.60	0.35-2.95	>65	>65	>65
WHO/FAO	21 – 23	-	-	0.6 – 1	-	>65	>65	>65
World Standards	18 – 23	3.2 - 4.5	-	0.25 – 1	0.33-2.90	60 – 70	60 – 70	60 – 70

The moisture content of honey samples collected from Nekemte town ranged between 11.05 and 13.65 g/100 g, indicating relatively low moisture levels. These values fall within acceptable ranges for high-quality honey suitable for international markets, though they are slightly below the national and global standards set by WHO/FAO and other regulatory bodies (Buba et al., 2013; Areda, 2015). Additionally, the study revealed that titratable acidity levels of the honey samples ranged from 0.6% to 2.86%, which is within the acceptable range established by national and international standards.

Antimicrobial activity of honey

Seven honey samples were tested for their antibacterial efficacy against various microorganisms. The results demonstrated significant antibacterial activity, especially at higher concentrations. At 100% concentration, all honey samples exhibited stronger antibacterial action against the pathogens than at 75% concentration due to the dilution effect. Notably, Ebantu (Qello) honey showed the largest inhibitory zone (30 mm), indicating a potent antimicrobial effect. The antibacterial activity of Ebantu honey was demonstrated against several pathogens, including *S. flexneri* (34 mm), *P. aeruginosa* (40 mm), E. coli (40 mm), *S. typhimurium* (40 mm), and *S. aureus* (39 mm). Similar to Qello honey, honey samples from Limu and Haro Limu districts also exhibited strong antibacterial activity against all the tested pathogens.

Table 2

Antibacterial activity and Zone Diameters of Inhibition (ZDI) of Honey sold at Nekemte Town, 2022

Honey types	Concentration	*E.coli*	*S.typhimurium*	*S.aureus*	*P.aeruginosa*	*S.flexneri*
	100%	20.05±0.02	21.00±0.0^{e}	22.00±0.02^{e}	25.25±0.85^{d}	25.25±0.3^{cd}
Arjo Guddatu	75%	16.00±0.02^{ef}	16.00±0.25^{f}	17.00±096f	19.00±0.36^{ef}	19.36±0.2^{cd}
	50%	13.05±0.05^{f}	0.00^{h}	0.00^{h}	0.00^{h}	0.00^{h}
	100%	36.02±0.00^{b}	37.00±.07^{bc}	36.05±0.5^{b}	33.05±0.25^{c}	30.25±0.25^{b}
Mettu	75%	19.0±0.05^{de}	20.02±0.5^{e}	20.25±0.52^{ef}	20.36±0.66^{e}	20.36±3.69^{cd}
	50%	0.00^{h}	12.02±0.2^{g}	0.00h	12.36±0.36^{g}	0.00^{h}
	100%	40.05±0.2^{a}	40.00±0.05a	39.00±0.05^{a}	40.25±0.36^{a}	34.05±0.25^{a}
Beddele	75%	20.05±0.5^{d}	17.00±0.05^{f}	17.05±0.5^{f}	25.3±0.36^{d}	17.00±0^{c}
	50%	0.00^{h}	12.00±0.02^{g}	22.25±0.25^{e}	14.25±0.25^{g}	0.00^{h}
	100%	39.05±0.5^{a}	39.00±0.05^{a}	35.00±0.69^{b}	34.3±0.25^{c}	30.25±0.36^{b}
Gidda Ayena	75%	19.05±0.5^{de}	18.00±0.05^{f}	19.00±5.8^{ef}	18.00±0.25^{ef}	17.025±0.36^{c}
	50%	12.05±0.0^{fg}	13.05±0.5^{g}	16.00±0.25^{f}	0.00^{h}	0.00^{h}
	100%	20.05±0.08^{d}	20.36±0.36^{e}	22.58±.58^{e}	35.05±0.58^{c}	30.0±0.2^{b}
Limu	75%	16.05±0.3^{ef}	17.00±0.15^{f}	15.522±0.22^{f}	18.025±0.2^{ef}	19.00±0.6^{cd}
	50%	0.00^{h}	0.00^{h}	0.00^{h}	15.05±0.5^{g}	14.00±00.2^{d}
	100%	39.0±0.2^{a}	35.00±0.01^{c}	30.02±0.25^{c}	35.25±0.25^{c}	34.00±0.00^{a}
Haro	75%	20.02±0.25^{d}	20.00±0.2^{e}	16.00±0.25^{f}	17.25±0.25^{ef}	16.00±0.0^{c}
	50%	12.00±0.58^{fg}	0.00^{h}	0.00^{h}	0.00^{h}	0.00^{h}
Ebantu	100%	14.2±0.69^{f}	21.00±0.2^{e}	23.69±0.25^{e}	30.25±0.32^{c}	27.00±0.03^{c}
	75%	17.05±0.69^{e}	18.00±0.00^{f}	19.25±0.58^{ef}	20.36±0.2^{e}	17.00±0.03^{c}
	50%	0.00^{h}	0.00^{h}	0.00^{h}	0.00^{h}	0.00^{h}
Control		28.02±0.3^{c}	29.00±0.01^{d}	30.00±0.01^{c}	30.35±0.3^{c}	25.25±0.02^{cd}

Note: SD - Standard Deviation. Means are compared by One Way ANOVA-Post Hoc Tukey comparisons. Figures in the same column followed by the same letter superscript show that there is no significant variation, and in different letters, there is significant variation.

Arjo Guddatu honey showed the highest antibacterial activity against S. flexneri at 100% concentration, with an inhibition diameter of 35 mm. In contrast, Bedelle honey exhibited lower antimicrobial activity at 75% concentration but showed potent antibacterial effects at 100%, particularly against *E. coli* (36 mm), *S. typhimurium* (37 mm), *S. aureus* (36 mm), and *P. aeruginosa* (33 mm). Haro Limu honey demonstrated strong antibacterial activity at 100% concentration against *S. typhimurium* and *E. coli*, both with inhibition zones of 39 mm, although all strains exhibited resistance at 75%. Matu honey showed notable antibacterial effects against S. flexneri (20 mm) and *P. aeruginosa* (25 mm) at 100% concentration, although resistance was observed at 75%.

Overall, the honey samples tested in this study exhibited inhibition zones ranging from 12 mm to 40 mm. The most promising antimicrobial activity was observed in Ebantu (Qello) honey. The variations in antibacterial activity could be attributed to differences in vegetation, honey processing methods, and the types of microorganisms tested, as well as the honey and diluent concentrations used in each study (Table 2).

Discussions

The acidic nature of the honey samples is in line with previous findings reported for kinds of honey from Tigray (Kebede et al., 2014). The acidity, reflected in the pH values ranging from 3.32 to 3.96, is mainly attributed to the presence of organic acids. These acids not only enhance the flavor of honey but also play a crucial role in preserving its quality by inhibiting microbial growth and spoilage (Adebiyi et al., 2004). The variation in electrical conductivity observed among the honey samples could be due to differences in the botanical origin and mineral content of the nectar sources in the collection areas. Qello honey, with the highest EC, likely originates from flora with higher mineral content, whereas Limu honey, which showed the lowest EC, may come from nectar sources with less mineral diversity. These findings are consistent with the observations of Moniruzzaman et al. (2013), who reported that floral and geographical variations significantly affect the electrical conductivity of honey.

The observed acidity across all honey samples is consistent with findings from previous studies on Tigray and Tepi kinds of honey (Kebede et al., 2014). The acidic pH is attributed to the presence of organic acids, which enhance the flavor profile of honey and contribute to its antimicrobial and preservative properties (Adebiyi et al., 2004). The variation in electrical conductivity among samples likely reflects differences in mineral content and botanical origin, which are influenced by the geographical and floral diversity of the collection areas (Moniruzzaman et al., 2013). Additionally, the total sugar content showed substantial variation, ranging from 50% to 83%. According to local honey practitioners and honey-keeping sources, this discrepancy is influenced by factors such as the maturity of the honey and the type of nectar collected by bees. The results align with findings by Ibrahim et al. (2012), though they also highlight the need for further investigation into how environmental factors, floral sources, and honey-harvesting practices contribute to sugar composition. Understanding these factors is essential for improving honey quality and ensuring compliance with national standards.

This shows that while some honey samples fall within the QSAE range, some surpass the QSAE (2005) minimum limit of 65%. This has a comparable total sugar level to that reported by Ibrahim et al. (2012). It is crucial to remember, though, that variables like processing techniques, floral sources, and environment can also affect sugar concentration. To completely comprehend the elements influencing the variations in sugar concentration across various honey varieties, further investigation is required.

The relatively low moisture content observed in the honey samples is a significant indicator of good quality and suggests that the honey is less susceptible to microbial contamination and fermentation during storage. According to international standards set by Codex Alimentarius (FAO/WHO) and national quality guidelines, the maximum allowable moisture content in honey should not exceed 20 g/100 g. In this study, all samples were well below this threshold, demonstrating that they meet the basic quality requirements for both local consumption and export markets (Buba et al., 2013; Areda, 2015).

The variation in moisture content among the honey samples may be influenced by several factors. One of the primary contributors is environmental conditions during honey production, particularly temperature and humidity levels, which affect the rate of water evaporation from the nectar. Regions with dry climates are more likely to produce honey with lower moisture content due to faster dehydration. Additionally, harvesting practices such as the timing of collection (ensuring the honey is mature and fully capped) can significantly influence moisture levels. Harvesting immature honey, which still contains high water content, often results in poor-quality honey that is more likely to ferment.

Another important factor is the floral source of the nectar. Different plant species produce nectar with varying moisture levels, and this variability can directly affect the final moisture content of the honey. Similarly, post-harvest practices such as honey processing and storage conditions play an essential role. Honey stored in a dry, sealed environment tends to retain its low moisture level, while improper storage can lead to moisture absorption from the surrounding environment, especially in humid conditions (Ibrahim et al., 2012). The findings of this study are consistent with those reported by other researchers. For instance, Buba et al. (2013) and Gebreegziabher et al. (2013) also observed low moisture content in high-quality honey samples from different Ethiopian regions. Such characteristics not only enhance the microbial stability of honey but also contribute to improved physical properties such as viscosity and texture.

The relatively low moisture content observed in the honey samples is likely due to several environmental and management-related factors. Conditions such as dry climates, warm environmental temperatures, and extended ripening time in the hive likely promoted water evaporation. Moreover, traditional harvesting practices, hive types, and floral sources also influence moisture retention in honey (Areda, 2015; Ibrahim et al., 2012). Lower moisture content is advantageous, as it contributes to better stability, reduces the risk of fermentation, and improves honey's shelf life, making it more desirable for both local and global markets (Gebreegziabher et al., 2013).

The acidity of honey, influenced by the presence of organic acids and inorganic ions, contributes to its antimicrobial activity and flavor profile. The observed low titratable acidity values suggest that the honey is fresh and has undergone minimal fermentation. According to Moussa et al. (2012), higher levels of free acidity in honey are typically the result of fermentation processes initiated by osmophilic yeasts, which convert glucose and fructose into alcohol and carbon dioxide. These byproducts can further oxidize into organic acids, increasing the free acidity of honey. However, the relatively low acidity values found in this study indicate that the honey was likely well-ripened, properly harvested, and adequately stored, minimizing microbial degradation. Maintaining appropriate moisture and acidity levels is essential for ensuring honey's quality, marketability, and consumer satisfaction. Therefore, producers should continue to adopt good harvesting and storage practices to meet both domestic and international standards.

The results highlight the variation in antibacterial efficacy between different honey samples, which may be attributed to differences in their physicochemical properties, floral sources, and production methods. The higher concentrations (100% and 75%) exhibited a greater antibacterial action, likely due to the increased concentration of antimicrobial compounds, such as hydrogen peroxide, flavonoids, and phenolic acids, which are known for their antimicrobial properties (Molan, 1992). Haro and Bedelle honey samples were particularly effective, possibly due to their higher levels of these compounds. The broad antibacterial activity observed in Ebantu (Qello) honey suggests the presence of diverse phytochemicals that provide strong protection against a wide range of pathogens. Limu honey, despite showing moderate antibacterial effects at 75% concentration, had significant efficacy against several key bacterial strains, indicating that its antibacterial properties might be more selective. The potent action of Arjo Guddatu honey against *S. flexneri* suggests that specific honey varieties may have targeted activity against certain pathogens.

The resistance observed at the 50% concentration can likely be explained by the dilution effect, which reduces the concentration of active compounds in the honey. The finding that all strains exhibited resistance at the 75% concentration in some samples, such as Haro Limu honey, also suggests that the antibacterial efficacy of honey varies not only with its concentration but also with its unique composition and the types of pathogens being tested. Further research is needed to isolate and identify the specific antimicrobial compounds in each honey variety and to evaluate their effectiveness against a broader range of bacterial and fungal pathogens. Such studies provide valuable insights into the potential of honey as a natural antimicrobial agent in clinical and agricultural settings.

The study's findings align with previous research that highlights the antimicrobial properties of honey. The antibacterial effects observed in the samples tested are primarily due to the physicochemical characteristics inherent to honey, including its hydrogen peroxide content, organic acids, and high sugar concentration. Hydrogen peroxide, produced by the glucose oxidase enzyme in bees' hypopharyngeal glands, is a known antimicrobial agent that inhibits the growth of pathogens by producing reactive oxygen species (ROS) that damage microbial cells (Khan et al., 2007).In addition to hydrogen peroxide, the acidic nature of honey, with pH values ranging from 3 to 4.5, contributes to its antimicrobial properties by creating an unfavorable environment for the growth of many microorganisms (Weston, 2000). Organic acids, such as gluconic acid, play a significant role in lowering the pH and further inhibiting microbial growth. Furthermore, honey's high sugar content, which is typically around 80% (w/w), exerts an osmotic effect that dehydrates microorganisms, thereby limiting their growth and survival.

Non-peroxidic compounds, including polyphenols, are also believed to contribute to honey's antimicrobial activity. Polyphenols have antioxidant properties that help neutralize free radicals and protect cells, and they are known to possess direct antimicrobial effects by interfering with bacterial cell walls (Molan, 1992). These compounds are especially important for kinds of honey that exhibit strong antibacterial activity even in the absence of significant levels of hydrogen peroxide.

The results from this study support the antimicrobial potential of honey samples from various districts of Nekemte town, showing their ability to inhibit both Gram-positive and Gram-negative bacteria. However, while the antibacterial activity of these honey samples is promising, further research is needed to confirm their suitability for treating bacterial infections. Specifically, additional studies are required to examine the role of peroxidase activity and the potential interference of wound-produced catalase in diminishing honey's antimicrobial effectiveness. The potential for honey to be used as a therapeutic agent must also be investigated in clinical settings to fully understand its potential in medical applications.

Furthermore, the variability in antibacterial activity across different honey samples highlights the importance of understanding the factors that influence honey's antimicrobial properties, including its botanical origin, processing methods, and environmental conditions. As this study only assessed a limited number of honey samples, further research with a broader range of honey varieties will help clarify the extent of honey's antibacterial potential and its practical applications in health care.

CONCLUSIONS

The findings of this study confirm again that Nekemte honey, East Wollega Zone, is extremely physico-chemically heterogeneous and has immense antimicrobial power. pH varied between 3.32 and 3.96, whereas electrical conductivity varied from 0.48 mS/cm to 1.17 mS/cm, showing diversity perhaps due to floral source and environment. The sugar content varied from 50 g/100 g to 83 g/100 g, as per the standards of honey quality prescribed. The antibacterial activity of honey samples was outstanding against common pathogenic bacteria, i.e., *Staphylococcus aureus, Escherichia coli, Salmonella enterica serovar Typhimurium, Shigella flexneri*, and *Pseudomonas aeruginosa.* Among the samples attempted, Ebantu (Qello) honey was at its maximum at higher concentrations and thus is an excellent prospect as a natural antimicrobial agent. Jemoa honey was effective at high concentration but failed when used at a concentration level of 50%, where the resistance of all the pathogens was noted.

Recommendations

Based on these results, honey derived from Nekemte is concluded to have the potential to be utilized as a natural medicine for wound healing and infection prevention. The physicochemical property variability reflects the abundance of the surrounding environment but suggests a requirement for the standard process of assessment. Therefore, it is recommended,

1. Additional research is conducted to clinically test the medicinal quality of local honey under in vivo conditions.

2. Regulated quality control tests for the evaluation of honey should be introduced and governed in the region.

CRediT authorship contribution statement

Temesgen Tafesse: Formal analysis, Investigation, Resources, Data Curation, Visualization, **Desalegn Amenu:** Writing - Original Draft, Writing - Review & Editing, **Ayantu Nugusa:** Conceptualization, Methodology, Validation

Declaration of competing interests

The authors affirm that there is no conflict of interest.

Ethical approval

The authors declare that no human participants, their data, or biological material were used in this study.

Data availability statement

Adequate data is available and will be presented upon request.

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