RESEARCH ARTICLE

Physicochemical and antibacterial activity of honey sold in Nekemte, East Wollega Zone, Western Ethiopia

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Background: In regions like Nekemte, located in the East Wollega Zone of Western Ethiopia, honey production is not only a traditional practice but also a source of livelihood for many communities. Understanding the physicochemical attributes and biological activities of honey from this region is essential for assessing its quality, safety and potential health benefits. This study evaluated the physicochemical characteristics and antibacterial activities of honey sold at Nekemte, East Wollega Zone.

Materials and methods: Honey samples were collected from various vendors in Nekemte and analyzed for their physicochemical characteristics, including moisture content, pH, total acidity, color and sugar composition. The antibacterial activity of the honey samples was assessed using agar well diffusion and broth micro dilution methods against selected bacterial strains.

Results: Antimicrobial activity of honey tested against five bacteria: *S. aureus*, *E. coli*, *S. typhimurium*, *S. flexneri* and *P. aeruginosa*. The results showed that the honey had a pH and EC value of 3.5 to 3.96 and 0.48 m/Scm to 1.17 m/Scm, respectively. Honey has been known to possess antimicrobial properties and wound healing activity. The total sugar and ash contents varied from 65 g/100 g to 83.00 g/100 g and 0.28 g/100 g to 0.75 g/100 g. The antibacterial activity assay was conducted at concentrations of 100%, 75% and 50%. Jemoa honey showed more antibacterial effects at 100%, while at 75%, all pathogens had fewer effects below 10 mm. At 50%, all strains were found to be resistant.

Conclusion: Overall, the study demonstrated that Ebantu (Qello) honey exhibited significant antibacterial properties against common pathogens, particularly at higher concentrations. These findings support the potential use of honey as a natural antimicrobial agent in wound care and infection control.

Key Words: *Honey; Antimicrobial activities; Physiochemical property*

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 [1]. 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 [2]. Antimicrobial agents reduce infectious diseases globally, but resistance to pathogens weakens their effectiveness, posing a serious threat to public health and all antibiotics, including major last-option drugs [3].

Honey, a natural food with a sweet taste, has antimicrobial properties due to its high concentration of monosaccharide sugars. Its low pH and presence of hydrogen peroxide preserve its antimicrobial properties, but too high a pH can make it unpalatable. Honey has been used in traditional medicine for its antimicrobial properties, including wound healing [4]. There haven't been many studies on natural honey from Ethiopia to assess its antibacterial properties. When tested in vitro, honey produced by honeybees (A*pis mellifera*) demonstrates both bacteriostatic and bactericidal activity. However, before using honey as a preventive and curative measure for common diseases related to the tested bacterial species, pharmacological standardization and clinical evaluation of the effect of honey are essential [5].

The study examined various honeys for their antibacterial activity and their effects on pathogenic microbes. It was found that when honey was introduced to harmful bacteria, the samples' development was suppressed, indicating antibacterial activity. The in Nekemte town, East Wollega Zone, known for its natural resources and diverse flora, has not yet been studied for its physicochemical properties and antimicrobial activity. The investigation aims to fill this knowledge gap.

MATERIALS AND METHODS

Study area and design

The study was conducted in East Wollega Zone, Nekemte town and the honey samples sold at Nekemte town were collected from different zones, Buno Bedelle, Illuababora, East Wollega districts. This experimental laboratory study investigated the antimicrobial activity of honey against various human pathogenic bacteria. Physiochemical analysis of honey was conducted and the minimum inhibition concentration, inhibitory concentration and bactericidal concentration were also investigated. The research design was experimental.

Samples and sampling techniques

Samples of bee honey were gathered from places that sold it in Nekemte tow and kept at room temperature for the night before being subjected to microbial and physiochemical analysis in the biology department of Wollega University. The Ethiopian Public Health Institute in Addis Ababa, Ethiopia provided the cultures of pathogenic bacteria. Among these are *S. aureus* (ATCC 25923), *S. flexneri* (ATCC 12022), *S. typhimurium* (ATCC 14028), *P. aeruginosa* (ATCC 27853), and *E. coli* (ATCC 25922).

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Physicochemical analysis

A homogenized honey solution was prepared by dissolving 5 g of honey in 50 ml of distilled water. The pH was measured using a digital pH meter, calibrated every time. The conductivity cell was used to determine the pH. The honey solution was then diluted and heated until boiling. The reaction was completed with a methylene blue indicator and titration. Ash content was determined using the AOAC official method 942.05. The stainless steel crucibles were washed, rinsed and oven dried. Ten grams of honey samples were weighed and placed in a furnace at 110°C for half an hour and 600°C for two hours. After cooling, the mass was measured [6]. The relative amount of lactic acid was determined using mathematical formulas. The honey samples were then titrated against 0.1 N NaOH using phenolphthalein as an indicator. To determine, carbohydrates, a honey sample was weighed, stirred and diluted with distilled water and chloric acid reagent. The extract was diluted and pipetted into test tubes, using different sugar standards. Anthrone reagent was added to the tubes and they were placed in a boiling water bath for 12 minutes. The solutions were cooled and absorbance at 630 nm was read against blanks. Results were calculated using Agbagwa et al.'s formula [7].

Antimicrobial activity

The study used five bacterial strains and evaluated the antimicrobial activity of honey using the agar diffusion method. The honey sample was added to wells, and chloroamphinicole was used as a positive control. The antimicrobial activity was measured by measuring the inhibition zone around each tested substance. The one-way ANOVA and descriptive statistics were used to compare the antibacterial effects (inhibitions) of honeys. Version 20 of the Statistical Package of Social Science (SPSS) was used for all statistical analysis.

RESULTS

Physicochemical analysis

The physico-chemical properties of honey significantly influence its quality and antimicrobial activity. The pH values of honey, ranging from 3.2 to 3.96 (Table 1), indicate that the honeys analyzed were acidic, in line to this Tigray honey and Tepi honey having higher pH values which is more similar with this study. The study found all samples had a pH value between 3.5 and 3.98, with an overall mean of 3.9. This acidity is due to the presence of organic acids, which contribute to honey flavor and stability against spoilage [8]. The study found that the Electrical Conductivity (EC) of nectar honey ranged from 0.48 m/Scm (Limu) to 1.17 m/Scm (Qello), with higher values in Ebantu district (Qello) honey. This indicates different flora species in each locality, similar to results from Malaysia were reported, where honeys from different floral sources had varying EC values [9].

Furthermore, the study found that the total sugar content of honey Sold in Nekemte town, ranged from 50 g/100 g (Haro Limu) to 83.00 g/100 g (Bedelle), with sucrose levels varying based on maturity and nectar compound origin (Table 2). This indicates that some honey samples are within the QSAE range, while others exceed the minimum limit of 65% set by QSAE. The total sugar content of this is similar to those reported. However, it is important to note that variations in sugar content can also be influenced by factors such as climate, floral sources and processing methods. Further research is needed to fully understand the factors contributing to the differences in sugar content among different types of honey.

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/100 g) (Mean ± SD)	Reducing sugars g/100 g) (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^{b}	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^{b}
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	-	-	0.6	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

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 significance variation and different letters there is significant variation

The study found that the mean moisture content of honey samples was between 11.05 and 13.65 g/100 g, which are acceptable for the world honey market. However, this value is lower than previous studies, which reported higher moisture content. The lower moisture content in the honey samples analyzed in this study may indicate a higher quality product with better stability and shelf life. It is important for producers to maintain consistent moisture levels to meet market standards and consumer expectations [10]. Honey moisture content varies based on harvesting season, maturity, hive type, environmental temperature and plant moisture content, ranging from 13% to 23%, depending on source and climatic conditions [11]. The study found a low titrable acidity of 0.6 to 2.86% in honey, which may be due to the presence of organic acids and inorganic ions. This acidity is within acceptable limits of international and national recommended values for honey free acidity. High free acidity values may indicate yeast fermentation, where glucose and fructose are converted into carbon dioxide and alcohol, contributing to the level of free acidity in honey. He found high free acidity values in honey due to yeast fermentation, where glucose and fructose are converted into carbon dioxide and alcohol [12-15].

Antimicrobial activity of honey

The study evaluated the antimicrobial activity of seven different honey samples against pathogenic bacteria. The results showed that at 100% and 75% concentrations, Arjo Guddatu, Limu, Qello and Haro honey showed high activity against some human pathogens, but almost all strains showed resistance at 50% concentration.

Honey samples from showed potent antimicrobial activity against almost all pathogens at 100% concentration, possibly due to dilution factors. Ebantu honey had the widest mean zone of inhibition, while Haro and Bedelle had high antimicrobial activity. Arjo Guddatu, honey had more antibacterial effects on *S. flexneri*, while Limu honey had high effects on *E. coli*, *S. typhimuriu*, *S. aureus* and *P. aeruginosa* at 100% concentration but had low antimicrobial activity at 75% concentration. Haro Limu honey had high antibacterial effects on *S. typhimurium* and *E. coli*, but all strains were resistant at 75% concentration (Table 2).

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*
Arjo Guddatu	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}
	75%	16.00 ± 0.02^{ef}	16.00 ± 0.25^{f}	17.00 ± 096^{f}	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}
Mettu	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}
	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}	0.00^{h}	12.36 ± 0.36^{g}	0.00^{h}
Beddele	100%	40.05 ± 0.2^{a}	40.00 ± 0.05^{a}	39.00 ± 0.05^{a}	40.25 ± 0.36^{a}	34.05 ± 0.25^{a}
	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}
Gidda Ayena	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}
	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}
Limu	100%	20.05 ± 0.08^{d}	20.36 ± 0.36^{e}	22.58 ± 0.58^{e}	35.05 ± 0.58^{c}	30.0 ± 0.2^{b}
	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}
Haro	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}
	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}

DISCUSSION

For the most parts, all honey samples showed potent antimicrobial activity against all pathogens at 100% concentration than 75% concentration and this may be due dilution factors. Among all honey samples, Qello (Ebantu district) honey demonstrated the highest inhibition zone (30 mm). Hence, honey collected from Ebantu Woreda showed potential inhibitory action against *E. coli* (40 mm), *S. typhimurium* (40 mm), *S. aureus* (39 mm) *P. aeruginosa* (40 mm) and *S. flexneri* (34 mm). Similarly, honey samples collected from Haro Limu and Limu district had shown high antimicrobial activity against all selected pathogens next to Qello honey (Table 2). In addition, Arjo Guddatu honey had more antibacterial effects at 100% aganist *S. flexneri* with 35 mm inhibition diameter. Onother hand, Beddele honey had demonstrated potential antibacterial effects high against *E. coli* (36 mm), *S. typhimuriu* (37 mm), *S. aureus* (36 mm), *P. aeruginosa* (33 mm) at 100% and but it had low antimicrobial activity at 75% concentration (Table 2). Haro Limu honey has high antibacterial effects at 100% concentration on *S. typhimurium* and *E. coli* 39 mm and 39 mm respectively but both strains are resistant at 75% and the other strains have below 10 mm. Matu honey has high antibacterial effects on *S. flexneri* and *P. aeruginosa* 20 mm and 25 mm respectively but all strains are resistant at 75% concentration (Table 2).

In this study, the antimicrobial activity of honey samples was ranged from 12 mm-40 mm from with Ebantu (Qello) honey showed the most potential antimicrobial activity. The results of this study are greater than the result which is ranged from 13 mm-15 mm inhibition zone. Similarly, reported that, the honey samples from India showed potential antagonistic effects against both grams positive and negative bacteria, where inhibition zone ranged from 6.94 mm to 37.97 mm, which is more lined with the current study. This difference could be attributed duet differences in vegetation type widely cultivated in study araes, processing of honey and type of tested micro-organisms and this could probably be due to the concentration of honey used and concentration of diluent used to dilute the honey.

The general characteristics of honey to prevent bacterial growth have been explained by various scientists who reported inhibition of pathogenic microbial growth from presence of hydrogen peroxide resulting from the action of glucose oxidase enzyme produced from hypopharyngeal glands of worker's bees on glucose in presence of oxygen that inhibits microbial and fungal growth. Presence of inherent physical-chemical properties such as high sugar content (about 80% w/w) that results into high osmotic effect that dehydrate the micro-organisms has been reported to contribute inhibition of microbial growth and inhibition of microbial growth to be due to presence of diverse organic acids such as gluconic acids that remarkably creates an acidic micro-environment (pH 3-4.5) that prevents growth of many micro-organisms. Apart from hydrogen peroxide as a factor that inhibits microbial growth. Elucidated inhibition of microbial growth to be due to presence of non-peroxidic substances such as polyphenols which possess anti-microbial activity.

The results of the present study indicated that almost all of the honey quality parameters analyzed from all locations of the study area revealed that, all the physicochemical parameters in line with-in limits of national and international standards set by Ethiopian Conformity Assessment Enterprise for analyzing all honey samples (ECAE), Codex Alimentarius Commission and EU Council. It is possible to conclude that although honey samples sold at Nekemte town were found to possess antibacterial activity against gram positive and gram negative bacteria, further research needs to be done to confirm the usefulness of those honeys for different

bacterial infection treatments since, all the honeys were found to possess peroxidal activity which brings questions about the role of catalase produced by wounds in the elimination of this antimicrobial effect.

CONCLUSION

The comprehensive assessment of the physicochemical properties and antibacterial activity of honey sold in Nekemte, East Wollega Zone, Western Ethiopia, sheds light on its quality, potential therapeutic value and suitability for various applications. Through this study, several key conclusions can be drawn.

The honey samples from Nekemte exhibited a range of physicochemical characteristics, including moisture content within acceptable limits, varying pH levels indicative of acidity, total acidity values reflecting differences in floral sources and processing, and a spectrum of colors from light to dark hues. The predominant presence of glucose and fructose in the honey samples underscores their nutritional value and sweetness, contributing to the sensory appeal of the honey sourced from this region. The antibacterial testing revealed notable activity against both gram-positive and gram-negative bacteria, highlighting the potential therapeutic relevance of Nekemte honey in combating microbial infections. The mechanisms underlying this antibacterial activity, such as the production of hydrogen peroxide, low pH, and presence of bioactive compounds, contributes to its antimicrobial efficacy. The findings suggest that honey from Nekemte meets essential quality parameters and safety standards, making it suitable for consumption and potential use in healthcare settings. The documented physicochemical and antibacterial properties of Nekemte honey have significant implications for its utilization in traditional medicine, food preservation, natural remedies and possibly pharmaceutical applications. Its local availability also promotes sustainable beekeeping practices and economic development in the region.

ETHICS APPROVAL

Not applicable.

CONSENT FOR PUBLICATION

Not applicable.

DATA AVAILABILITY

The data used and analyzed during the current study are available within the manuscript.

CONFLICT OF INTEREST

The authors have no any conflict of interest.

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AUTHORS' CONTRIBUTION

DA, RD, CT, AN, AT, DD and TT participating on Data collection, field work and data conceptualization, in addition, they are participating 1st, 2nd and 3rd author. All authors were participating on data analysis, edition and conceptualization; furthermore, DA is the corresponding author. Copy editing, translation, paraphrasing and finally edition was supervised and processed by DA.

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