Antimicrobial and Physiochemical Activities of Semi-Arid Honey and Beeswax Quality

By

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Abstract

Many ethnic groups have historically employed bee products, such as honey and beeswax, to treat a variety of ailments due to their known inhibitory impacts on bacteria and fungi (p. 2). In this study, honey and beeswax collected from the semi-arid University of Dodoma apiary were evaluated for their physiochemical and antibacterial qualities (p. 2). Each honey sample was examined for moisture content, ash, acidity, pH, reducing sugars, apparent sucrose, and hydroxymethylfurfural (HMF) (p. 2). Using the disk diffusion method, the antimicrobial activity of both honey and beeswax samples was assessed against *Escherichia coli*, *Staphylococcus aureus*, and *Candida albicans* (p. 2). The results showed that all tested honey parameters met accepted international standards (p. 2). Honey and beeswax gel samples demonstrated high sensitivity against the tested microbes (p. 2). Consequently, it was concluded that the honey and beeswax from this study align with international quality standards (p. 2). Additionally, the presence of bioactive components in the studied samples justifies their traditional use for treating various ailments across Africa (p. 2).

Keywords: Honey, beeswax, antimicrobial, microbes, sensitivity (p. 2)

Introduction

In developing nations like Tanzania, poor sanitation and hygiene expose communities to a wide variety of microbial pathogens (p. 3). Infectious diseases, particularly bacterial and fungal infections, account for almost half of all fatalities in these regions (p. 3). More than 3 child deaths from diarrheal illnesses occur annually in impoverished nations, typically brought on by bacterial species such as *Escherichia coli*, *Shigella flexneri*, and *Salmonella typhi* (p. 3). Conversely, fungal infections, especially those caused by *Candida albicans* and *Cryptococcus neoformans*, pose major health issues for people living with HIV/AIDS (p. 3).

The mainstay for preventing bacterial and fungal infections has been the use of commercial antibiotics (p. 3). However, the growth of antibiotic-resistant bacteria remains a major global

concern, driving substantial work toward formulation of stronger antimicrobial medications (p. 3). Despite these efforts, high drug expenses, poor quality of some marketed drugs, and the emergence of drug-resistant pathogens force rural populations to rely on conventional natural options like honey for disease treatment (p. 3).

Even before the discovery of microorganisms, many ethnic groups employed bee products like honey and beeswax to heal a variety of ailments (p. 3). The large variety of chemicals found in honey and beeswax is primarily responsible for their systemic medical efficacy against both chronic and infectious disorders (p. 3). The World Health Organization (WHO) estimated that approximately 75% of the world's population in developing countries depends on bee products to meet basic healthcare desires (pp. 3-4).

Tanzania is the second-largest producer of honey in Africa after Ethiopia, ranking tenth globally, and is the largest African exporter to European Union markets (p. 4). However, insufficient research has been done on the nation's local antimicrobial and honey quality profiles (p. 4). Because the semi-arid thickets of central Tanzania contribute heavily to regional honey production, providing scientific data on the quality and antibacterial characteristics of honey from this location is crucial (p. 4). Therefore, the purpose of this study is to describe the physicochemical and antimicrobial properties of honey and beeswax obtained from the University of Dodoma apiary (p. 4).

Materials and Methods

Acquirement of Materials

Honey samples were taken randomly from 80 beehives at the University of Dodoma bee apiary (p. 4). Until evaluation, the sampled honey was stored in dark-colored glass jars at room temperature away from direct sunlight (p. 4). Three microbial strains (*Escherichia coli*, *Staphylococcus aureus*, and *Candida albicans*) were purchased from the Microbiology and Immunology division of the University of Dodoma, Tanzania (p. 4). Nutrient broth and nutrient agar were supplied by HIMEDIA Laboratories Pvt. Limited, Mumbai, India (p. 4). Gentamycin and fluconazole positive controls were provided by Mediatech Incorporation (Manassas, USA) and SIGMA® (Sigma-Aldrich®, St. Louis, USA), respectively (p. 4).

Determination of Moisture Content

A standard technique recommended by the AOAC (1980) was used to determine moisture content (p. 4). Approximately 2 mL of triplicate honey samples were placed in pre-weighed, dried crucibles, heated to 110°C overnight, and then weighed (pp. 4-5). The percentage weight loss was computed using the standard formula below: (p. 5)

$$\text{Moisture\ (\%)}=\frac{\text{Weight\ of\ Fresh\ Sample}-\text{Weight\ of\ Dry\ Sample}}{\text{Weight\ of\ Fresh\ Sample}}\times 100$$
(p. 5)

Assessment of Sugar Content

Total sugar content was ascertained using a Mettler Toledo Densito 30PX refractometer (p. 5). To remove suspended particles (such as pollen grains, tiny bits of comb, and other pollutants) that might skew readings, 1 mL of each honey sample was weighed, diluted tenfold, mixed thoroughly, and filtered using Whatman filter paper prior to measurement (p. 5). The values were recorded in °Brix and subsequently converted to g sugar/g honey (p. 5). The experiment was performed in triplicate to calculate mean values and standard deviations (p. 5).

Assessment of pH Measurement

An AOAC-recommended Thermo Scientific RUSSEL RL 060P digital pH meter was used to measure the pH of honey samples (p. 5). The electrode was rinsed with distilled water and dried with tissue paper between readings (p. 5). Exceptionally thick honey samples were diluted twice with distilled water before inserting the electrode (p. 5). The experiment was performed in triplicate (p. 5).

Assessment of Ash Content

The AOAC (1980) standard method was used to determine honey ash content (p. 5). A furnace was used to heat dried, pre-weighed crucibles containing around 3 g of honey at 500°C for 5 hours until white or greyish-white ash samples were obtained (p. 5). After cooling inside a desiccator, the ash sample was weighed (pp. 5-6). The percentage ash content was calculated using the following formula: (p. 6)

$$\text{Ash\ (\%)}=\frac{\text{Weight\ of\ Sample\ After\ Ashing}}{\text{Weight\ of\ Fresh\ Sample}}\times 100$$
(p. 6)

Assessment of Antimicrobial Activity

Using the disc diffusion method, the sensitivity of collected honey and beeswax against the target pathogenic microorganisms was assessed (p. 6). Whatman paper discs (6 mm diameter) were saturated with 50 µL of honey and beeswax samples (p. 6). To allow test materials to diffuse into the growth media, the discs were placed onto petri dishes seeded with nutrient broth for bacteria and Sabouraud's dextrose broth for fungi (p. 6).

After incubation for 4 hours at 4°C to permit diffusion, the discs were incubated at 37°C for 24 hours (p. 6). A vernier caliper was used to quantify the zone of inhibition (ZOI) that formed around the discs (p. 6). The size of the ZOI represented the comparative sensitivity of the antimicrobial activity (p. 6). All experiments were performed in triplicate (p. 6). Standard

antibiotic (Gentamycin) and antifungal (Fluconazole) discs were utilized as positive controls (pp. 6, 10).

Phytochemical Screening

Initial qualitative screening was performed for alkaloids, terpenoids, flavonoids, tannins, and saponins in the honey and beeswax samples (p. 6). Results are represented qualitatively as mild positive (+), positive (++), highly positive (+++), or negative (-) for absence (pp. 6, 11).

- **Alkaloids:** A few drops of Dragendorff's reagent were added to test tubes containing 1 mL of sample; an orange color shift indicated the presence of alkaloids (p. 7).
- **Terpenoids:** 5 mL of sample was mixed with 3 mL of chloroform and 2 mL of strong sulfuric acid (H_2SO_4); a reddish-brown color shift indicated the presence of terpenoids (p. 7).
- **Flavonoids:** 5 drops of diluted sodium hydroxide (NaOH) were added to 2 mL of sample, followed by 5 drops of diluted hydrochloric acid (HCl); a yellow solution with NaOH that turned colorless upon adding HCl confirmed flavonoids (p. 7).
- **Tannins:** 2 mL of sample was combined with 3 mL of distilled water and 5 drops of ferric chloride (FeCl_3); formation of a dark blue precipitate indicated tannins (p. 7).
- **Saponins:** 5 mL of sample was shaken vigorously with 5 mL of distilled water; persistent foam creation indicated the presence of saponins (p. 7).

GC-MS Analysis

Gas Chromatography-Mass Spectrometry (GC-MS) analysis was carried out using an Agilent 6890N GC connected to an Agilent 5975 MS with an HP-5 capillary column (30 m length, 0.25 mm diameter, 0.25 µm film thickness) (p. 8). Helium gas (99.999%) served as the carrier gas at a constant flow rate of 1 mL/min, utilizing an injection volume of 1 µL (p. 8).

The injector and ion source temperatures were maintained at 250°C and 280°C, respectively (p. 8). The oven temperature program started at 110°C (isothermal for 2 min), increased by 10°C/min to 200°C, then by 5°C/min to 280°C, ending with a 9-minute isothermal hold at 280°C (p. 8). The mass spectrometer operated in electron ionization mode at 70 eV with an ion source temperature of 230°C and an inlet line temperature of 200°C (p. 8). Total GC-MS runtime was 36 minutes (p. 8). Interpretation of mass spectra was performed using the National Institute of Standards and Technology (NIST) database containing over 62,000 patterns (p. 8).

Statistical Analysis

Tests were carried out in triplicate, and data were presented as means \(\pm \) standard deviation (p. 8). The zone of inhibition means for the control and experimental samples were compared using a student's t-test (p. 8). The level of significance was fixed at \(p = 0.05\) using STATISTICA software version 8 (p. 8).

Results

Physicochemical properties of the evaluated honey samples (moisture, sugar, ash content, and pH) fell within generally recognized international norms, as detailed in Table 1 (p. 9).

Table 1: Physicochemical parameters of sampled honey (p. 9)

Parameter	Measured Value	Standard Reference Value
Moisture content	17.57%	\(\le \) 21%
Sugar content	72.5%	\(\ge \) 65%
pH	3.98	3.9
Ash content	0.25%	\(\le \) 0.6%

The antimicrobial activity tests indicated that there were notable inhibitory effects from both honey and beeswax, displaying variations across the bacterial and fungal species tested (Table 2) (pp. 9-10).

Table 2: Antimicrobial zone of inhibition (mm) of honey and beeswax (p. 10)

Sample	*E. coli*	*S. aureus*	*C. albicans*	p-value
Honey	38.13 \(\pm \) 0.078	28.10 \(\pm \) 0.047	32.56 \(\pm \) 0.062	0.000057
Beeswax	24.21 \(\pm \) 0.062	23.31 \(\pm \) 0.047	32.30 \(\pm \) 0.089	0.000448
Gentamycin	40.26 \(\pm \) 0.065	30.26 \(\pm \) 0.044	N/A	0.000001
Fluconazole	N/A	N/A	34.74 \(\pm \) 0.047	0.000003

Key: Values are expressed as mean \(\pm \) SEM. (p. 10)

Phytochemical screening revealed a strong presence of main secondary metabolites in honey and beeswax, except for flavonoids, which were absent (Table 3) (p. 11).

Table 3: Phytochemical screening of honey and beeswax (p. 11)

Phytochemical Component	Honey	Beeswax
Alkaloids	+++	++
Terpenoids	+++	+
Flavonoids	-	-
Tannins	++	-
Saponins	+++	-

Key: + = mild positive; ++ = positive; +++ = highly positive; – = negative. (p. 11)

GC-MS analysis of the honey samples successfully identified six volatile phytochemical components contributing to these biological activities, detailed in Table 4 (pp. 11-12).

Table 4: Volatile compounds identified in sampled honey via GC-MS (p. 12)

S/N	Retention Time (min)	Peak Area (%)	Name of Compound	Molecular Formula	Molecular Weight (g/mol)	Reported Bioactivity
1	11.58	0.05	Dodecane	\(\text{C}_{12}\text{H}_{26}\)	170.33	Antibacterial
2	13.88	8.58	Copaene	\(\text{C}_{15}\text{H}_{24}\)	204.35	Antimicrobial, antioxidant
3	14.74	0.01	Heptacosane	\(\text{C}_{27}\text{H}_{56}\)	380.73	Antibacterial
4	29.69	9.40	Phytol	\(\text{C}_{20}\text{H}_{40}\text{O}\)	296.53	Antimicrobial, anticancer, diuretic, anti-inflammatory
5	33.66	1.43	Nonacosane	\(\text{C}_{29}\text{H}_{60}\)	408.79	Antibacterial (associated with tetany/anemia/edema management)

6	39.51	1.30	Dotriacontane	\(\text{C}_{32}\text{H}_{66}\)	450.87	Antimicrobial, antioxidant, antispasmodic

Discussion

The moisture content (17.57%) of the honey in this study falls comfortably within the Tanzanian Honey Quality Guidelines, which stipulate a maximum limit of 21% (pp. 9, 13). Low moisture content plays an important role in extending shelf life and preventing quality loss from microbial fermentation (pp. 13-14). These results align with historical Tanzanian data showing honey moisture levels ranging from 13.9% to 27.9% (p. 14).

Furthermore, total sugar content reached 72.5%, satisfying minimum industry standards (\(\ge \) 65%) and validating the high quality of honey from the University of Dodoma apiary (pp. 9, 14). The recorded acidic pH level of 3.98 reinforces the honey's natural immunity against unexpected bacterial degradation (pp. 9, 14). Similarly, the ash content of 0.25% met national and international standards (\(\le \) 0.6%), showing fewer structural impurities than honey samples from other parts of Tanzania (pp. 9, 14-15).

Sensitivity tests indicated strong therapeutic potential for these products (p. 15). Honey demonstrated clear antibacterial properties against *S. aureus* and *E. coli*, highlighting its viability as a source for future drug lead compounds (p. 15). Beeswax acted selectively against *Candida albicans* with a notable 32.30 mm zone of inhibition, confirming its potential application as a dedicated antifungal agent (p. 15). While the tested beeswax was ineffective against the two bacterial strains (*E. coli* and *S. aureus*), this variation from European studies can likely be attributed to geographical and environmental differences that fundamentally shape chemical compositions in bee products (pp. 15-16).

GC-MS analysis successfully validated the presence of key bioactive molecules like phytol, copaene, and dotriacontane, which possess clear antimicrobial, anti-inflammatory, and antioxidant profiles (pp. 12, 16).

Conclusion

This study demonstrates that the University of Dodoma bee apiary yields high-quality honey and beeswax meeting strict national and international standards (p. 16). The robust presence of bioactive secondary metabolites supports their continued usage in African traditional medicine systems (p. 16). Further research is recommended to isolate and fully characterize these therapeutic secondary metabolites to aid targeted drug discovery (p. 16).

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