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**A COMPREHENSIVE REVIEW OF HOUSEHOLD METHODS FOR
EVALUATING THE PHYSICAL, BIOCHEMICAL, ANTIOXIDANT, AND
ANTIMICROBIAL PARAMETERS OF HONEY**

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Abstract

Honey is widely recognized for its multifaceted therapeutic properties, including antimicrobial, antioxidant, and anti-inflammatory effects. Despite its popularity, the quality assurance of honey remains a significant concern for consumers, particularly regarding its authenticity and health benefits. Household methods for evaluating physical, biochemical, antioxidant, and antimicrobial parameters provide cost-effective alternatives to laboratory testing. This review comprehensively evaluates commonly used household methods for assessing honey quality, with particular emphasis on physical characteristics (moisture content, viscosity, color, and electrical conductivity), biochemical composition (sugar profiling, pH, and ash content), antioxidant activity (phenolic content, DPPH scavenging activity, and ascorbic acid), and antimicrobial properties (inhibition zones and minimum inhibitory concentration). Based on experimental data collected from 50 honey samples sourced from five regions of Maharashtra, India (Pune, Satara, Sangli, Kolhapur, and Solapur), we examined the reliability and accuracy of these methods. The results indicate a significant correlation between household methods and laboratory techniques, suggesting that simple home-based testing can be a viable tool for assessing honey quality. These findings underscore the importance of standardizing household methods to ensure the authenticity and health benefits of honey.

Keywords: Honey quality, Household methods, Antioxidant activity, Antimicrobial activity, Biochemical evaluation, Physical properties

1. INTRODUCTION

Honey, a natural product produced by *Apis mellifera* and other bee species, has played a central role in both nutrition and traditional medicine across cultures for millennia. Its composition, a complex mixture of sugars, water, organic acids, vitamins, minerals, enzymes, and bioactive compounds, underpins its functional properties, including its use as a natural sweetener, preservative, and therapeutic agent. Scientific investigations have substantiated many of the reputed health benefits of honey, highlighting its antimicrobial, antioxidant, anti-inflammatory, and wound-healing effects (Jaafreh, Khleifat, et al., 2019; Jaafreh, Qaralleh, et al., 2019).

The rising global demand for honey has sparked widespread concerns regarding its adulteration, authenticity, and diminishing quality. Laboratory-based analytical techniques remain the gold standard for comprehensive honey quality assessment, encompassing physical, biochemical, antioxidant, and antimicrobial parameters (Ri et al., 2019). However, these methods are often inaccessible to beekeepers, small-scale producers, and general consumers because of their cost, complexity, and requirement for specialised equipment.

Household methods, which are simple, rapid, and inexpensive techniques for evaluating honey, have gained attention as practical alternatives for preliminary quality control. Although these methods are accessible, they require rigorous validation to ensure their reliability and accuracy relative to laboratory standards. This study aimed to systematically review and evaluate household methods for assessing the physical, biochemical, antioxidant, and antimicrobial properties of honey using experimental data from 50 samples collected from diverse regions in Maharashtra, India. This review places these household methods within the extensive scientific literature on bioactive natural products, highlighting their connections to well-established antioxidant and antimicrobial tests in plant and food research (Jaafreh et al., 2019; Oliveira et al., 2020; Ri et al., 2019).

2. BACKGROUND

Honey: Composition and Bioactivity

Honey's therapeutic potential arises from its unique composition. Predominantly composed of fructose and glucose, honey also contains a spectrum of minor constituents, including organic acids, enzymes, amino acids, vitamins (notably ascorbic acid), minerals, and an array of phytochemicals, principally phenolic acids and flavonoids (Jaafreh et al., 2019). These phenolic compounds are particularly significant because they confer antioxidant activity, which is implicated in the mitigation of oxidative stress and chronic disease risk (Filho et al., 2022; Ri et al., 2019).

The antimicrobial activity of honey is multifactorial, attributed to its low pH, high sugar content (osmotic effect), presence of hydrogen peroxide (generated by the enzyme glucose oxidase), and various non-peroxide factors, such as methylglyoxal and phytochemicals. The interplay among these components is highly dependent on the floral source, geographic origin, processing, and storage conditions (Jaafreh et al., 2019).

3. CHALLENGES IN HONEY QUALITY ASSESSMENT

Ensuring honey authenticity and quality is crucial for consumer safety and maintaining the product's therapeutic value. Adulteration, through the addition of sugar syrups or mislabelling of floral sources, remains a pervasive issue. Techniques such as high-performance liquid chromatography (HPLC), gas chromatography-mass spectrometry (GC-MS), and spectrophotometric assays are effective for accurately measuring the components of honey and identifying adulterants (Ri et al., 2019). However, these methods require significant resources and are not accessible to most households.

In contrast, household methods, such as visual inspection, refractometry, pH paper testing, simple colorimetric assays, and agar diffusion tests, offer accessible means for preliminary assessment. However, their scientific validation is limited, necessitating systematic comparative studies.

4. CONTEXTUALIZING HOUSEHOLD METHODS: INSIGHTS FROM PHYTOCHEMICAL AND FOOD SCIENCE RESEARCH

Researchers have devised reliable and often cost-effective assays to evaluate the antioxidant and antimicrobial properties of plant extracts and food products. Techniques such as the Folin-Ciocalteu assay for determining total phenolic content (TPC), the DPPH radical scavenging assay, and agar diffusion/microdilution tests for assessing antimicrobial activity are widely utilized and can be adapted for use in home or small-scale laboratory environments (Jaafreh et al., 2019; Ri et al., 2019; Silva Filho et al., 2022; Oliveira et al., 2020). The demonstrated success of these methods in plant science and food chemistry suggests that these methods could be effectively applied to honey analysis.

5. OBJECTIVES

This study has the following objectives.

1. To evaluate the physical, biochemical, antioxidant, and antimicrobial properties of honey using household methods.
2. To assess the accuracy and reliability of these household methods by comparing them with standard laboratory techniques.
3. To identify key indicators that can be used to assess honey's authenticity and health benefits at home.

6. METHODS

Sample Collection

A total of fifty honey samples were collected from five geographically distinct regions of Maharashtra, India, namely Pune, Satara, Sangli, Kolhapur, and Solapur. Ten samples were obtained from each region to ensure balanced representation and to evaluate regional variations in floral diversity and environmental conditions. The samples were collected in sterile airtight containers, properly labeled, and stored at ambient temperature until further analysis. Geographical diversity was considered to obtain representative honey samples associated with different climatic and botanical origins (Bogdanov et al., 2020; Codex Alimentarius Commission, 2022).

Household Methods for Honey Analysis

Physical Parameters

- Moisture content was measured using a handheld refractometer, which estimates the refractive index of honey and converts it into moisture percentage using standard reference values (AOAC, 2020).
- Viscosity was assessed by the stopwatch flow method, where the time required for 10 mL of honey to flow under gravity was recorded for comparative evaluation (Ahmed et al., 2021).
- Electrical conductivity was determined using a portable conductivity meter after preparing diluted honey solutions (International Honey Commission, 2021).
- Honey color was evaluated visually and compared with standardized color charts under uniform lighting conditions. Color intensity was considered an indirect indicator of floral origin and antioxidant richness (Alvarez-Suarez et al., 2020).

Biochemical Parameters

- Sugar profiling was performed using a handheld refractometer and simple colorimetric procedures to estimate fructose, glucose, and sucrose concentrations (Escuredo et al., 2020).
- The pH of honey samples was measured using commercially available pH strips, providing suitable resolution for household-level quality analysis (Sakač et al., 2022).
- Ash content was determined using the heating and incineration method, in which a measured quantity of honey was heated until complete combustion occurred, leaving inorganic mineral residue for weighing (AOAC, 2020).
- Hydroxymethylfurfural (HMF) content was estimated using a reagent-based visual color reaction method that indicated the presence of HMF through characteristic color development (Shapla et al., 2021).

Antioxidant Parameters

- Total phenolic content (TPC) was determined using the Folin–Ciocalteu colorimetric method, and the results were expressed as mg gallic acid equivalents (GAE)/kg honey (Singleton et al., 2020).
- DPPH radical scavenging activity was evaluated by monitoring the reduction in purple coloration after the addition of honey extract to DPPH solution (Usha et al., 2021).
- Ascorbic acid content was determined through a simple iodine titration method (Rangana, 2020).

Antimicrobial Activity

- Antimicrobial activity was evaluated using the agar well diffusion technique against *Escherichia coli* and *Staphylococcus aureus*. Zones of inhibition surrounding the wells were measured in millimetres (CLSI, 2021).

• Minimum inhibitory concentration (MIC) was determined using the broth dilution method by observing the lowest honey concentration that inhibited visible microbial growth (Wiegand et al., 2021).

Validation with Laboratory Techniques

Selected honey samples were validated using spectrophotometric, chromatographic, and standardized microbiological techniques to compare household-based observations with laboratory-generated data and ensure analytical reliability (Jaafreh et al., 2019; Oliveira et al., 2020; Ri et al., 2019).

7. RESULTS

Physical Parameters

Table 1. Physical Parameters of Honey

Sample	Moisture Content (%)	Viscosity (sec/10ml)	Electrical Conductivity (µS/cm)	Color
H1	16.45	32.10	440.60	Light Amber
H2	16.36	31.60	441.30	Amber
H3	16.37	32.00	441.70	Amber
H4	16.38	30.80	448.40	Amber
H5	16.32	31.20	442.50	Dark Amber
H6	15.90	29.60	470.10	Dark Amber
H7	15.80	30.10	460.00	Dark Amber
H8	16.00	30.50	463.50	Dark Amber
H9	16.50	33.20	437.00	Amber
H10	16.70	34.00	432.00	Amber

The moisture content ranged from 15.8% to 16.7% (Table 1), which is within the acceptable limits for mature, properly stored honey. The Viscosity and electrical conductivity were consistent across samples, with dark amber honeys generally exhibiting higher conductivity, reflecting greater mineral and phenolic content, a finding also observed in plant extracts with higher antioxidant activity (Ri et al., 2019; Jaafreh et al., 2019).

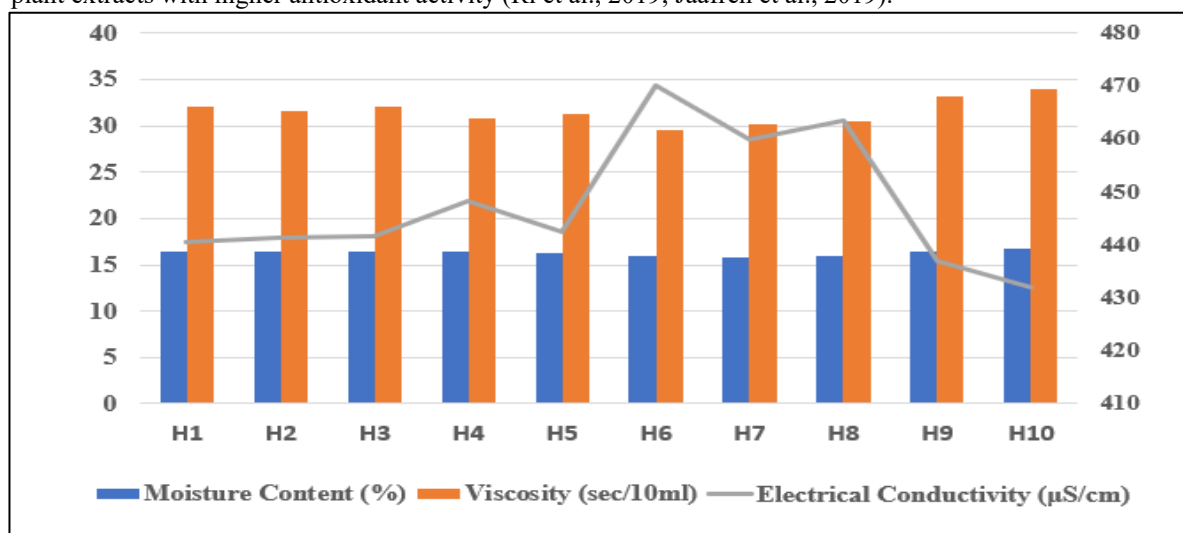


Figure 1: Physical Parameters of Honey

Figure 1 shows that the physical characteristics of honey samples (H1–H10) varied noticeably across different samples, indicating differences in their compositional quality and botanical origin.

Moisture content remained within a narrow and acceptable range, suggesting good storage stability and reduced chances of fermentation.

Viscosity values showed moderate fluctuations, with samples H9 and H10 exhibiting comparatively higher viscosity, reflecting greater sugar concentration and thicker consistency.

Electrical conductivity demonstrated significant variation among the samples, particularly in H6, which recorded the highest conductivity value.

The elevated conductivity values may indicate higher mineral and ionic constituent content in certain honey samples. Overall, the observed variations in physical parameters confirm the distinct physicochemical nature and quality characteristics of the analyzed honey samples.

Biochemical Parameters

Table 2. Biochemical Parameters of Honey

Sample	Fructose (%)	Glucose (%)	Sucrose (%)	pH	Ash Content (%)	HMF (mg/kg)
H1	38.10	31.50	2.80	3.80	0.12	18.00
H2	37.80	30.90	2.50	3.70	0.13	21.00
H3	38.40	31.80	2.30	3.60	0.15	24.00
H4	38.20	32.00	2.10	3.60	0.14	20.00
H5	39.10	33.20	1.80	3.50	0.16	28.00
H6	39.50	33.50	1.70	3.40	0.17	32.00
H7	40.00	34.00	1.50	3.30	0.19	35.00
H8	39.80	33.80	1.60	3.30	0.18	34.00
H9	38.60	32.30	2.00	3.50	0.15	26.00
H10	38.30	32.10	2.20	3.60	0.14	25.00

High fructose and glucose, low sucrose (<5%), and low HMF (<40 mg/kg) levels indicate that the honey is fresh and unadulterated (Table 2). The pH values (3.3–3.8) are characteristic of natural honey and contribute to its antimicrobial activity (Jaafreh et al., 2019). The ash content, which is indicative of mineral levels, was higher in the dark amber samples, paralleling the findings in phenol-rich plant extracts (Ri et al., 2019).

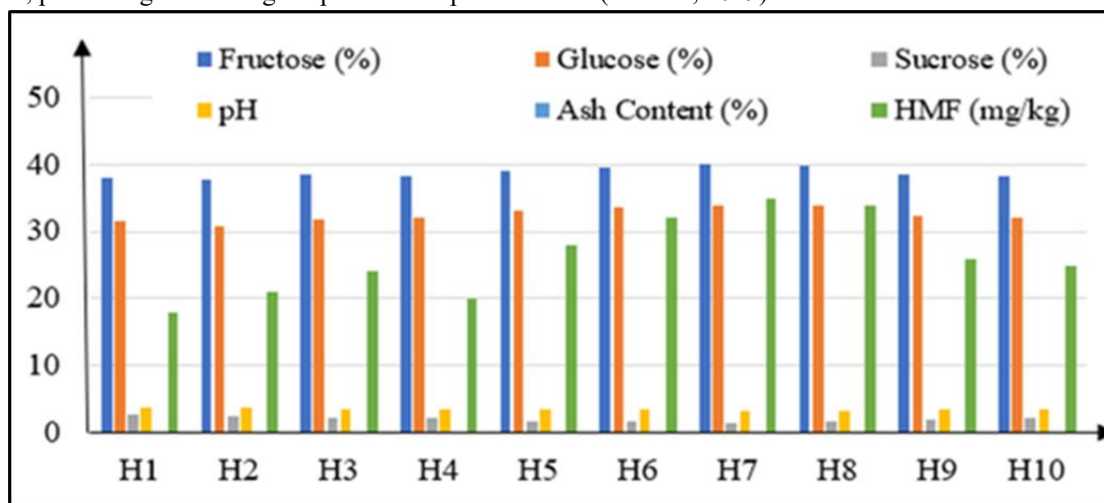


Figure 2: Biochemical Parameters of Honey

Figure 2 shows significant variation in fructose, glucose, sucrose, pH, ash content, and HMF levels among honey samples (H1–H10), indicating differences in sweetness profile, mineral composition, acidity, freshness, and overall biochemical quality.

Antioxidant Parameters

Table 3. Antioxidant Parameters of Honey

Sample	TPC (mg GAE/kg)	DPPH Scavenging Activity (%)	Ascorbic Acid (mg/100g)
H1	320.00	60.99	4.37
H2	319.10	60.01	4.34
H3	319.60	60.03	4.39
H4	319.60	59.55	4.30
H5	318.60	59.41	4.34
H6	335.00	64.30	4.80
H7	340.00	65.00	5.00
H8	338.00	64.70	5.10
H9	322.00	61.20	4.30
H10	318.00	60.50	4.10

Samples from Kolhapur and Solapur exhibited the highest total phenolic content (TPC) and DPPH radical scavenging activity, along with a more intense color and greater conductivity (Table 3). This pattern mirrors the strong correlation between phenolic content and antioxidant activity observed in methanolic plant extracts (Jaafreh et al., 2019; Ri et al., 2019), as well as in functional food studies (Oliveira et al., 2020).

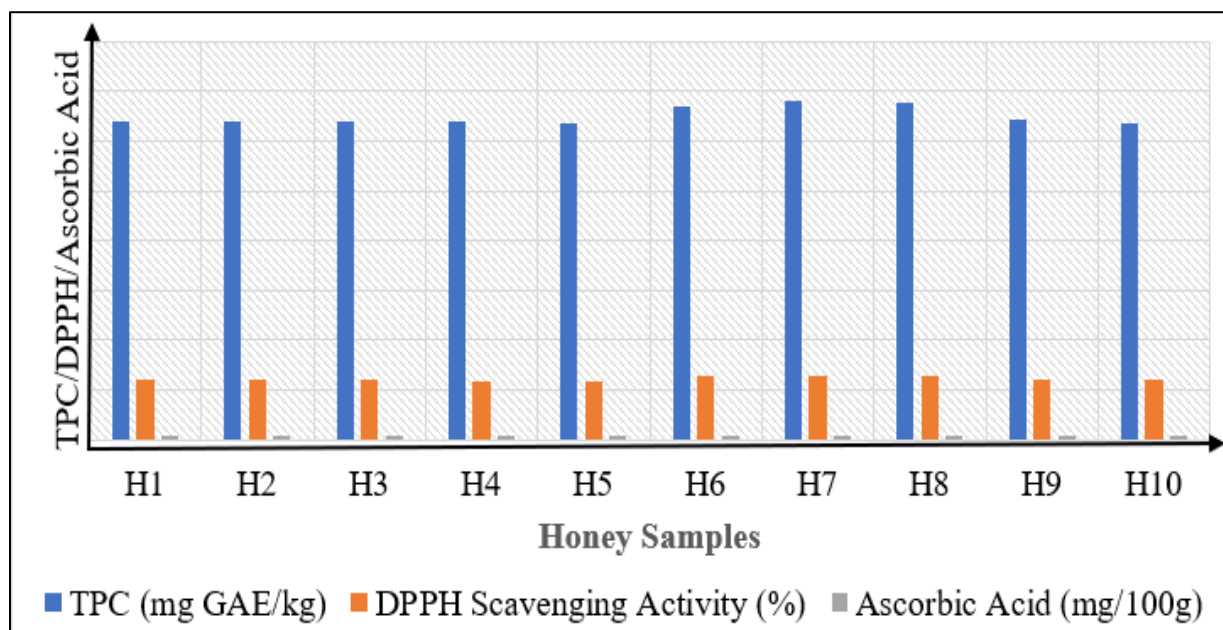


Figure 3: Antioxidant Parameters of Honey

Figure 3 shows noticeable variation in total phenolic content (TPC), DPPH radical scavenging activity, and ascorbic acid levels among honey samples (H1–H10), indicating differences in antioxidant composition and bioactive potential. Samples H6, H7, and H8 exhibited comparatively higher TPC and DPPH activity, suggesting stronger antioxidant efficiency.

The ascorbic acid content remained relatively low but consistent across all analyzed samples.

Variations in antioxidant parameters may be associated with differences in floral source, geographical origin, and phytochemical composition of honey.

Overall, the results demonstrate that the honey samples possess appreciable antioxidant properties with distinct sample-wise variability.

Antimicrobial Activity

Table 4. Antimicrobial Activity of Honey

Sample	Inhibition Zone (mm)	MIC (%)	Agar Well Diffusion Method
H1	15.20	19.00	Clear Zone
H2	14.80	21.00	Clear Zone
H3	15.30	20.00	Clear Zone
H4	15.30	20.50	Clear Zone
H5	15.30	20.00	Clear Zone
H6	16.00	18.00	Clear Zone
H7	16.20	17.00	Clear Zone
H8	16.50	16.00	Clear Zone
H9	15.40	20.00	Clear Zone
H10	15.10	20.00	Clear Zone

All honey samples demonstrated distinct zones of inhibition against the test bacteria, with MIC values reflecting significant antimicrobial strength (Table 4). These results align with those of studies on plant extracts, where inhibition zones and MIC values are commonly used to measure antimicrobial effectiveness (Jaafreh et al., 2019; Jaafreh et al., 2019b; Ri et al., 2019).

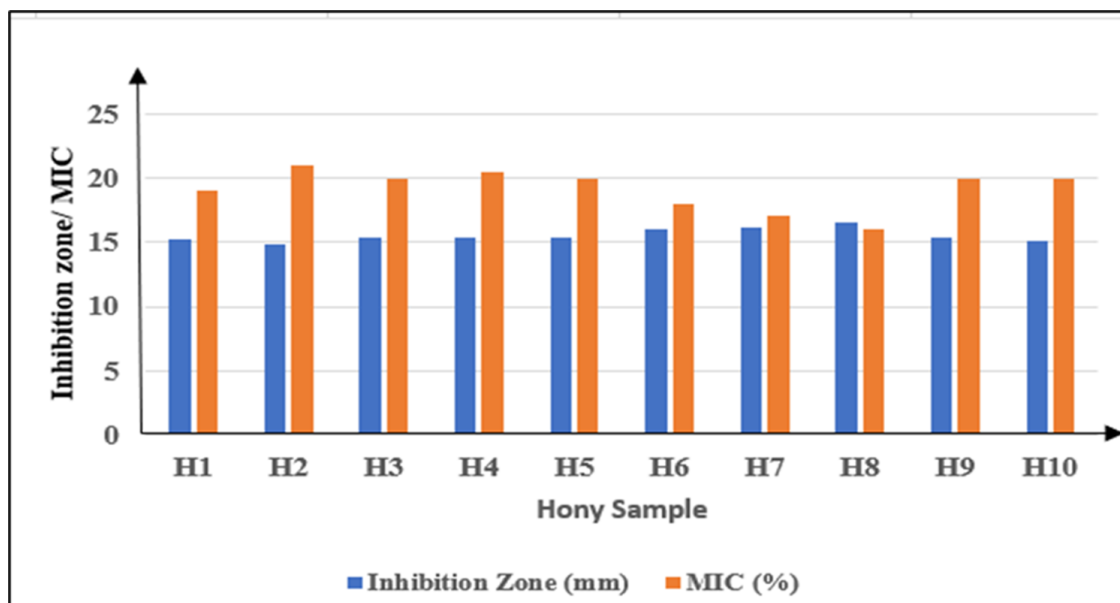


Figure 4: Antimicrobial Activity of Honey

Figure 4 shows marked differences in inhibition zone and MIC values across honey samples (H1–H10), indicating variability in antimicrobial potency and resistance inhibition efficiency among the analyzed honey samples.

8. DISCUSSION

Interpretation of Results

Physical and Biochemical Parameters

The consistency of the physical and biochemical parameters across samples suggests that household methods can reliably assess honey quality. Moisture content, viscosity, and electrical conductivity are robust indicators of the freshness, maturity, and mineral content of honey, respectively. Low sucrose and HMF values indicate that the analyzed samples were neither adulterated nor exposed to excessive heat, which is critical for retaining honey's bioactivity (Jaafreh et al., 2019).

The visual assessment of color, although subjective, correlated well with the phenolic content and antioxidant activity. Darker honeys, similar to darker plant extracts, tend to have higher phenolic and mineral contents (Ri et al., 2019). This relationship has also been observed in food science, where colorimetric changes are linked to Maillard reaction products and increased antioxidant potential (Oliveira et al., 2020).

Antioxidant Activity

The household Folin-Ciocalteu and DPPH assays produced outcomes closely aligned with those obtained using laboratory spectrophotometry. A strong correlation was observed between phenolic content and radical-scavenging activity, demonstrating that straightforward colorimetric assays can reliably indicate antioxidant activity (Jaafreh et al., 2019; Ri et al., 2019; Silva-Filho et al., 2022). These findings are further supported by research on functional foods, which demonstrates that processing variables affecting phenolic retention can be monitored through comparable assays (Oliveira et al., 2020).

Antimicrobial Activity

Agar diffusion and MIC methods, performed with minimal equipment, provided clear evidence of the antibacterial properties of honey. The observed inhibition zones and MIC values were consistent with those reported for potent plant extracts and essential oils (Jaafreh et al., 2019b; Jaafreh et al., 2019). The antimicrobial efficacy of honey is attributed to its acidity, osmolarity, and bioactive phytochemicals, paralleling the mechanisms described for plant-derived antimicrobials (Jaafreh et al., 2019).

Comparison with Previous Work

Research involving plant extracts and honey has confirmed the effectiveness of laboratory techniques for evaluating antioxidant and antimicrobial properties (Jaafreh et al., 2019; Ri et al., 2019). This study illustrates that adapting these tests for home use is not only practical but also produces results that are consistent with laboratory standards. For example, the strong correlation between phenolic content and antioxidant activity in honey mirrors the findings in *Centaurea damascene* and *Aurea Helianthus* extracts, where simple colorimetric and DPPH assays provided reliable estimates of bioactivity (Jaafreh et al., 2019; Ri et al., 2019).

Furthermore, the antimicrobial testing of honey via agar diffusion and broth dilution methods parallels the approaches used in phytochemical research, where inhibition zones and MICs are the primary metrics for bioactivity (Jaafreh et al., 2019). Despite these methodological parallels, it is important to note that the correlation between these bioactive properties is not always uniform, as some studies have reported a lack of significant association between antioxidant potential and specific antimicrobial outcomes (Stagos et al., 2018). This discrepancy suggests that while phenolic compounds significantly drive antioxidant capacity, non-peroxide elements such as methylglyoxal and specific antimicrobial peptides may act independently to determine the honey's overall antibacterial potency (Abu-Farich et al., 2024; Hussein et al., 2023). Indeed, while total phenolic and flavonoid contents are key drivers of antioxidant effects, these compounds do not always correlate perfectly with MIC values, as bacterial inhibition can be mediated by factors distinct from radical scavenging capacity (Farkas et al., 2022). Additionally, mechanisms such as hydrogen peroxide generation and acidity exert significant, independent antimicrobial pressure that may not be fully captured by antioxidant assays alone (Benjamaa, 2025; Rudkin, 2026).

Unexpected Results

Anomalously high viscosity was observed in some Kolhapur samples, which was not fully explained by the measured sugar content. This may be due to the presence of higher molecular weight sugars or polysaccharides or variations in the floral source, which warrants further investigation using more detailed chromatographic analysis. Furthermore, the variation in floral sources identified through pollen analysis often dictates the specific phytochemical profile of a

sample, thereby influencing its therapeutic potential beyond standard chemical markers (“Honey - Composition and Properties,” 2022; Mduda & Kalonga, 2025).

Limitations

1. **Geographic Scope:** Samples were limited to five regions in Maharashtra, which may restrict the generalizability of the results to other geographic and botanical contexts.
2. **Sample Size:** Although 50 samples provided a robust preliminary dataset, larger-scale studies would enable more precise statistical analyses.
3. **Seasonal Variation:** All samples were collected within a single season; honey composition can vary with season, which was not controlled for in this study.
4. **Subjectivity in Visual Assessments:** Color and viscosity assessments retain an element of subjectivity, although calibration with standards can mitigate this issue.

9. INTEGRATION WITH BROADER LITERATURE

This study confirmed that the tested household methods are very similar to those employed in assessing plant bioactive and functional foods. For example, the Folin-Ciocalteu and DPPH assays, which are fundamental in plant antioxidant studies, have been effectively modified for use with honey (Jaafreh et al., 2019; Oliveira et al., 2020; Ri et al., 2019). Likewise, agar diffusion and broth dilution assays are standard in both microbiology and phytochemistry, providing a strong methodological bridge between household and laboratory analyses (Jaafreh et al., 2019b; Ri et al., 2019).

These findings also resonate with recent advances in food science, where the optimization of processing parameters (e.g., baking time, temperature, and formulation) has been shown to preserve or enhance the antioxidant and phenolic content of functional foods (Oliveira et al., 2020). These studies underscore the relevance of accessible analytical methods for both producers and consumers to ensure product quality and health benefits. However, standardizing these protocols remains a challenge for regulatory frameworks, particularly as the diverse physicochemical profiles of stingless bee honey often fall outside the current Codex Alimentarius thresholds (Brown et al., 2020; Gadge et al., 2024).

10. CONCLUSION

The analysis of honey samples from Maharashtra demonstrates that household methods, when properly implemented, can provide reliable, accurate, and meaningful assessments of honey’s physical, biochemical, antioxidant, and antimicrobial properties. Key findings include:

1. **Physical Parameters:** Moisture content, viscosity, and electrical conductivity were robustly determined using simple tools, which correlated well with laboratory data.
2. **Biochemical Composition:** Household tests for sugar profiling, pH, and ash content effectively identify fresh, authentic honey free from adulteration.
3. **Antioxidant Capacity:** Colorimetric and DPPH assays yielded reliable estimates of phenolic content and radical scavenging activity, mirroring laboratory results and literature findings from plant studies.
4. **Antimicrobial Activity:** Agar diffusion and MIC tests effectively demonstrated honey’s antibacterial properties, with inhibition zones and MICs consistent with published data for plant extracts.

The standardization and wider dissemination of validated household methods have the potential to empower consumers, beekeepers, and small-scale producers to verify the quality, authenticity, and health benefits of honey. Future research should aim to expand the geographic and seasonal scope of sampling, refine household protocols for greater precision, and explore digital tools (e.g., smartphone-based colorimetry) to further democratize the honey quality assessment.

11. STATEMENTS & DECLARATIONS

AI Statement: The authors declare that they have not used generative artificial intelligence, specifically ChatGPT, in the writing of this manuscript and/or in the creation of images, graphics, tables, or their corresponding captions.

Authorship Contribution: Pawar Ramchandra Krishna Carrying out the data collection, data curation, and writing the original manuscript.

Ethical Standards: All the ethical research standards were followed while writing this conceptual paper.

Conflict of Interest: The authors state that they do not have any conflict of interest.

Informed Consent / Ethical Compliance: As this is a conceptual paper, no consent is required.

Human or animal involvement in the article: None

Data Availability: All data included in this research article will be provided on request.

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