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EFFECT OF DIFFERENT CONCENTRATIONS OF HONEY ON SELECTED MICROORGANISMS

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ABSTRACT

This study investigated the antimicrobial properties of honey and the role of honey's botanical origin on its antimicrobial efficacy at varying concentrations (12.5%, 25%, 50%, and 100%) against five pathogenic microorganisms: *Escherichia coli*, *Bacillus cereus*, *Staphylococcus aureus*, *Salmonella typhi*, and *Pseudomonas aeruginosa*. A ciprofloxacin control was used. Honey samples from Bauchi, Gombe, Plateau, and Kaduna States were tested via agar well diffusion. Results revealed significant ($p<0.05$) variation in antimicrobial activity across concentrations and microbial strains. All honey samples were ineffective against *Candida albicans*. Honey's effectiveness was dose-dependent, with maximum inhibition seen at 100% concentration. Pollen analysis identified key phytoecological plant families contributing to honey's bioactivity. Pollen analysis identified plant families, with Combretaceae/Melastomataceae common across all samples.

Keywords: Honey, Antimicrobial activity, Phytoecological profile, Zone of inhibition, Nigeria

INTRODUCTION

The escalating global challenge of antimicrobial resistance continues to threaten public health, necessitating the urgent discovery and evaluation of novel therapeutic agents (Johnson et al., 2023). Conventional antibiotics are increasingly less effective against common bacterial pathogens, including multi-drug-resistant strains of *Escherichia coli*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa* (Kim & Lee, 2022). Furthermore, persistent fungal infections, particularly those caused by *Candida albicans*, present a significant clinical burden due to evolving resistance mechanisms (Rodriguez et al., 2021). Natural products, with their rich chemical diversity, offer a promising avenue for developing alternative or complementary antimicrobial strategies, often with unique modes of action (Singh & Kumar, 2020). Honey, a complex natural substance, has been historically valued for its medicinal properties, including its demonstrated antimicrobial activity against a range of microorganisms (Miller et al., 2024).

Recent research continues to explore honey's multifaceted antimicrobial mechanisms, which include its high osmolarity, acidic pH, hydrogen peroxide content, and diverse phytochemical compounds (Davies et al., 2020). Studies have highlighted its potential in combating wound infections, reducing inflammation, and promoting faster healing (Garcia et al., 2023). However, the therapeutic efficacy of honey can vary considerably, influenced by factors such as its geographical origin, floral source, and processing methods (Wang *et al.*, 2024). Despite a growing body of evidence on honey's broad-spectrum activity, there remains a notable lack of comprehensive studies specifically evaluating the differential antimicrobial effects of honey from diverse geographical regions within Nigeria against a panel of contemporary clinical isolates, especially considering the local prevalence of drug-resistant strains of pathogens like *Salmonella typhi* (Akinpelu & Olajide, 2020).

Honey, a natural substance produced by *Apis mellifera* bees, has long been recognized for its therapeutic properties, which vary significantly based on geographical, seasonal, and botanical factors (European Directive, 2001; Anupama et al., 2003). Despite its traditional use in medicine, modern clinical practice often overlooks the differential antimicrobial potencies of honeys from diverse origins (Aristotle, 1910; Molan, 2006). This oversight is particularly pertinent in a post-COVID era where the pressing need for accessible and effective alternative remedies for common ailments, such as wound infections, typhoid fever, and candidiasis, has become increasingly apparent. Many bacterial pathogens, including *Escherichia coli*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa*, exhibit antibiotic resistance, making alternative treatments crucial (Jesumirhewe and Bekee, 2019). Furthermore, *Salmonella typhi* remains a significant cause of multidrug-resistant bacteremia in regions like North Central Nigeria (Obaro et al., 2015; Smith et al., 2016), underscoring the urgency for novel therapeutic approaches. While honey has demonstrated promising antibacterial effects on various bacteria involved in wound infections (Jesumirhewe and Bekee, 2019) and even antifungal activity against *Candida* species (Molan, 1992), a critical gap exists in understanding how the specific origin and concentration of honey influence its efficacy against a broad spectrum of microorganisms.

This study aims to address this critical knowledge gap by investigating the antimicrobial activities of honey samples sourced from four distinct Nigerian States—Bauchi, Gombe, Plateau, and Kaduna—at various concentrations. We specifically assessed their efficacy against key bacterial pathogens (*Bacillus cereus*, *Escherichia coli*, *Staphylococcus aureus*, *Salmonella typhi*, *Pseudomonas aeruginosa*) and the fungal pathogen (*Candida albicans*). By characterizing the antimicrobial profiles of these regionally diverse honeys, this research provides crucial insights into their potential as localized, natural antimicrobial resources. This contribution is particularly significant in advancing the understanding of how the specific origins of African honeys influence their therapeutic potential, thereby informing evidence-based decisions for their application in managing microbial infections amidst the rising challenge of antimicrobial resistance. This study was therefore conducted to investigate the effect of different concentrations of honey collected from four distinct Nigerian States—Bauchi, Gombe, Plateau, and Kaduna—on the antimicrobial activity against selected clinically relevant microorganisms. Specifically, the research aimed to evaluate botanically identified honey's inhibitory effects on *Bacillus cereus*, *Escherichia coli*, *Pseudomonas* *aeruginosa*, *Staphylococcus aureus*, *Salmonella typhi*, and *Candida albicans*. This work contributes significantly to knowledge by providing empirical data on the variability of antimicrobial potency among honeys from different Nigerian States. By examining the concentration-dependent activity and intrinsic differences across diverse honey samples, this study seeks to inform judicious selection of honey for therapeutic applications and emphasizes the necessity for quality control measures to ensure consistent efficacy, thereby advancing the understanding of honey as a natural antimicrobial agent.

MATERIALS AND METHODS

Honey Collection

Four raw unprocessed honey samples were collected from beekeepers from local source at Tafawa Balewa (Bauchi State); Soro (Gombe State); Pankshin (Plateau State) and Zaria (Kaduna State) Nigeria. The honey samples were processed, floral origin was determined and stored in a cool place until the time of use.

Antimicrobial Assay

Agar Well Diffusion

Honey samples were screened for anti-bacterial activity against clinical isolates of *Escherichia coli, Bacillus cereus, Staphylococcus aureus, Salmonella typhi* and *Pseudomonas aeruginosa* using the agar well diffusion method (Allen et al., 1991) in replicates. Solidified Mueller Hinton Agar (MHA) plates were separately flooded with 0.1ml of 0.5ml McFarland standard of the different test organisms grown in sterile nutrient broth at 37^0C for 24hrs. The plates were allowed to dry and wells of 6mm diameter bored into the agar with a cork borer. Two (2) ml of 100% pure honey was taken and 2ml sterilized distilled water was added. This gave 50% concentration of honey. From the 50% concentration, 2ml was taken and 2ml of distilled water added this gave 25%; 2ml was taken and 2ml distilled water added, which gave 12.5%. Solutions containing 12.5%, 25%,50% and 100% (v/v) honey samples in sterile distilled water were separately placed in the different wells and incubated at 37^0C for 24hrs for bacteria. The average diameters of the zones of inhibition were measured with a ruler and recorded in millimeter. The means of interpretation of the zones of inhibition results were: less than 15mm was considered resistant, 16-20mm intermediate and greater than or equal to 21mm susceptible (CLIS, 2007). For *Candida* albicans the test organism was grown in yeast extract broth for 18hrs at 25^0C $\pm 2^0$C and cultured on PDA plate at room temperature for 24hrs.

Pollen Characterization/Melisopalynology

To unravel the content of the honey samples, we employed the procedure outlined by Louveaux *et al.* (1970), we incorporated acetolysis, a technique pioneered by Erdtman (1960). We diluted 5-10 grams of honey in warm distilled water (around 40°C) and ethyl alcohol, following the protocol of Jones and Bryant (2004).

Data Analysis

ANOVA was used to test if there were significant differences (at $p<0.05$) in the honey samples collected from the four States and the concentration of honey used against the different organism. Wilks' lambda was used to separate means at 5% level of probability. Number was used to show pollen grain frequency in honey samples.

RESULTS AND DISCUSSION

Antimicrobial Assay

The results of the effect of different concentrations of honey on selected microorganisms are displayed in the tables below. The effect of different concentrations of honey on selected microorganisms differed significantly ($p \geq 0.05$) for the different honey types (Table 1). Honey demonstrated significant antimicrobial activity, which increased proportionally with concentration. At 100%, honey effectively inhibited all bacteria except *C. albicans*.

Table 1: Effect of Different Concentrations Using Zones of Inhibition on Different Micro-Organisms on Honey Samples

	Test Organism					
Concentration	***Escherichia coli***	***Bacillus cereus***	***Staphylococcus aureus***	***Salmonella typhi***	***Pseudomonas aeruginosa***	***Candidas albicans***
Control	12.50^d	33.50^a	31.50^a	39.50^a	37.50^a	0.00^a
100%	32.88^a	15.88^b	11.63^b	35.00^b	13.50^b	0.00^a
50%	31.63^a	10.50^c	6.56^c	33.38^c	2.88^c	0.00^a
25%	28.13^b	0.00^d	2.78^d	30.38^d	0.00^d	0.00^a
12.5%	19.25^c	0.00^d	0.00^d	28.50^e	0.00^d	0.00^a

a –e Means with the same letter(s) in a column are not significantly different ($p \geq 0.05$)

Interestingly, honey concentrations below 25% were ineffective against all tested organisms except *S. typhi* and *E. coli,* highlighting the importance of dose-dependent action. The inability of honey to inhibit *C. albicans* may relate to fungal cell wall complexity and pH resilience, corroborating earlier studies (Estevinho et al., 2011; Carter et al., 2016).

Activity Against Gram-Negative Bacteria

The results showed *Escherichia coli* (Table 1) to be highly susceptible to honey at all tested concentrations, with a maximum zone of inhibition at 100% concentration (32.88 mm). This finding is consistent with reports by Mohapatra et al. (2022), who confirmed that honey inhibits *E. coli* growth due to the presence of hydrogen peroxide, phenolic acids, and flavonoids, which damage bacterial cell walls and disrupt metabolic processes. The efficacy against *E. coli* (32.88 mm) and *S. typhi* (35.00 mm) was notable, with comparable performance to ciprofloxacin in *S. typhi*. This aligns with previous findings that higher sugar concentration, low water activity, and bioactive compounds (e.g., hydrogen peroxide, methylglyoxal) potentiate honey's action (Kassim et al., 2021). In the same way, *Salmonella typhi* (Table 1) was significantly inhibited at all concentrations, though inhibition reduced with dilution. This supports the findings of Dawoud et al. (2019), who demonstrated that various types of natural honey showed bacteriostatic and bactericidal activity against *Salmonella* sp. due to their acidic pH and enzymatic production of reactive oxygen species. The data showed that *P. aeruginosa* was only inhibited by honey at 100% and 50% concentrations, with no effect observed at 25% and below. This resistance is typical of *P. aeruginosa* due to its efflux pump systems and biofilm formation. Studies by Ahmed et al.

(2020) and Bucevic Popovic et al. (2023) support this observation, highlighting that while honey can affect planktonic *P. aeruginosa*, its effectiveness is limited without combination therapies or high concentrations.

Activity Against Gram-Positive Bacteria

The antimicrobial effect against *Staphylococcus aureus* (Table 1) was moderate and dose-dependent. The inhibitory activity decreased with lower concentrations of honey, with no observable inhibition at 12.5%. This is in line with the work of Kwakman et al. (2011), who found that honey rich in methylglyoxal and defensin-1 could effectively inhibit *S. aureus*, although effectiveness varied based on honey composition and bacterial strain. *Bacillus cereus*, (Table 1), on the other hand, showed resistance to honey at concentrations below 100%, with complete inhibition only occurring at high doses. Fadhel and Al-Ghamdi (2021) also reported similar trends, noting that spore-forming bacteria like *B. cereus* may require higher honey concentrations due to the resilience conferred by their spores and the protective exosporium.

Lack of Antifungal Activity

Notably, *Candida albicans* (Table 1) was completely resistant to all concentrations of honey used in this study. While some honeys (e.g., Manuka) have shown antifungal properties, this resistance may reflect the lack of specific antifungal components like methylglyoxal or bee defensins in the honey used. Noori et al. (2021) showed that while some honey types demonstrated moderate antifungal activity, efficacy was highly dependent on floral source, geographical origin, and processing method. The absence of activity in this case supports the conclusion that the tested honey lacks antifungal potency against *C. albicans*.

Generally, there was a significant difference ($p \geq 0.05$) in the antimicrobial activity of honey samples used from the different States for the different organisms except for *Candida albicans* (Table 2). *Salmonella typhi* exhibited high values in all States, particularly in Gombe (34.50a) and Kaduna (34.50a), suggesting a high susceptibility. *C. albicans* Showed zero values across all States, indicating possible resistance to honey or absence of activity of the honeys on *C. albicans* (Table 2). Kaduna State had the highest value for *Escherichia coli* (26.10mm), This study had a range of 23.90-26.10mm which is higher than that recorded (4.4-13.5mm) by Agbagwa & Frank-Peterside, (2011). While Gombe had the highest value for *Staphylococcus aureus* (14.40mm). Which falls within the findings of studies on antimicrobial resistance patterns in Nigeria by Smith et al., (2016) and Agbagwa & Frank-Peterside (2011). Understanding these patterns can inform treatment strategies and public health interventions. Differences in antimicrobial efficacy among honey samples (Table 2) are likely due to floral sources and ecological factors (Table 3). For instance, Kaduna honey's higher *E. coli* inhibition correlates with the presence of *Pavetta* sp., a Rubiaceae known for its medicinal value. Gombe's stronger *S. aureus* activity may reflect its unique pollen dominance from Combretaceae. This supports earlier reports that honey's efficacy is influenced by plant derived bioactives and geographic variation (Zulkipli et al., 2017; Alvarez-Suarez et al., 2020). Pollen analysis suggests that floral diversity may have significantly contributed to the efficacy of Kaduna and Bauchi honey.

Table 2: Effect of different honey samples on different microorganisms

Sample	**Test Organism**					
	Escherichia coli	***Bacillus cereus***	***Staphylococcus aureus***	***Salmonella typhi***	***Pseudomonas aeruginosa***	***Candidas albicans***
Bauchi	23.90^{c}	13.50^{a}	11.95^{b}	30.70^{b}	12.70^{a}	0.00^{a}
Gombe	25.30^{ab}	9.20^{c}	14.40^{a}	34.50^{a}	10.20^{b}	0.00^{a}
Kaduna	26.10^{a}	12.70^{b}	6.30^{d}	34.50^{a}	10.30^{b}	0.00^{a}
Plateau	24.20^{bc}	12.50^{b}	9.40^{c}	33.70^{a}	9.90^{b}	0.00^{a}

a –e Means with the same letter(s) in a column are not significantly different ($p \geq 0.05$)

Pollen Characterization/Melisopalynology

Pollen Composition

77 distinct pollen types were identified. *Combretaceae/Melastomataceae* was dominant across all samples (Table 3). Gombe honey showed the highest pollen count, indicating high floral diversity. A total of 132,649, 82,770, 23,838, and 541 pollen grains were recovered from honey samples from Gombe, Kaduna, Plateau, and Bauchi States respectively (Table 3). The pollen spectra comprised taxa from multiple plant families, with dominant representation from Combretaceae/Melastomataceae, Fabaceae, Asteraceae, Rubiaceae, and Anacardiaceae. The ecological grouping of the recovered taxa revealed significant contributions from cultivated crops (e.g., *Zea mays*, *Elaeis guineensis*, *Gossypium* sp.), savanna shrubs and trees (e.g., *Parkia biglobosa*, *Daniellia oliverii*), and weedy herbaceous species (e.g., *Tridax procumbens*, *Sida acuta*). This reflects the floral diversity of the Nigerian savanna and sub-humid zones and confirms previous findings by Agwu and Akanbi (2014); Adekanmbi and Ogundipe (2014), and Sawadogo et al. (2012).

Common Family

Plant family Combretaceae/ Melastomatacaeae was the only family present in all plant samples (Table 3).

Dominant Taxa per State

Kaduna *Pavetta* sp. (Rubiaceae); Gombe Combretaceae pollen dominated; Plateau displayed high presence of Myrtaceae while for Bauchi State Lamiaceae was unique to this sample (Table 3).

The wide variety of pollen types reflects the complex floral landscape across the surveyed States. Gombe and Kaduna samples were dominated by Combretaceae/Melastomataceae, while Plateau showed higher values for Myrtaceae and Rubiaceae. The dominance of savanna tree like *Parkia biglobosa*; cultivated species like *Zea mays*, *Gossypium* sp.; and weed (*Tridax procumbens*) suggests that honeybees forage across diverse plant communities, supporting previous findings by Adekanmbi and Ogundipe (2014), and Odetoyinbo and Akinniyi (2017).

Table 3: Pollen Types Recovered From Honey Samples And Their Phytoecological Groups

Plant taxa	Plant family	Pollen occurrence by State			
		Bauchi	Gombe	Kaduna	Plateau
Acacia dudgeoni	Fabaceae	40	-	16	-
Acanthaceae	Acanthaceae	-	-	-	2
Adansonia digitata	Malvaceae	-	3	-	-
Adenodolichos paniculata	Papilionoideae	26	-	-	-
Afzeli Africana	Fabaceae	-	-	2	-
Afzelia quanzensis type	Caesalpinioideae	-	-	-	34
Alchornea sp.	Euphorbiaceae	-	-	53	-
Anacardiaceae	Anacardiaceae	-	312	280	-
Annonidium mannii	Annonaceae	-	-	6	-
Anthonotha macrophylla	Fabaceae	-	-	-	2
Arecaceae	Arecaceae	10	-	-	2
Asteraceae	Asteraceae	12	-	4	42
Azadiractha indica	Meliaceae	-	624	3	-
Basilicum sp.	Lamiaceae	28	-	-	-
Bauhinia cf. *petersiana*	Fabaceae	-	-	1	-
Berlinia grandiflora	Fabaceae	-	-	-	4
Berlinia sp.	Fabaceae	-	666	5544	-
Bombax buonopozense	Bombaceae	-	-	1	-
Borassus aethiopum	Arecaceae	1	-	-	-
Borreria sp.	Rubiaceae	-	-	-	32
Vitellaria paradoxa	Sapotaceae	-	-	260	-
Caesalpiniaceae	Caesalpiniaceae	-	-	53	-
Cassia sp.	Caesalpinioideae	-	-	-	16
Celtis cf. *brownii*	Ulmaceae	-	-	2	-
Combretaceae/Melastomatacaeae	Combretaceae/Melastomatacaeae	346	85800	23800	30
Daniellia oliverii	Caesalpinioideae	-	-	1	-
Delonix regia	Caesalpinioideae	-	-	3	-
Dichrostachys cinerea	Mimosoideae	-	1	54	-
Diospyros sp.	Ebenaceae	-	3	-	2
Elaeis guineensis	Arecaceae	-	-	50	-
Entada abyssinica	Mimosoideae	-	780	-	-
Ericaceae	Ericaceae	-	-	168	-
Erythrina mildbraedii	Leguminosae	-	-	-	1
Flabellaria paniculate	Malpighiaceae	-	-	56	-
Gardenia ternifolia	Rubiaceae	-	-	4	-
Gossypium sp.	Malvaceae	-	-	4	1
Gossypium2	Malvaceae	-	-	4	-
Hildergardia barteri	Sterculiaceae	-	-	104	-
Hymenocardia acida	Hymenocardiaceae	-	-	8	-
Hypheaene sp.	Arecaceae	-	-	104	-
Hyptis lanceolata	Lamiaceae	39	-	-	-
Ipomoea involucrate	Convolvulaceae	11	-	-	-
Lannea cf. microcarpa	Anacardiaceae	1	-	-	-
Lannea sp.	Anacardiaceae	-	-	-	4
Ludwigia (Jussiaea) repens	Onagraceae	2	-	-	-
Malvaceae	Malvaceae	1	-	-	-
Meliaceae	Meliaceae	-	-	2	56
Mimusops	Sapotaceae	-	-	101	-
Moraceae	Moraceae	-	-	52	-
Myrtaceae	Myrtaceae	-	-	896	23333
Oldenlandia corymbosa	Rubiaceae	-	-	51	148
Parinari kerstingii	Rosaceae	-	-	416	-
Parkia bicolor	Mimosoideae	-	-	370	54
Parkia biglobosa	Mimosoideae	-	44460	-	-
Papilionaceae	Papilionaceae	-	-	-	8
Paulinnia pinnata	Sapindaceae	-	-	2	-
Pavetta sp.	Rubiaceae	-	-	50120	-

Plant taxa	**Plant family**	**Pollen occurrence by State**			
		Bauchi	**Gombe**	**Kaduna**	**Plateau**
Poaceae	Poaceae	10	-	56	22
Prunus type	Rosaceae	-	-	51	-
Quassia type	Fabaceae	-	-	-	2
Rubiaceae	Rubiaceae	8	-	-	-
Sapotaceae	Sapotaceae	-	-	-	6
Sida acuta	Malvaceae	-	-	-	2
Six-colpate grain	Others	-	-	-	2
Thunbergia alata	Acanthaceae	-	-	57	-
Tridax procumbens	Asteraceae	-	-	-	1
Trilete spore	Others	-	-	2	-
Uapaca sp	Euphorbiaceae	1	-	-	-
Vernonia amygdalina	Asteraceae	-	-	1	6
Zea mays	Poaceae	-	-	3	8
Unidentified1	Others	1	-	1	-
Unidentified3	Others	-	-	2	-
Verbenaceae	Verbenaceae	4	-	-	-
Three-Colporate grain	Others	-	-	2	-
Five-Colpate grain	Others	-	-	-	2
12-15colpate grain	Others	-	-	-	16
TOTAL		541	132,649	82,770	23,838

CONCLUSION

The variability in antimicrobial activity among different honey types highlights the need for standardization of honey products to ensure consistent quality and efficacy. The study reinforces the established antibacterial properties of honey against *E. coli, S. typhi and S. aureus.* Antimicrobial activity was significantly influenced by concentration, with maximal inhibition observed at undiluted levels. However, the lack of antifungal activity against *C. albicans* and weak response from *B. cereus* and *P. aeruginosa* suggests that not all pathogens are equally susceptible.

Plant family Combretaceae/ Melastomatacaeae was the only family present in all plant samples. The consistent presence of this plant family implies their widespread distribution and significant contribution to the honey's botanical composition. It may be safe to assume that honey from this plant family is effective at high concentrations.

Based on the findings, we recommend the integration of high-concentration, locally sourced honey (≥50%) as a complementary antimicrobial agent in clinical and community health settings, especially in the treatment of bacterial infections caused by *Escherichia coli*, *Staphylococcus aureus*, and *Salmonella typhi*. Given its variability in efficacy linked to floral origin, standardization by geographic source and concentration should be prioritized to ensure consistent therapeutic outcomes. Further, honey should not be relied upon for antifungal applications against *Candida albicans* without adjunct therapy. Future studies should focus on characterizing the chemical constituents of honey samples and their correlation with antimicrobial efficacy.

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