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Nature's Golden Elixir: Exploring the Potency of Sokoto Honey's Zn, Fe, Se, I, Phytochemicals and Antibacterial Power against *Staphylococcus aureus* and *Escheria coli* in Wound CARE

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ABSTRACT In Sokoto State, challenges such as poor healthcare services, increasing antimicrobial resistance, prevalence of infectious diseases, and micronutrient deficiencies persist, particularly among vulnerable populations. This study aimed to investigate the potential of locally sourced honey as a natural therapeutic agent through the evaluation of its micronutrient content, phytochemical constituents, and antibacterial activity against *Staphylococcus aureus* and *Escherichia coli*, two common pathogens in wound infections. Honey samples from different zones in Sokoto were assessed for iron (Fe), zinc (Zn), selenium (Se), and iodine (I) using Atomic Absorption Spectroscopy (AAS). Phytochemicals were screened using standard biochemical methods, and the antibacterial efficacy was examined using agar well diffusion at concentrations ranging from 10% to 100%. Results revealed that the honey contained varying but significant levels of essential micronutrients: Fe (7.3–10.11 ppm), Zn (2.6–11.0 ppm), Se (0.50–0.60 ppm), and I (0.05–1.30 ppm). Phytochemical analysis indicated a rich presence of alkaloids, tannins, saponins, flavonoids, and phenols. The antibacterial assay demonstrated that all concentrations of honey exhibited clear zones of inhibition against both test organisms, with activity increasing proportionally with concentration. These findings validate the therapeutic potential of Sokoto honey not only in addressing bacterial infections but also in contributing to nutritional support due to its micronutrient content.

INDEX TERMS Honey, antibacterial activity, micronutrients, phytochemicals, *Staphylococcus aureus*, *Escherichia coli*

I. INTRODUCTION

Honey, a natural sweetener produced by *Apis mellifera*, has long been valued not only for its nutritional properties but also for its therapeutic applications. Globally, honey is known for its energy content, antioxidant capacity, and antimicrobial properties [1]. In recent decades, there has been a resurgence of interest in natural remedies, including honey, particularly in low-resource settings where access to modern healthcare is limited [2], [3].

Sokoto State in northwestern Nigeria is characterized by high rates of malnutrition, poor sanitation, and limited access to quality healthcare services, especially in rural areas [4], [5]. These challenges have been compounded by the rise in antimicrobial resistance, making the treatment of infectious diseases increasingly difficult [6]. In this context, locally available natural substances like honey offer a promising alternative due to their reported antimicrobial, wound-healing, and immune-modulating effects [7], [8].

State-of-the-art methods for evaluating the bioactive properties of honey include atomic absorption spectroscopy (AAS) for mineral profiling and agar well diffusion for assessing antimicrobial activity [9], [10]. Previous studies have confirmed the presence of beneficial micronutrients such as zinc (Zn), iron (Fe), selenium (Se), and iodine (I) in honey [11], [12]. These micronutrients are essential for immune function, antioxidant defense, thyroid regulation, and cellular metabolism [13], [14]. Moreover, phytochemicals like flavonoids, phenols, and tannins

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contribute significantly to the antibacterial effects of honey [15], [16].

Despite global acknowledgment of honey's therapeutic potential, there remains a gap in localized research that quantifies its composition and verifies its efficacy against prevalent pathogens in specific regions. Studies specific to Sokoto honey are sparse, particularly those that simultaneously explore micronutrient content, phytochemical profile, and antibacterial performance [17], [18]. Such comprehensive analysis is critical to establish the safety and efficacy of honey as a low-cost therapeutic option.

This study thus aims to evaluate the biochemical and antimicrobial characteristics of honey collected from Sokoto State. Specifically, we investigate the concentration of essential micronutrients (Zn, Fe, Se, I), screen for phytochemical compounds, and determine antibacterial activity against *Staphylococcus aureus* and *Escherichia coli*, two common bacteria implicated in wound infections [19], [20]. The objectives are to:

1. Analyze the levels of Fe, Zn, Se, and I using AAS.
2. Identify major phytochemicals present in honey samples using standard biochemical assays.
3. Assess the antibacterial activity of honey at different concentrations using the agar well diffusion method.

This study contributes to the scientific understanding of Sokoto honey in three significant ways. First, it provides region-specific data on the nutritional and therapeutic value of honey. Second, it establishes a baseline for evaluating honey's potential to combat antimicrobial-resistant pathogens. Third, it promotes the use of natural, locally available health interventions in rural healthcare systems. The findings of this study will inform further clinical trials and contribute to integrated public health strategies.

The rest of this paper is organized as follows: Section II presents the methods used in sampling, microbial isolation, and chemical analyses. Section III outlines the results, while Section IV discusses the implications of the findings. The conclusion and future recommendations are presented in Section V.

II. METHOD

A. STUDY DESIGN

This study utilized an experimental laboratory design to investigate the bioactive composition and antibacterial potential of locally sourced honey from Sokoto State, Nigeria. The research was structured to evaluate the micronutrient content, phytochemical constituents, and antibacterial activity of the honey samples using validated and replicable protocols. The study was not randomized nor controlled through a clinical setting but followed standardized in vitro assessments based on microbiological and biochemical analyses.

B. SAMPLE COLLECTION AND PREPARATION

Honey samples were obtained directly from local commercial beekeepers located in three primary geopolitical zones within Sokoto State: Sokoto East, Sokoto West, and Sokoto Central. Only raw, unprocessed honey that had not been subjected to heating, dilution, or adulteration was selected. Each sample was collected in sterile glass containers, labeled according to its zone of origin, and transported to the laboratory for analysis under controlled storage conditions (i.e., protected from light and stored at room temperature).

Wound swab samples were collected from patients presenting with infected wounds at the Specialist Hospital in Sokoto. Ethical clearance was obtained from the hospital's review board, and informed verbal consent was secured from each participant. Sterile swab sticks were used to collect specimens, which were promptly placed into sterile sample bottles and transported to the microbiology laboratory within 30 minutes of collection for immediate culturing and analysis.

C. CULTURE MEDIA PREPARATION

Two types of media were employed in this study: Nutrient Agar (NA) and Eosin Methylene Blue (EMB) Agar. NA was used for the growth of general bacteria, while EMB was used to selectively isolate and identify Gram-negative organisms, particularly *Escherichia coli*. Media were prepared in accordance with manufacturer instructions. Specifically, 5.6 g of NA powder was dissolved in 200 mL of distilled water, and 36 g of EMB powder was dissolved in 1000 mL of distilled water. Both were autoclaved at 121°C for 15 minutes to ensure sterilization. After autoclaving, the media were poured into sterile Petri dishes under aseptic conditions and allowed to solidify at room temperature.

D. ISOLATION AND IDENTIFICATION OF TEST ORGANISMS

The wound swab samples were streaked directly onto NA and EMB plates and incubated at 37°C for 24 hours. Colonies that developed were purified through sub-culturing. Standard biochemical tests were used to identify the bacterial isolates, including Gram staining, catalase, and coagulase tests.

Gram staining involved the application of crystal violet, iodine solution, decolorization with alcohol, and counterstaining with safranin. The stained slides were examined under oil immersion at 100x magnification. Catalase testing was performed by adding a drop of 3% hydrogen peroxide to a slide containing a smear of the isolate. Bubble formation indicated a positive reaction. The coagulase test was conducted using rabbit plasma to differentiate *Staphylococcus aureus* (coagulase-positive) from other staphylococci.

E. DETERMINATION OF MICRONUTRIENTS

Atomic Absorption Spectrophotometry (AAS) was employed to determine the concentrations of four essential micronutrients in the honey samples: iron (Fe), zinc (Zn), selenium (Se), and iodine (I). Approximately 5 mL of honey was subjected to wet digestion using a mixture of nitric acid and perchloric acid in a microwave digestion system. The digested sample was filtered and diluted appropriately. AAS was calibrated using standard solutions, and each sample was analyzed in triplicate. Results were reported as mean ± standard deviation in parts per million (ppm).

F. PHYTOCHEMICAL SCREENING

Qualitative screening was conducted to identify the presence of alkaloids, flavonoids, phenols, saponins, and tannins in

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the honey samples. Standard biochemical tests were used: alkaloids were detected using Mayer's reagent, saponins by the frothing method, flavonoids using lead acetate, phenols with ferric chloride, and tannins using gelatin precipitation. The presence and relative abundance of each compound were visually assessed and recorded.

G. ANTIBACTERIAL ACTIVITY ASSAY

The antibacterial activity of the honey samples was assessed using the agar well diffusion technique. The isolates of *Staphylococcus aureus* and *Escherichia coli* were prepared by adjusting turbidity to match 0.5 McFarland standards. Each organism was evenly spread on separate NA plates using sterile cotton swabs. Wells of 6 mm diameter were created using a sterile cork borer.

Different concentrations of honey (10%, 20%, 30%, 40%, and 100%) were prepared by diluting with sterile distilled water. Each well was filled with 100 µL of the respective concentration. As controls, standard antibiotic discs such as ciprofloxacin (for *E. coli*) and azithromycin (for *S. aureus*) were placed on the same plates. All plates were incubated at 37°C for 24 hours, after which zones of inhibition were measured in millimeters using a transparent ruler. The experiment was conducted in triplicate for accuracy and reproducibility.

III. RESULTS

TABLE 1 shows the results depicting the levels of iron, selenium, zinc, and iodine micronutrients assessed in honey samples collected from three different zones of Sokoto state, Nigeria. 7.3 ± 0.5 to 10.11 ± 0.15, 0.50 ± 0.01 to 0.60 ± 0.01, 2.6 ± 0.1 to 11.0 ± 0.05 ppm, 0.05 ± 0.001 to 1.30 ±0.01 ppm are concentration ranges of Fe, Se, Zn, and I respectively assessed from samples of honey obtained from 3 zones of Sokoto. However, determination of micronutrients in materials such as honey is essential because micronutrients (such as honey Fe, Zn, se, and I) are essential, our body cannot make them, rather the body relied on import from food materials like honey [5]. Honey contents vary according to various factors, and the intake of micronutrients in very low level or high-level food materials can affect health of the body. This motive will help in checking and maintaining quality; and avoidance of contamination as well [5, 12] Therefore, the concentrations of micronutrients in food samples need proper monitoring and quality checkup [5, 12, 16]. More especially, Fe, Zn, are essential when in minute amount, but when in excess act as heavy metals and in turn leading to toxicity [5, 12]. Trace iron amount is involved in hemoglobin biosynthesis, redox reactions etc. zinc plays vital riles in metabolic processes, and is involved in enzymes (as cofactor, signal transduction, and structural components [5, 15, 17]. Noteworthy, iodine determined in the sampled honey is trace (because iodine recommended dietary allowance for adults is >150 g/day). Iodine is vital, because it is needed in thyroid hormones and iodine outcomes are regarded as iodine deficiency disorders (IDD) enzymes [15]. Similarly, selenium in minute amount is needed necessarily for cellular function for instance as component of antioxidant enzymes (gluthathione and thioredoxin reductase. In fact, selenium and iodine are linked; that is why human biological system have 13-20 mg range of selenium content, which is above what was found in the analyzed honey (Table 1) [15]. Low levels of zinc, selenium, iron, and iodine in the analyzed honey from Sokoto is an indication of good quality and lack of pollution [18].

TABLE 1
Showing the levels of Zinc, Iron, Selenium, and Iodine present in honey collected from Sokoto, Nigeria

SOKOTO ZONE	I (ppm)	Fe (ppm)	Selenium (ppm)	Zinc (ppm)
Sokoto East	0.06 ± 0.001	10.11 ± 0. 15	0.50 ± 0.01	11.0 ± 0.05
Sokoto West	0.05 ± 0.001	9.0 ± 0.5	0.60 ± 0.01	5.5 ± 0.01
Sokoto Central	1.30 ± 0.05	7.3 ± 0.5	0.51 ± 0.05	2.6 ± 0.1

Key: Values are expressed as mean ± standard deviation

TABLE 2
Showing the levels of phytochemicals present in honey collected in Sokoto, Nigeria

Serial number	Phytochemical	Concentration
1	Alkaloids	+++
2	Saponins	++
3	Flavonoids	++
4	Phenols	++
5	Tannins	++

Key: ++ concentrated, +++ very concentrated

TABLE 2 shows the result of phytochemical investigation in honey collected from Sokoto. It shows the presence of tannins, saponins, flavonoids, and phenols (as concentrated contents). This has become in agreement with the Rahman etal., (2013) [1] finding that reported various phytochemical metabolites in different honey samples. This has been the major reason that supports the application of honey as an antibacterial therapeutic agent by many [1, 2, 4, 19]. Other possible strategy of honey to neutralize microbes is its ability to contain oxidase that produces hydrogen peroxide. The release of H_2O_2 chemical kills bacteria in the wound and at certain level, the available catalase in the honey acts to neutralize the H_2O_2 [4]. Thus, honey has been demonstrated based on this study to act as a nutritionally rich food containing Fe, Zn, Se, and I, an indicator to measure environmental pollution, and a substance to be utilized against some bacteria species in a safe, and harmonic fashion [1].

IV. DISCUSSION

A. INTERPRETATION OF RESULTS

The analysis of honey samples from Sokoto State confirmed the presence of key micronutrients Fe, Zn, Se, and I in concentrations beneficial to human health. Iron was present at concentrations ranging from 7.3 to 10.11 ppm, zinc from 2.6 to 11.0 ppm, selenium from 0.50 to 0.60 ppm, and iodine from 0.05 to 1.30 ppm. These values fall within acceptable

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dietary intake limits, indicating that Sokoto honey is safe and potentially valuable as a supplementary nutritional source.

The presence of Fe and Zn is particularly significant, given their roles in hematopoiesis, immune function, and enzyme catalysis. Iron facilitates oxygen transport via hemoglobin, while zinc supports tissue repair and acts as a cofactor in over 300 enzymatic processes [26], [27]. Selenium and iodine are essential for thyroid hormone metabolism and antioxidant defense, and their detection in Sokoto honey suggests further health benefits [28], [29].

Phytochemical screening revealed the presence of flavonoids, phenols, alkaloids, tannins, and saponins. These compounds are well-documented for their antimicrobial, anti-inflammatory, and antioxidant activities. For instance, flavonoids can disrupt bacterial cell walls and impede DNA synthesis, while tannins are known to denature bacterial proteins, resulting in growth inhibition [30].

Antibacterial testing demonstrated a clear correlation between honey concentration and bacterial inhibition. Higher concentrations resulted in larger zones of inhibition, with undiluted (100%) honey producing the most significant antibacterial effects against both *Staphylococcus aureus* and *Escherichia coli*. This finding is consistent with previous studies that have documented honey's ability to inhibit both Gram-positive and Gram-negative bacteria [31], [32].

B. COMPARISON WITH PREVIOUS RESEARCH

The antimicrobial activity observed in this study is consistent with previous findings in different geographical contexts. Guruvu et al. [33] evaluated the antibacterial properties of various honey types and reported that inhibition was most prominent at higher concentrations. Similarly, Suerdem and Akyalcin [34] found that honey samples from Kosovo exhibited comparable antibacterial spectra against *E. coli* and *S. aureus*.

Furthermore, Wahid et al. [35] documented trace mineral profiles in honey collected from Sabah, Malaysia, noting levels of Zn and Fe similar to those found in Sokoto honey. This suggests that environmental and botanical factors contribute to mineral content, yet some consistencies exist across regions. Conversely, Saghaei et al. [36] reported the presence of hazardous heavy metals such as lead and cadmium in honey from polluted zones in Iran. The absence of such contaminants in Sokoto honey underscores its ecological purity.

Phytochemical profiles also align with global findings. Amabye and Mekonen [37] documented the presence of similar bioactive compounds in Ethiopian wild honey, attributing antibacterial activity to phenols and flavonoids. The presence of hydrogen peroxide, an additional antimicrobial factor, was confirmed in other works and may act synergistically with phytochemicals to enhance honey's effectiveness [38].

A more recent study by Aljaghwani et al. [39] demonstrated that honey exhibits inhibitory action against multidrug-resistant strains, supporting the claim that honey could serve as an adjunct therapy in antimicrobial resistance (AMR) management. This aligns with global health priorities aimed at reducing dependency on synthetic antibiotics.

C. LIMITATIONS AND IMPLICATIONS

Despite promising results, this study has some limitations. First, the investigation focused solely on two bacterial strains *S. aureus* and *E. coli* which, although clinically relevant, do not represent the full spectrum of pathogenic organisms. Future studies should include fungal species, anaerobes, and drug-resistant variants to establish broader antimicrobial efficacy.

Second, the phytochemical screening was qualitative. While it successfully identified the presence of bioactive compounds, it did not quantify their concentrations. Spectrophotometric and chromatographic analyses in future work could provide detailed insight into the contribution of each compound to antimicrobial activity.

Third, the study was confined to a limited geographical region. Although samples were taken from three zones within Sokoto State, seasonal variation and floral diversity may influence honey composition. A larger sample pool covering different seasons and additional states would strengthen generalizability.

In terms of public health, the findings have several implications. First, the detection of essential micronutrients suggests that honey could be promoted as a functional food, especially in areas with prevalent micronutrient deficiencies. Second, the demonstrated antibacterial activity supports its integration into traditional medicine and topical applications for wound care, particularly in resource-limited settings. Third, honey's potential role in combating antimicrobial resistance highlights the importance of supporting further research and policy development surrounding natural alternatives to antibiotics.

In conclusion, the results affirm that Sokoto honey is both nutritionally valuable and therapeutically effective. Its phytochemical and mineral composition, coupled with its significant antibacterial activity, positions it as a candidate for further development in integrative health care. Future studies should aim to expand the microbiological spectrum, employ quantitative assays for bioactive compounds, and explore synergistic interactions between honey and conventional antibiotics.

V. CONCLUSION

This study aimed to investigate the phytochemical composition, micronutrient profile, and antibacterial activity of honey collected from different zones in Sokoto State, Nigeria, with a specific focus on its efficacy against *Staphylococcus aureus* and *Escherichia coli*. The findings confirmed that Sokoto honey contains essential micronutrients such as iron (7.3–10.11 ppm), zinc (2.6–11.0 ppm), selenium (0.50–0.60 ppm), and iodine (0.05–1.30 ppm), which are important for immune function, metabolism, and cellular health. In addition to its nutritional content, the honey exhibited significant phytochemical presence, including alkaloids, flavonoids, phenols, tannins, and saponins, which are known for their antimicrobial and antioxidant properties. The antibacterial assay revealed a concentration-dependent inhibition pattern, with the highest zone of inhibition recorded at 100% honey concentration against both test organisms. These results suggest that Sokoto honey possesses strong antibacterial properties that could support its application in managing bacterial

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infections, particularly wound pathogens, and enhance dietary micronutrient intake in populations vulnerable to deficiencies. The absence of toxic metal contamination further supports its safety and ecological quality. This study provides a scientific basis for promoting the therapeutic and nutritional use of raw honey in public health interventions, particularly in regions with limited access to modern healthcare services. However, the study also identified limitations, such as the exclusion of resistant bacterial strains, lack of quantitative phytochemical analysis, and a narrow sample origin. Therefore, future research should expand microbial scope to include a broader range of pathogenic strains, employ advanced analytical techniques like HPLC for phytochemical quantification, and assess seasonal and geographical variations of honey composition across Nigeria. Additionally, exploration of synergistic effects between honey and conventional antibiotics may provide new insights into managing antimicrobial resistance. The potential of Sokoto honey as a functional food and alternative therapeutic agent warrants deeper investigation through in vivo models and clinical trials to establish safety, efficacy, and dosage standards applicable in medical practice.

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DATA AVAILABILITY

No datasets were generated or analyzed during the current study.

AUTHOR CONTRIBUTION

All authors contributed significantly to the development of this research. Nura Maiakwai Salah and Asiya Gidado Yabo conceptualized the study and designed the experimental framework. Yusuf Sarkingobir and Aminu Umar Imam were responsible for sample collection, microbial testing, and data analysis. Malami Dikko and Atiku Yari Dogon Daji performed the laboratory-based phytochemical and elemental analyses. Rilwanu Umar and Yusuf Yahaya Miya contributed to literature review, manuscript writing, and editing. All authors reviewed and approved the final version of the manuscript for publication.

DECLARATIONS

ETHICAL APPROVAL

The authors declare that there are no conflicts of interest regarding the publication of this paper. This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors. All procedures performed in the study involving bacterial isolates were carried out in accordance with standard ethical and biosafety guidelines. Informed consent was obtained from all participants for the collection of wound samples used for microbial analysis. The data generated or analyzed during this study are included in this published article and are available from the corresponding author upon reasonable request.

CONSENT FOR PUBLICATION PARTICIPANTS.

Consent for publication was given by all participants

COMPETING INTERESTS

The authors declare no competing interests.

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