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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** The challenges of poor healthcare, antimicrobial resistance, infectious diseases spread, and malnutrition are concerns in Sokoto state. Thus, a natural honey was studied for micronutrients (Zn, Fe, Se, I), phytochemicals, and antibacterial activity on *Staphylococcus aureus* and *Escherichia coli* isolated from wound using standard methods and reagents of analytical grade. Atomic absorption spectroscopy determined Fe, Zn, Se, and I as follow:  $7.3 \pm 0.5$  to  $10.11 \pm 0.15$  ppm,  $0.50 \pm 0.01$  to  $0.60 \pm 0.01$  ppm,  $2.6 \pm 0.1$  to  $11.0 \pm 0.05$  ppm,  $0.05 \pm 0.001$  to  $1.30 \pm 0.01$  ppm are ranges of concentrations in honey in Sokoto. When agar well diffusion method was used to determine the antibacterial activity of the honey on the test microorganisms. The result revealed that, the honey samples have strong antibacterial activities against the test organisms and zones of inhibition obtained showed high antibacterial activity. The antibacterial activity increased proportionally with the concentrations of honey. At all concentrations, the honey had a high antibacterial activity (clearer zone) on *Staphylococcus aureus* and *Escherichia coli*. The presence of phytochemicals in honey, is one of the reasons for using honey in therapy on more recent times is vindicated. This is the reason why the honey is accepted particularly in the treatment of ulcers and bed sores, and other infectious problems or burns and wounds. The presence of honey Zn, Se, Fe, and I as micronutrients is a great gift for curbing malnutrition.

INDEX TERMS Honey, antibacterial, iron, selenium, micronutrients, iodine

## I. INTRODUCTION

Honey is a sweet substance synthesized by honey bees from plant materials or the likes. The product is a good source of energy, and related nutrients [1]. Additionally, many people utilized honey for purposes other than nutritionally-based applications. For instance, there has been reported

application of honey in the traditionally medical therapy to cure infections cause by microbial agents [1,2, 3]. This activity of using honey as an antibiotic/ antibacterial agent against has been speculated to be possible, because the honey is composed of diverse array of phytochemicals that are

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naturally antimicrobials. Moreover, the honey possesses two important significant enzymes, viz, oxidase and catalase. The oxidase is saddled with the task of gushing out hydrogen peroxide ( $H_2O_2$ ), a potent toxic compound with ability to kill bacteria. And the catalase is saddled with the control of  $H_2O_2$  production by neutralizing the  $H_2O_2$  to minimize overactivity of oxidase enzyme therein [4].

Noteworthy, minerals naturally occur in the biosphere as obtained from inorganic substances and gather due to organisms' actions, many of these minerals have to be present in bioavailable fashion for life to smoothly go on because cells and tissues require them for biological functions, maintenance of normal physiological reactions, and many more important activities. These metals require by the body are classified severally. Therewith, zinc, iron. selenium, and iodine are needed by the body in small amount and are therefore dubbed as essential micronutrients [5]. Fortunately, honey elsewhere has been reported to contain many mineral elements along with energy source, and antimicrobial agents [5]. This is not an absurdity, because the honey bee is an animal that utilizes several plant materials, the producers of foods and major source of minerals in the biosphere [6, 7]. However, element such as iodine, selenium, zinc and iron (potentially toxic metals under slight concentration elevation) when in excess in food materials such as honey indicate pollution and can invariably cause harm to human consumers [8, 9, 10]. Thus, it is imperative to assess the levels of elements such as Zn, Se, Fe, and I in honey; phytochemicals, and antimicrobial activity on microbes to maintain quality, safeguard public health, aid in disease therapy, and monitor environmental pollution [7]. Because, honey compositions are affected by their diversity of geography, environment, food intake, genetics, etc [11, 12]. The objective of this study was to determine the Fe, Zn, Se, and I level in honey collected in Sokoto; determine the phytochemicals and antibacterial activity of honey on Staphylococcus aureus and Escherichia coli isolated from wound.

## II. METHODS

#### A. SOURCE OF SAMPLE

The wound sample used in this work was collected /obtained from the medical department of Specialist Hospital, Sokoto. The honey used was obtained from local commercial producers in Sokoto State, Nigeria. It did not contain any diluents or additives and had not been heated [2].

# B. MEDIA PREPARATION

# 1. NUTRIENT AGAR PROCEDURE:

Nutrient agar powder of 5.6g was measured on a weighing balance following manufacturer's instruction. The nutrient agar powder was dissolved in 200ml of distilled water and total dissolution was ensured before autoclaving. The sterilized medium was allowed to cool a little after which it was dispensed aseptically into Petri dishes and allowed to gel [2].

#### 2. EOSIN METHYLENE BLUE PROCEDURE:

Eosin Methylene Blue agar powder of 36g was measured on a weighing balance following manufacturer's instruction. The EMB agar powder was dissolved in 1000ml of distilled water and total dissolution was ensured before autoclaving. The sterilized medium was allowed to cool a little after which it was dispensed aseptically into Petri dish and allowed to gel [2].

#### C. SAMPLE PROCESSING

The wound samples which was collected from Specialist Hospital using a sterilized swap sticks were inoculated directly on the freshly sterilized media and incubated at room temperature for 24hrs.

## D. IDENTIFICATION OF TEST ORGANISMS

The test organism which was collected from the medical department of Specialist Hospital, Sokoto was purified and verified by sub-culturing the test organisms on fresh agar plates (NA, and EMB) and carrying out biochemical tests such as Gram staining, catalase and coagulase test to identify the organisms.

#### 1. GRAM STAINING

A smear of the isolate was made on a clean grease free slide, air dried and heat fixed. The slide was flooded with 0.5% solution of crystal violet and allowed for 1 minute. The stain was washed off with water and flooded again with iodine solution (mordent) and allowed for 30 seconds after which it was washed off.

The slide was decolorized with alcohol/acetone for 10 seconds and washed off immediately with distilled water. The slides were counter stained with saffranin for 30 seconds and rinsed with distilled water and allowed for air dry. The stained slide was viewed under the microscope using x100 objective lens (immersion oil) [2].

#### 2. BIOCHEMICAL TEST

Catalase Test. This was performed by dropping a loopful of hydrogen peroxide on a clean grease free slide followed by the mixing of the loopful of isolate with the hydrogen peroxide on the slide. The production of gas bubbles from the mixture which occurred almost immediately, is a positive reaction  $2H_2O_2$   $2H_2 + O_2$ 

Coagulase Test. This test is used to differentiate staphylococcus aureus from streptococcus species.

Antibacterial Sensitivity Test. The antibacterial activity of honey collected from Sokoto State against the two pathogens was tested in-vitro using agar well diffusion method (Kirby Bauer's method). The test materials were prepared by diluting each honey in sterilized double distilled water at different dilution (concentration) 10%, 20%, 30% and 40% also net honey (100%).

Nutrient agar plates were prepared and each plate was properly inoculated with each test organism using streaking method with the help of a sterile wire loop. Wells were made

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	$0.06 \pm 0.001$	$10.11 \pm 0.15$	$0.50 \pm 0.01$	$11.0 \pm 0.05$
Sokoto West	$0.05 \pm 0.001$	$9.0 \pm 0.5$	$0.60 \pm 0.01$	$5.5 \pm 0.01$
Sokoto Central	$1.30 \pm 0.05$	$7.3 \pm 0.5$	$0.51 \pm 0.05$	$2.6 \pm 0.1$

Key: Values are expressed as mean  $\pm$  standard deviation

using a sterile cork borer and each well was filled with different concentrations of the honey. The plates were incubated at 37°C for 24hrs and observed for zone of inhibitions.

This in-vitro experiment was compared with the use of a control sensitivity disc which served as a control.

often leads to confusion because equations do not balance dimensionally. If you must use mixed units, clearly state the units for each quantity in an equation [2].

# Elemental and phytochemical analysis

Fe, Zn, Se, and I were determined using atomic absorption spectroscopy (AAS) as reported in [13, 14]. Phytochemicals were determined using standard methods related in [13, 14].

#### **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.  $7.3 \pm 0.5$  to  $10.11 \pm 0.15$ ,  $0.50 \pm 0.01$  to  $0.60 \pm 0.01$ ,  $2.6 \pm 0.1$  to  $11.0 \pm 0.05$  ppm,  $0.05 \pm 0.001$  to  $1.30 \pm 0.01$  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 2
Showing the levels of phytochemicals present in honey collected in Sokoto. Nigeria

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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<sub>2</sub>O<sub>2</sub> chemical kills bacteria in the wound and at certain level, the available catalase in the honey acts to neutralize the H<sub>2</sub>O<sub>2</sub> [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**

In TABLE 3, this study investigated the possible antibacterial activity of honey from Sokoto State on two pathogenic microorganisms (*Staphylococcus aureus* and *Escherichia coli*) isolated from wound demonstrated antibacterial spectrum and efficiency against the test bacterial. From the result represented on TABLE 4, it was observed that the zone of inhibition was increased with the concentration of the honey i.e. an increase in the honey concentration increases the zone of inhibition. This observation agrees with [20]. In the same vein, the

TABLE 3

Shows the isolation of samples collected from Special Hospital Sokoto. Nigeria so as to identify organisms in guestion

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	Samples	Gram	Morph.	Catalase	Coagulase	Slant	Butt	H <sub>2</sub> S	Gas	Bacterial	1
		Reaction								identified	
	A	(+Ve)	Cocci	(+Ve)	(+Ve)	K	K	K	K	Staphylococcus aureus	
	В	(-Ve)	Rod	(-Ve)	(-Ve)	A	A	K	K	Escherichia	
										coli	

TABLE 4
The biochemical test carried out and the zone of the inhibition measured in honey in Sokoto, Nigeria

		EXTRACT		CONTROL		
S/N	Test organisms	Concentration (mg/ml)	Zone of inhibition (mm)	Antibiotics (mg)	Zone of inhibition (mm)	
1.	Staphylococcus aureus	10	1.8	RD	3.0	
		20	2.3	CTZ	1.5	
		30	2.6	S	1.5	
		40	3.0	AZM	-	
2.	Escherichia coli	10	1.0	CFX	1.0	
		20	1.3	PEF	3.0	
		30	2.2	CN	3.0	
		40	2.5	AU	-	

Key:

The result above shows that honey has antibacterial potentials. Different concentration of the honey produced different zones of inhibition.

antibacterial activity of honey was recently investigated [22] by microbiologists looking at the effect of honey on their collection of MRSA – strains of Staphylococcus aureus that cause ward closures in hospitals because they are resistant to most or all of the commonly used antibiotics. All the strains they studied were found to have their growth halted completely by the honeys at 5 – 10%. Also it has been demonstrated [21] that honey can accelerate wound healing and also possess bacterial properties [20]. From the findings of this work, it has now been depicted that, the honey would serve as a good substance in counteracting the activities of the two microbes (*Staphylococcus aureus* and *Escherichia* 

## VII. CONCLUSION

Sokoto state is in a number of challenges affecting the public health. For instance, the issues of micronutrient deficiency especially among children, infectious diseases (especially among rural dwellers), and poor or expensive healthcare are pronounced. Thus, the search for effective natural and cheap substances like honey begins. This study aimed to discover the available phytochemicals that can be effective in preventing microbes especially the Staphylococcus aureus and Escherichia coli) in the state. Likewise, there is intention to unveil the concentrations of some micronutrients (Fe, Zn, Se, and I) so as to help in the prevention of malnutrition among residents. The honey collected from Sokoto contains phytochemicals and possess antibacterial activity against Staphylococcus aureus and Escherichia coli. Likewise, the honey was found to be rich in Zn, Fe, Se, and I micronutrients that are essential in human life especially among the young ones.

coli) when more technical tests and studies are observed. Likewise, the presence of the phytochemicals is an indication that, the substance could be effective on other forms of microbes. This can be ascertained through more tests and this a good portend in the state where there are challenges regarding healthcare especially in the rural areas. Similarly, the presence of micronutrients (Zn, Fe, Se, and I) is a good portend that, the substance could be used by the public to address the issues of micronutrients deficiencies in the state especially among the young ones, but further scientific observations need to be taken into cognizance as well [23, 24, 25].

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<sup>+ =</sup> Positive

<sup>- =</sup> Negative

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