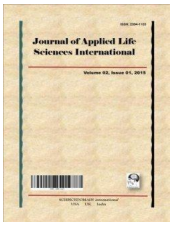

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In-Vitro Antimicrobial Effect of Different Honey Samples against Selected Micro-organisms Marketed in Abuja Nigeria

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Authors' contributions

This work was carried out in collaboration between all authors. Author KTO designed the study, collected the samples, performed the statistical analysis and wrote the draft of the manuscript. Authors MA and PO managed the analyses of the study and also the literature search. All the authors read and approved the final manuscript.

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ABSTRACT

Aim: This study aimed to investigate the antimicrobial effect of honey samples sold in different markets in Abuja, Nigeria against some selected microbial isolates.
Study Design: Five honey samples obtained from different markets in Abuja metropolis, Nigeria were used for this study against some medically important micro-organisms viz; *Staphylococcus aureus*, *Escherichia coli*, *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Salmonella paratyphi*, *Klebsiella pneumonia* and *Candida albicans*.
Place and Duration of Study: The study was conducted in Abuja, Nigeria at the Department of Microbiology and Biotechnology, National Institute for Pharmaceutical Research and Development [NIPRD], from January 2018 to February 2018.
Methodology: Agar diffusion technique of Microbiological standard was employed. Each of the 5 honey samples was diluted in two-fold dilutions to have 100, 50, 25 and 12.5% concentrations respectively and tested against the test organisms. A fixed-dose concentration of chloramphenicol

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at 30 µg/mL was used as a control drug against the bacterial isolates.
Results: All the honey samples showed varying degrees of inhibitory effect against all the test organisms. It inhibited all the organisms at 100% concentration while some samples showed activity at 50% concentration. However, only *Salmonella paratyphi* was susceptible at 25% while no effect was observed at 12.5% concentration for all the honey samples.
Conclusion: The study has shown that honey has an antimicrobial effect on certain micro-organisms, and could be used as an adjunct therapy against certain infections to minimise proliferation of multi-drug resistant micro-organisms in the society.

Keywords: Honey; antimicrobial; bacteria; resistance; susceptibility.

1. INTRODUCTION

Honey is one of the oldest traditional medicines considered as a remedy for different microbial infections and also recognised as an effective antimicrobial agent in the treatment of burns and wounds [1]. Honey is a sweet and viscous substance which is produced by bees and some related insects. Honey is produced by bees from the nectar (floral) or honeydew (aphid) through regurgitation, enzymatic activity and water evaporation [2]. Honey is produced by bees collecting nectar for use as sugars consumed to support the metabolism of muscle activity during foraging or to be stored as a long-term food supply [3].

The antimicrobial activity of honey was first recognised in 1892, by Van Ketel [4], which was then followed by extensive research conducted to substantiate this claim further and to demonstrate factors that contribute to the antimicrobial activity [5].

Honey is made from many different floral sources, and its antimicrobial activity varies greatly with origin and processing [5]. Honey is produced when the nectar and sweet deposits from plants are assembled, modified and kept in the honeycombs by honeybees of the genus *Apis mellifera* [6,7]. Honey has shown very strong antimicrobial effects against pathogenic and non-pathogenic organisms (yeasts and fungi) and also against those that developed resistance to many antibiotics which could be bacteriostatic or bactericidal depending on the concentration used [8]. Honey also inhibits great bacteria diversity such as *Staphylococcus a*ureus, *Escherichia coli* and *Pseudomonas spp* [9]. These organisms are the common causes of nosocomial infections in humans. There are reports of *S. aureus, E. coli* and *P. aeruginosa* as multi-drug resistant bacteria against existing antibiotics thus, honey based on reported antimicrobial effects could provide adjunct therapy against resistant bacteria. *Pseudomonas aeruginosa* emerged as an important human pathogen which is tolerant of temperatures as high as 50°C and is capable of growing under aerobic conditions, as well as anaerobic conditions [10]. The organism is a challenging pathogen in the hospital setting as it is resistant to many antibiotics and also capable of forming hardy biofilms, both within the body and on the surfaces of medical instruments [11,12,13]. However, *P. aeruginosa* is an opportunistic organism infecting; burn, cystic fibrosis, leukaemia, transplant, neutropenic, long-term urinary catheters, and diabetic patients as well as intravenous drug abusers. *Staphylococcus aureus* is also considered to be a major pathogen found naturally on the skin and in the nasopharynx of the human body that colonises and infects both hospitalised patients with decreased immunity, and healthy immuno-competent people in the community. The organism causes infections of the skin, nose, urethra, vagina and gastrointestinal tract, most of which are minor and not life-threatening [14]. *Escherichia coli* remain one of the most frequent causes of several common bacterial infections in humans and animals responsible for a broad spectrum of diseases. It is the prominent cause of enteritis, urinary tract infection, septicemia, other clinical infections, such as neonatal meningitis and also prominently associated with diarrhoea [15]. *Salmonella paratyphi*, a causative agent of enteric fever is capable of causing serious and often life-threatening infections like infective endocarditis, pericarditis, Sino-venous thrombosis, osteomyelitis, meningitis and bone marrow infiltration [16]. There are also reports in the literature of abscesses caused by *S. paratyphi*, including renal abscess [17].

There are basically two main types of honey, apiary and forest honey. Apiary honey is produced by the honeybees in apiaries, *Apis cerana indica* and *Apis mellifera*, which are gathered together by the modern extraction

method whereas forest honey is produced in forests by rock bee, *Apis dorsata*, or wild nests of *A. cerana indica*, and are gathered together by the crude method of squeezing the comb [18]. Honey is an essentially concentrated aqueous solution of inverted sugars, and also contains a complex mixture of other saccharides, proteins, enzymes, amino acids, organic acids, polyphenols, and carotenoid like substances, Maillard reaction products, vitamins and minerals [19]. Honey constitutes about 95 to 97% of carbohydrate [20]. The most common sugars present and which are responsible for most of the physical and nutritional characteristics of honey are fructose and glucose [21]. The antibacterial effect of honey has been linked with strong osmolarity, low pH [22], ability to produce hydrogen peroxide and gluconic acid which originates from the dissolution of sugar by honey's glucose oxidase [5,9] and other honey components such as aromatic acids or phenolic compounds [23].

Therefore, this study investigates the antimicrobial effect of different honey samples marketed at different locations in Abuja metropolis against some selected micro-organisms implicated in human infections in the society.

2. MATERIALS AND METHODS

2.1 Sample Collection

A total of five commercial honey samples were collected into sterile containers from five different markets in Abuja, Nigeria and were stored in a dark place at room temperature (24 - 26°C). Four of the honey samples were collected from different markets in Abuja Municipal Area Council (Karmo, Gwa-Gwa, Dei-Dei and Kado) while one of the honey samples was collected from Bwari market in Bwari Area Council, Abuja FCT, Nigeria.

2.2 Description of the Survey Areas

Abuja Municipal Area Council (AMAC) is the largest and most developed of the six (6) area councils. The bulk of the built-up area of AMAC is made up of the Federal Capital City (FCC). AMAC is located between latitude $8^0\ 40^1$ and $9^0\ 20^1$ north of the equator and longitude $6^0\ 40^1$ and $7^0\ 40^1$ east of the Greenwich meridian. The Abuja Federal Capital Territory has a land mass of approximately 8000 sq km of which the FCC occupies about 250 sq km with population recent census at 778,567 for AMAC [Federal Republic of Nigeria Official Gazette, 2007]. The four markets (Karmo, Gwa-Gwa, Dei-Dei and Kado) are situated on the same plain area that spans about 25 kilometres of 5 kilometres distance from each market. Bwari Area Council is located in the north-eastern part of FCT. It is approximately fifteen kilometres north of Abuja city and twenty-five kilometres north-east of Suleja in Niger state. The northern expressway of Abuja is the boundary between Abuja Municipal Area Council and the Bwari Area Council [24].

2.3 Test Organisms

Pure clinical isolates of *Bacillus subtilis, Klebsiella pneumoniae*, *Candida albicans* collected and biochemically confirmed from Diagnostic Laboratory of NIPRD clinic and American Typed cultures of *Escherichia coli* [ATCC 25952], *Staphylococcus aureus* [ATCC 25923], *Pseudomonas aeruginosa* [ATCC 27853], *Salmonella paratyphi* [ATCC 9150] were used in this study.

2.4 Determination of Anti-microbial Activity

Prepared concentrations [100%, 50%, 25% and 12.5% v/v] of honey samples were tested against the organisms using Agar well diffusion method [25]. Test organisms were suspended into 5 ml of Mueller Hinton broth [MHB, Fluka] and incubated at 37°C. Following incubation at 37°C for 24 hrs, organisms were diluted with normal saline to a turbidity that was equivalent to 0.5 Mc Farland standard [10^6 CFU)/ml] [26]. One hundred microliter of the suspension of standardised microorganisms was inoculated into sterile molten Mueller Hinton agar, swirled and poured into sterile Petri dishes and allowed to solidify. Holes for each concentration of the honey sample and positive control were bored aseptically using a sterile cork borer of 6 mm. The bottom of the bored holes was sealed using a drop of Mueller Hinton agar. One hundred microliters of different concentrations of the sample and a fixed dose [30 µg/mL] of the positive control, chloramphenicol being a drug of choice as a broad spectrum antibiotic was dispensed into appropriately labelled wells respectively. The plates were allowed to dry inside the biosafety cabinet as well as allowing the honey to diffuse for about 2 hrs and then incubated at 37°C for 24 – 48 hours.

Antibacterial activity was assessed by measuring the size of the zone of inhibition surrounding wells and taking the average of the readings of each duplicate plate post incubation.

2.5 Statistical Analysis

Data were analysed using graph pad prism version 6.04. Results were expressed as means ± standard deviation and differences between means were analysed statistically using an analysis of variance (ANOVA).

3. RESULTS AND DISCUSSION

The five honey samples from different markets in Abuja evaluated for antimicrobial activity against Gram-positive bacteria viz; *Staphylococcus aureus, Bacillus subtilis* and Gram-negative bacteria such as *Escherichia coli, Pseudomonas aeruginosa, Salmonella paratyphi, Klebsiella pneumoniae* and *Candida albicans* [yeast like fungi] showed varying degrees of activity. From Table 1, *Salmonella paratyphi* was most susceptible to the antibacterial efficacy of the honey sample from Karimo market with an average zone of inhibition of 29 mm at 100% concentration. The activity was reduced to 26 mm at 50% and 19 mm at 25% concentration, indicating that the efficacy of the honey is dose dependent. The lowest average zone of inhibition was observed against *Escherichia coli* [9 mm] at 100% concentration while *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* were resistant to the sample at all concentrations tested.

Honey samples from Dei-Dei market also showed varying antimicrobial activity against *Salmonella paratyphi* and *Candida albicans* having inhibitory zones of 31 mm and 7 mm respectively at 100% concentration.

Furthermore, the sample from Gwagwa market recorded a high antimicrobial efficacy against *Salmonella paratyphi* at 100% [33 mm] while its activity against *Staphylococcus aureus* plummeted to 7.5 mm. However, no activity was observed against *Pseudomonas aeruginosa* at all the tested concentrations. *Salmonella paratyphi* was the most susceptible microorganism to samples from Bwari and Kado markets with an average zone of inhibition of 27.5 mm and 33 mm at 100% respectively while *Klebsiella pneumoniae* was resistant at all concentrations to honey sample from Bwari market.

Among the microbial isolates tested, *Salmonella paratyphi* was the most susceptible to all the honey samples, whereas *Pseudomonas aeruginosa* was resistant to all the honey samples except for Dei-Dei sample which had activity at 100% concentration. This observation implies that the honey samples tested are therapy specific. These results are in agreement with a report by Bilal et al. [27] who found that honey exhibited a fairly good antimicrobial activity against both Gram-negative and positive bacteria and a remarkable activity was observed with *Salmonella paratyphi* and *Bacillus subtilis*. A study [28] previously showed that honey inhibited the growth of *S. aureus* and *E.coli* at 100% concentration and this is in conformity with our present findings on the effect of honey against micro-organisms. A work by Tan et al. [29] on honey samples, showed activity against *E. coli, S. aureus, B. subtilis* and *P. aeruginosa.* Also, El-Sukhon et al. [30] reported that Gram-negative bacteria are more sensitive to the action of honey than Gram-positive bacteria which is in agreement with our present study. The antimicrobial effect of honey on Gram-negative bacteria by Taormina et al. [31] attributed it to the presence of high content of tetracycline derivatives, hydrogen peroxide and powerful antioxidants, as also to a naturally low pH, and to the presence of phenolic acids, lysozyme and flavonoids. A report by Zumla and Lulat [32], showed that honey is an excellent inhibitor to *E. coli*, *Salmonella* and *Shigella.* The present study showed antimicrobial activity against *S. aureus*, which is in agreement with a work done by Molan [33] who found *S. aureus*, as one of the bacterial species most susceptible to the antibacterial activity of honey. A study by Shyamapada et al. [34] showed that honey has less antimicrobial activity against *P. aeruginosa* as compared with the other test microorganisms*, S. typhi* and *E. coli*. The *in vitro* antimicrobial activity of honey reported by Radwan et al. [35], observed that honey stopped the growth of *Salmonella* and *Escherichia coli*. A study conducted by Nzeako and Hamdi [36], on six commercial kinds of honey found that *S. aureus, E.coli* and *P. aeruginosa* did not inhibit the honey concentrations at 40%.

It is worthy of note to mention that, further dilution of the honey below 25% concentration had no further effect on susceptible organisms (Table 1) similar to a previous study [28]. The differences observed in activity may be due to the osmotic pressure, low pH [33,37], nectar used in the production of the honey [38], growth

Table 1. Antimicrobial activities of different concentrations of the honey sample against microbial Isolate

			Zone of inhibition (mm)			
S/No.	**Location**	**Microbial isolates**	**100%**	**50%**	**25%**	**12.5%**
1	Karmo	*Staphylococcus aureus*	9.5±0.71	-	-	-
		Bacillus subtilis	12.0±0.00	7.5±0.71	-	-
		Escherichia coli	9.0±0.00	-	-	-
		Pseudomonas aeruginosa	-	-	-	-
		Candida albicans	10.0±0.00	-	-	-
		Salmonella paratyphi	29.0±0.00	26.0±0.00	19.0±0.00	-
		Klebsiella pneumoniae	-	-	-	-
2	Dei-Dei	*Staphylococcus aureus*	9.0±1.41	-	-	-
		Bacillus subtilis	10.0±2.83	-	-	-
		Escherichia coli	9.0±0.00	-	-	-
		Pseudomonas aeruginosa	10.0±1.41	-	-	-
		Candida albicans	7.0±0.00	-	-	-
		Salmonella paratyphi	31.0±0.00	28.0±0.00	22.0±0.00	-
		Klebsiella pneumoniae	17.0±0.00	-	-	-
3	Gwagwa	*Staphylococcus aureus*	7.5±0.71	-	-	-
		Bacillus subtilis	11.5±0.71	-	-	-
		Escherichia coli	8.5±0.71	8.0±1.41	-	-
		Pseudomonas aeruginosa	-	-	-	-
		Candida albicans	10.5±0.71	9.0±1.41	-	-
		Salmonella paratyphi	33.0±1.41	24.0±0.00	20.5±0.71	-
		Klebsiella pneumoniae	15.0±1.41	-	-	-
4	Bwari	*Staphylococcus aureus*	9.0±1.41	-	-	-
		Bacillus subtilis	11.0±1.41	-	-	-
		Escherichia coli	7.5±2.12	-	-	-
		Pseudomonas aeruginosa	-	-	-	-
		Candida albicans	8.0±0.00	-	-	-
		Salmonella paratyphi	27.5±2.12	22.0±1.41	16.0±1,41	-
		Klebsiella pneumoniae	-	-	-	-
5	Kado	*Staphylococcus aureus*	8.5±0.71	-	-	-
		Bacillus subtilis	10.0±0.00	-	-	-
		Escherichia coli	7.0±1.41	-	-	-
		Pseudomonas aeruginosa	-	-	-	-
		Candida albicans	8.0±1.41	-	-	-
		Salmonella paratyphi	33.0±1.41	25.5±0.71	14.5±0.71	-
		Klebsiella pneumoniae	8.0±0.00	-	-	-

Values are expressed as Mean ± S.D. - = No activity P<0.05

the rate of the micro-organisms, temperature, nutritional requirements and test methods [39]. The resistance of *Pseudomonas aeruginosa* to the honey samples might be due to the exo-pigmentation of the organism and environmental conditions [40]. Finally, different degrees of activity observed in the different honey samples may indicate different sources despite the closeness of the markets.

4. CONCLUSION

The study has shown that honey has an antimicrobial effect on certain micro-organisms, and could be used as an adjunct therapy against certain infections to minimise proliferation of multi-drug resistant micro-organisms in the society.

5. RECOMMENDATION

In future, the authors hope to obtain honey samples directly from the manufacturers within different states in Nigeria to access if the differences in climatic changes can affect its antimicrobial activity.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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