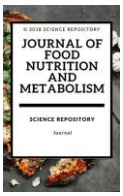

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Research Article

Antimicrobial Activity of Honey Samples from Ovia North East Local Government Areas in Edo State, Nigeria

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ABSTRACT

Background: Honey is one of the oldest natural products in our environment used as natural alternatives for emerging and re-emerging infectious diseases.

Objectives: This study was carried out to determine the antimicrobial activity of some honey samples collected from Ovia North-East local government area of Edo state Nigeria.

Materials and Methods: Three honey samples were obtained in April 2017 from local commercial producers in Ovia North-East local government area of Edo state Nigeria. Bacteria strains of *Staphylococcus aureus, Escherichia coli, Proteus spp, Klebsiella spp* and *Pseudomonas aeruginosa* were obtained from the University of Benin Teaching Hospital Edo state Nigeria. The antimicrobial activity of the honey samples was performed using the Kirby-Bauer disc diffusion method. The antimicrobial activity of Ciprofloxacin was also evaluated as a positive control.

Results: All the honey samples had antimicrobial activities against the pathogens tested. Most of the strains showed the highest inhibition zone and hence better antimicrobial activity when the sample from Ogbese was tested against the strains. An increase in the honey concentration increased the activity of the sample and hence the zone of inhibition against the pathogens tested. Among the Bacteria strains tested *Staphylococcus aureus* and *Pseudomonas aeruginosa* were the most sensitive against all the honey samples.

Conclusion: Our study shows the antimicrobial activity of honey samples from Ovia North east local government area in Edo state Nigeria. As bacterial resistance to antibiotics is on the increase, it is urgently required to discover alternative therapeutic agents.

Introduction

Honey is a sweet food produced by bees using nectar also gotten from flowers. The variety of honey produced by bees belonging to the genus *Apis* is most commonly referred to, which is collected by beekeepers and consumed by humans. Honey can also be referred to as a sweet substance naturally produced by *Apis mellifera* bees using plant's nectar or secretions or excretions of plant sucking insects on the parts of plants that are living which the honeybees collect and process until they can be harvested from honeycombs [1]. The flower from which bees gather nectar largely determines the colour, flavor and aroma of honey [2]. The chemical composition of honey also depends on the flower from which it is made. Antibacterial effect may therefore vary between different types of honey [2]. The sweet taste of honey is as a result of its fructose and glucose content and approximately it is relatively as sweet as granulated sugar [3]. Honey has a high nutritional composition containing other complex carbohydrates, different organic and amino acids, vitamins, minerals, proteins and other substances that influence its antimicrobial activity [4].

Previous reports show the ancient medicinal history of honey [5-7]. Honey is considered as one of natural products that serve as all-around remedies owing to its antibacterial and anti-inflammatory properties [7]. There are several reports of the antimicrobial activity of honey including its activity on oral pathogenic and food spoilage bacteria [8-10]. Honey can also be used in treating ulcers, bed sores and other skin infections as

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a result of burns and wounds [11, 12]. Honey has an osmotic effect, low pH, high content of reducing sugars, hydrogen peroxide, defensin- 1 as well as phytochemical factors which determines to a large extent the antimicrobial activity of honey [12-15]. The presence of hydrogen peroxide contributes majorly to the antimicrobial activity of honey [16, 13, 17, 18]. Catalase stems from the pollen of plants and it determines how much of peroxide is available in honey samples [19]. Light, temperature and oxygen also determine the quantity of hydrogen peroxide available. An earlier report showed the antibacterial activity of honey due to components such as phenolic compounds, lysozyme, flavonoids, acidity and osmolarity which are non-peroxide components [20]. Clinically, honey can take care of infections in the body, stimulate the growth of cells, strengthen the immune system and also act as anti-inflammatory and antioxidant agents [21].

Honey has antibacterial activity on bacteria commonly involved in wound infections like *Kleb spp, E. coli, S. aureus,* and *P. aeruginosa* despite their resistance to certain antibiotics [22-24]. In a previous report, honey had a better effect which included low rates of infection, less inflammation and faster healing when its activity was compared with silver sulphadiazine in treating patients with burns [25]. Honey also has antifungal effect especially on *Candida spp* [16, 26, 27]. There is a need to look for alternative remedies for infections caused by pathogenic bacteria especially before resistance will be developed against virtually all existing groups of antibiotics. There are a lot of natural products in our environment which we can explore to combat many emerging and re-emerging infectious diseases. Based on previous reports on honey as a promising antimicrobial agent, the antimicrobial activity of three different honey samples from three villages in Ovia North east Local Government Areas of Edo state Nigeria on *S. aureus, E. coli, Proteus spp, Klebsiella spp* and *P. aeruginosa* was investigated.

Materials and Methods

I Sample Collection

Three honey samples based on inclusion criteria were obtained from local commercial producers in Agbonmoba, Iguadolar and Ogbese villages in Ovia North-East Local Government Area of Edo state, Nigeria. Honey samples did not contain any diluents or additives and had not been heated. The samples were stored in the dark at room temperature (25-35ºC).

II Bacterial Strain

Strains of *S. aureus, E. coli, Proteus spp., Klebsiella spp.,* and *Pseudomonas aeruginosa* were obtained from the Department of Microbiology, University of Benin Teaching Hospital (UBTH), Edo State.

III Antimicrobial Susceptibility Test

The antimicrobial activities of honey samples collected from three different villages in Edo state were tested in-vitro against various pathogenic bacteria using the disc diffusion method [28]. The test materials were prepared by diluting each honey in sterilized distilled water at different dilutions (concentrations) of 1, ½, ¼, 1/8, 1/16, 1/32 and 1/64 that is 100%, 50%, 25%, 12.5%, 6.25%, 3.125% and 1.56% [12]. Mueller Hinton agar plates were prepared, and each plate was properly inoculated with 0.1ml of 10^{-1} concentration of each test organism. Wattman filter paper discs of about 6mm were impregnated with the different dilutions of honey for 30-45minutes. The discs were then placed properly at different points on the plates to prevent overlapping of the inhibition zones. The plates were incubated at 37ºC for 24hours and observed for zones of inhibition after the incubation period. This in-vitro experiment was compared with the use of an antibiotic disc (Ciprofloxacin) which served as a control.

IV Minimum Inhibitory Concentration (MIC) and Mininmu Bactericidal Concentration (MBC)

Minimum inhibitory concentration (MIC) is a measure of the minimum concentration of a chemical which will inhibit visible growth of a bacterium. Minimum bactericidal concentration (MBC) is the lowest concentration of an antibacterial agent that results in the total microbial death in a standard inoculum of a bacterial species [29]. Serial dilutions of the three honey samples were made in seven test tubes to give a final concentration of 1, ½, ¼, 1/8, 1/16, 1/32 and 1/64 that is 100%, 50%, 25%, 12.5%, 6.25%, 3.125% and 1.56%. 0.1ml of 10^{-1} dilution of each of the test organisms was dispensed into different dilutions of honey in the test tubes. The tubes were incubated at 37ºC for 24hours. After incubation, each tube was checked for visible growth. Clear test tube showed break points [30]. Tubes showing no visible sign of growth in the MIC determination were sub cultured on sterile nutrient agar plates by the streak plate method. The plates were then incubated at 37ºC for 24 hours. The lowest concentration that did not show growth of organisms was considered as the minimum bactericidal concentration (MBC).

Results and Discussion

Table 1 shows honey sample from Agbonmoba, Iguadolar and Ogbese at different concentrations with the respective zones of inhibition. At 100% (v/v) concentration the honey samples had maximum inhibition efficiency on all the isolates showing the highest zone of inhibition. At concentration 12.5% (v/v) and below the honey samples had no antimicrobial effect on the isolates (Data not shown). Results show that as dilution of the honey increases the antimicrobial efficacy reduced. This is in agreement with past works that reported the undiluted honey samples inhibited growth of test isolates but when dilutions of the samples were made, they had a reduced effect on the test isolates [31]. Taormina et al. also reported in their study that the development of zones of inhibition in testing the antimicrobial effect of honey depends on the concentration of honey used as well as the test isolates [32]. This correlates also with results obtained in this study where the antimicrobial activity of the samples depended on the type of honey and the concentration it was applied. This study also compares well with a previous report that screened for the antimicrobial efficacy of a type of honey obtained from Ogun state in Nigeria. It was reported that undiluted honey sample inhibited growth of all the test bacteria both gram negative and gram positive in a dose dependent manner [33].

Table 1: Antimicrobial Activity of the Honey samples.

Organism	**Honey Samples**	**Dilution 100**	**(%) 50**	**25**	**Control (Ciprofloxacin)**
Staphylococcus aureus 1	Agbonmoba	20[x]	15	10	19
	Iguadolar	17	14	10	18
	Ogbese	22	17	12	20
Staphylococcus aureus 2	Agbonmoba	15	11	6	16
	Iguadolar	12	8	-	15
	Ogbese	18	16	13	16
Staphylococcus aureus 3	Agbonmoba	18	13	6	17
	Iguadolar	15	10	6	15
	Ogbese	21	15	9	19
Staphylococcus aureus 4	Agbonmoba	15	10	6	15
	Iguadolar	12	10	6	13
	Ogbese	18	13	10	17
Escherichia coli 1	Agbonmoba	14	8	-	17
	Iguadolar	14	8	-	16
	Ogbese	17	14	-	14
Escherichia coli 2	Agbonmoba	14	10	8	18
	Iguadolar	14	10	8	17
	Ogbese	20	17	13	19
Escherichia coli 3	Agbonmoba	17	13	9	19
	Iguadolar	17	12	9	19
	Ogbese	20	16	12	18
Escherichia coli 4	Agbonmoba	14	11	6	16
	Iguadolar	14	11	6	15
	Ogbese	17	15	8	14
Proteus spp 1	Agbonmoba	15	10	-	16
	Iguadolar	12	10	-	14
	Ogbese	18	14	9	19
Proteus spp 2	Agbonmoba	16	12	-	19
	Iguadolar	13	10	-	15
	Ogbese	19	16	9	20
Proteus spp 3	Agbonmoba	13	9	-	14
	Iguadolar	10	7	-	12
	Ogbese	16	12	6	15
Proteus spp 4	Agbonmoba	11	9	6	14
	Iguadolar	10	6	-	13
	Ogbese	14	9	5	16
Klebsiella spp 1	Agbonmoba	10	8	6	12
	Iguadolar	10	8	6	12
	Ogbese	13	8	-	16
Klebsiella spp 2	Agbonmoba	12	9	7	14
	Iguadolar	12	9	7	14
	Ogbese	15	10	6	17
Klebsiella spp 3	Agbonmoba	11	7	-	13
	Iguadolar	15	13	10	18
	Ogbese	14	9	-	13
Klebsiella spp 4	Agbonmoba	15	11	7	17
	Iguadolar	13	10	7	17
	Ogbese	14	9	-	17
Pseudomonas aeruginosa 1	Agbonmoba	18	14	12	19
	Iguadolar	14	12	9	17
	Ogbese	21	17	12	20
Pseudomonas aeruginosa 2	Agbonmoba	12	8	6	20

	Iguadolar	12	8	6	16
	Ogbese	15	11	6	13
Pseudomonas aeruginosa 3	Agbonmoba	13	11	6	17
	Iguadolar	13	11	6	16
	Ogbese	17	10	5	15
Pseudomonas aeruginosa 4	Agbonmoba	19	15	13	20
	Iguadolar	15	10	-	17
	Ogbese	24	15	13	25

Key: (-)- No growth, (x)-zone of inhibition in mm

Table 2: MIC and MBC of Honey samples against the isolates.

Organism	**Honey Samples**	**Minimum Inhibitory Concentration(v/v)**	**Minimum Bactericidal Concentration (v/v)**
Staphylococcus aureus 1	Agbonmoba	25	50
	Iguadolar	25	100
	Ogbese	12.5	25
Staphylococcus aureus 2	Agbonmoba	12.5	25
	Iguadolar	50	100
	Ogbese	12.5	25
Staphylococcus aureus 3	Agbonmoba	12.5	25
	Iguadolar	50	100
	Ogbese	12.5	25
Staphylococcus aureus 4	Agbonmoba	12.5	25
	Iguadolar	50	100
	Ogbese	12.5	25
Escherichia coli 1	Agbonmoba	12.5	25
	Iguadolar	12.5	50
	Ogbese	12.5	25
*Escherichia col*i 2	Agbonmoba	12.5	25
	Iguadolar	50	100
	Ogbese	12.5	25
Escherichia coli 3	Agbonmoba	12.5	25
	Iguadolar	25	50
	Ogbese	12.5	25
Escherichia coli 4	Agbonmoba	12.5	25
	Iguadolar	50	100
	Ogbese	12.5	25
Proteus spp 1	Agbonmoba	50	100
	Iguadolar	25	50
	Ogbese	25	50
Proteus spp 2	Agbonmoba	25	50
	Iguadolar	25	50
	Ogbese	25	50
Proteus spp 3	Agbonmoba	50	100
	Iguadolar	50	100
	Ogbese	12.5	25
Proteus spp 4	Agbonmoba	12.5	25
	Iguadolar	50	50
	Ogbese	12.5	25
Klebsiella spp 1	Agbonmoba	50	100
	Iguadolar	50	100
	Ogbese	25	50
Klebsiella spp 2	Agbonmoba	12.5	50
	Iguadolar	25	100
	Ogbese	12.5	25

Klebsiella spp 3	Agbonmoba Iguadolar Ogbese	12.5 50 12.5	25 100 25
Klebsiella spp 4	Agbonmoba Iguadolar Ogbese	12.5 50 12.5	25 100 25
Pseudomonas aeruginosa 1	Agbonmoba Iguadolar Ogbese	12.5 25 25	25 50 50
Pseudomonas aeruginosa 2	Agbonmoba Iguadolar Ogbese	12.5 25 12.5	25 50 25
Pseudomonas aeruginosa 3	Agbonmoba Iguadolar Ogbese	12.5 50 12.5	25 100 25
Pseudomonas aeruginosa 4	Agbonmoba Iguadolar Ogbese	12.5 50 12.5	25 100 25

The MIC ranged between a concentration of 12.5% and 50% (v/v) dilution even though antimicrobial susceptibility concentrations ranged from 25-100% (v/v) (Table 2). Results in this study show that the range of antimicrobial susceptibility concentrations is the same as the minimum bactericidal concentration which is 25-100% (v/v). Adeoye-Isijola et al., reported the antimicrobial susceptibilities of different honey samples showing a MIC range at a lower concentration of 5 and 30% (v/v) dilution compared to results obtained from our study [34]. The report also showed antimicrobial susceptibility concentration range from 5-100% (v/v). The differences in the antimicrobial activities of honey samples may be due to differences in botanical source as well as geographical origin which consequently affect the chemical composition of the honey samples [35].

Previous reports have shown honey samples to have different antimicrobial efficacies against the same type of bacteria [36, 37]. Honey has also been shown to be effective against antibiotic resistant bacteria such as methicillin resistant *S. aureus*, Vancomycin resistant *Enterococcus spp* and Gram-negative rods that are multiresistant. Tan et al. reported in their study confirming the antimicrobial activity of honey samples [38-40]. Adeoye-Isijola et al. reported different honey samples exhibiting different antimicrobial effects against different test isolates [34]. These reports are similar with results from this study. The antimicrobial efficacy of the honey samples used in this study showed a remarkable antimicrobial spectrum and efficiency against the test bacteria. Results obtained show all the honey samples had antimicrobial efficiency against the test bacteria. Comparing the antimicrobial activity of the three honey samples, it was observed that honey from Ogbese produced the highest zone of inhibition (effect) at 100% (v/v) concentration. The *S. aureus* and *P. aeruginosa* isolates were more susceptible to the honey samples compared to the *E. coli, Proteus spp* and *Klebsiella spp* isolates. There are a number of variable factors that affect the antimicrobial effect of honey samples which include presence of an inhibine factor which is hydrogen peroxide, Osmotic property, Low protein content and non-peroxide components which include complex phenols and organic acids [13, 37, 41].

This study confirms the antimicrobial activity of honey. To the best of our knowledge, this study presents the first detailed report of the antimicrobial activity of honey samples from this locality. The antimicrobial effect against both Gram positive and negative bacteria is presented. It could be used as a therapeutic agent and also as an excellent alternative in treating bacterial infections especially in clinical cases caused by the tested microorganisms. Honey, a promising antimicrobial agent may also curtail the increasing prevalence of multiresistant microorganisms in our environment.

Conflicts of Interest

No Conflict of Interest is declared.

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