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Study on antibacterial efficacy of different honey types in South Western Nigeria against wound associated bacteria

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ABSTRACT

Aim: The study assessed and compared the antibacterial activities of different honey types in Southwest Nigeria. It also compared antibacterial potency of the honey with a standard antibiotic. This was with a view to ascertain and providing information on cheaper alternative potent antibacterial product of natural source as well to confirm the antibacterial efficacy of the honey in Southwest, Nigeria. **Materials and Methods:** The sensitivity testing of honey samples was determined using agar-well diffusion method. The minimum inhibitory concentration of honey samples was determined using broth tube dilution method. Minimum bactericidal concentration of honey samples was determined. The data obtained were analyzed with appropriate statistical methods. **Results:** The zone of inhibitions exhibited by all honey samples against the test bacteria ranged between 6 ± 0.0 and 30.7 ± 1.2 mm. The death rate ranges between 52.1% and 94.6% in the dark amber honey sample (H9) after 120 min of contact time at the same concentration. **Conclusion:** In this study, super dark amber honey shows the highest antimicrobial property which compared favorably with the standard antibiotic (streptomycin). This honey has shown to have a potent broad spectrum antibacterial activity. However, further studies are recommended to assess its practicality in terms of use in the clinical setting.

KEY WORDS: Antibacterial potency, death rate, honey, sensitivity

INTRODUCTION

Antimicrobial agents are very important in lowering the global burden of infectious diseases [1,2] however, as resistant disease causing agents develop and spread, the efficacy of the antibiotics is diminished [3]. This type of resistance to the antimicrobial agents poses enormous threat to public health, and for all kinds of antibiotics, including the major last-resort drugs [4].

The use of local medicine to treat infection has been in existence since the origin of mankind, and honey produced by *Apis* species is one of the oldest traditional medicines considered to be important in the treatment of several human ailments [5] and nutritional purposes [6,7]. Honey is also used in the inhibition of food borne and spoilage associated organisms [8,9]. The belief that honey is a nutrient, a drug, and an ointment has been supported in present day studies, and thus, an alternative of

medicine called apitherapy has been developed in recent years, treatments based on honey and other bee products against many diseases including bacterial infections [7]. In addition, honey is said to be hygroscopic which means that it can draw moisture from the environment thereby leading to dehydration [10,11] low pH and high sugar content (osmolarity) can also prevent microbial growth [12]. The healing properties of honey can be attributed to the fact that it offers antibacterial activity against many organisms [13,14], maintains protective barrier to prevent other infection [15]. Honey has been shown to have *in vivo* activity and is suitable for the treatment of ulcers [16].

Its immunomodulatory property is connected to wound repair [17,18]. Natural honey of different sources can vary in the potency of their antibacterial activities [10] which may be due to some intrinsic component [6].

MATERIALS AND METHODS

Materials

Honey samples

According to Swebasu Research Centre on African bee, Ile-Ife, honey was categorized into five based on their color and taste: Light amber honey, super light amber honey, dark amber honey, super dark amber honey, and bitter honey. Different samples were obtained directly from apiaries located within South Western Nigeria as shown in Table 1.

Production process of honey

Samples were harvested during the dry season of 2014 and raining season of 2015. African bee hive was used in the rearing of the bees. Harvesting is usually during the night because the bees were less active in the night. Honey samples were bottled aseptically and were transported to the laboratory for analysis.

Microorganisms

The test organisms for this research were wound associated bacteria and were obtained from Stock Collection Unit, Department of Microbiology, Obafemi Awolowo University, Ile-Ife, Nigeria. These organisms were typed cultures of National Collections of Industrial Bacteriology (NCIB) and locally isolated organisms. The bacterial isolates used were Gram-positive: *Staphylococcus aureus* (NCIB 8588), *Bacillus subtilis*, *Clostridium sporogenes* (NCIB 532), *Enterococcus faecalis* (NCIB 775), and *Listeria monocytogenes*. Gram-negative: *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Salmonella typhi*, and *Shigella flexneri*. *B. subtilis* involvement in wound infection [19]. Bacteria stock cultures were maintained at 4°C on nutrient agar slants and biochemical tests were used to confirm the identity of the locally isolated bacteria.

Table 1: Honey types and their sources

Code	Type	Source
H1	Dark amber	Ibadan
H2	Dark amber	Saki
H3	Super light amber	Ile-Ife
H4	Light amber	Ilese
H5	Light amber	Modakeke
H6	Super dark amber	Erin-Osun
H7	Dark amber	Oyo
H8	Super light amber	Ode-Omu
H9	Super dark amber	Akure
H10	Bitter	Ile-Ife
H11	Light amber	Oshogbo
H12	Dark amber	Ibadan
H13	Super dark amber	Idanre
H14	Super dark amber	Idanre

Methods

- Sensitivity testing of honey on bacterial isolates: The sensitivity testing of honey samples were determined using agar-well diffusion method as described by Irobi *et al*. [19].
- Determination of minimum inhibitory concentrations (MICs) of honey on the bacterial isolates: The MIC of honey was determined using broth tube dilution method as described by Ericsson and Sherris [20].
- Determination of minimum bactericidal concentrations (MBC) of honey on the bacterial isolates: The MBC of honey was determined according to the method of Olorundare *et al*. [21].
- Determination of death rate of bacteria isolates by honey: Assay for the death rate of bacterial isolates of honey was determined according to the method of Odenholt *et al*. [22].
- Phytochemical screening of honey: The honey samples were subjected to phytochemical screening using [23,24] methods to test for reducing sugar, tannins, proteins, flavonoids, steroids, saponins, and alkaloids.

Statistical Analysis

Statistical analysis of the results was done using *t*-test and analysis of variance.

RESULTS

Phytochemical Screening

Phytochemical screening of the honey samples showed the presence of flavonoids tannins, reducing sugar, protein, and carbohydrate this is shown in Table 1.

Sensitivity Test

Table 2 shows the diameters of the zone of inhibition (ZDI) of different honey samples against the bacterial isolates. All the isolates tested were susceptible to the activity of the honey at concentration of 50% dilution with sterile distilled water. The zones of inhibition measured ranged between 6.0 ± 0.0 mm and 30.7 ± 1.2 mm. On the other hand, organisms tested against

standard antibiotic-streptomycin at 1 mg/ml were sensitive to this compound with the exception of *P. aeruginosa* and *C. sporogenes*. The diameters of the zone of inhibition by streptomycin ranged between 10.0 ± 0.6 mm and 24.0 ± 0.6 mm.

The MICs and MBCs

The MICs of honey samples were found to be within the range of 3.13% and 50% this is shown in Table 3 and the MBCs of honey samples were found to be between 6.25% and 50% as shown in Table 4 and 5 respectively.

The Death Rate of Bacteria

The rate of killing of *B. subtilis* and *K. pneumoniae* by honey sample is shown in Tables 6 and 7, respectively.

Statistical analysis

Table 8 shows the linear model of sensitivity.

DISCUSSION

Antimicrobial activities of honey were investigated against some bacterial isolates, comprising Gram-positive and Gram-negative organisms. Honey at the concentration of 50% (v/v) dilution with sterile distilled water inhibited the growth of all isolates used in this study. The zones diameter of inhibition exhibited by honey ranged between 6.0 ± 0.6 mm and 30.7 ± 1.2.

E. coli which is one of the pathogen susceptible to the antibacterial activity of honey is known to be resistance to various convectional antibiotics [25]. A recognized strain, *E. coli* 0157:H7 produces high levels of toxins that can cause kidney damage, as well as septicemia, or blood poisoning. Symptoms can include diarrhea, chills, headaches, and high fever, and in some cases, the infection can lead to death even with medical intervention [2]. *S. typhi* the causative agent of acute infectious diseases such as typhoid and pneumonia, a respiratory infection in human was also susceptible to honey [26]. The result obtained establishes honey as a potent antimicrobial agent in the treatment of diseases caused by pathogens in human especially those that are becoming resistance to convectional antibiotics. Comparison of standard antibiotics streptomycin with honey points to the fact that honey possess antimicrobial agent. *S. aureus* and *E. coli* were resistance to streptomycin and both organisms were susceptible to honey.

Table 2: Phytochemicals in honey samples

Chemical test	Result
Alkaloids	−
Tannins	+
Saponins	−
Flavonoid	+
Protein	+
Reducing sugar	−

+: Positive, −: Negative

Table 3: The diameter zone of inhibition of the honey samples against test bacteria

Organisms	H1 (mm)	H2 (mm)	H3 (mm)	H4 (mm)	H5 (mm)	H6 (mm)	H7 (mm)	H8 (mm)	H9 (mm)	H10 (mm)	H11 (mm)	H12 (mm)	H13 (mm)	H14 (mm)	Streptomycin (mm)
P. aeruginosa	10.3±0.6	1.3±0.6	14.3±0.6	23.7±1.2	10.7±0.6	18.7±1.2	11.3±1.2	7.3±1.7	29.3±1.2	15.3±1.2	22.0±1.0	11.3±1.2	7.7±1.2	6.0±0.0	10.0±0.0
S. aureus	8.0±0.0	21.3±0.5	18.7±1.2	24.3±0.6	7.7±0.6	24.3±0.6	14.0±1.0	7.0±1.5	27.3±1.2	18.7±1.2	21.7±0.6	9.3±1.2	8.3±0.6	7.3±0.6	24.0±0.6
S. typhi	6.0±0.0	21.0±1.2	18.0±0.6	20.7±1.2	7.7±1.2	27.3±1.2	7.7±0.6	6.7±0.0	30.7±1.2	21.3±1.2	24.0±1.0	6.7±1.2	7.7±1.2	7.7±0.6	14.7±1.2
E. coli	12.7±1.2	24.3±0.6	18.7±0.6	22.7±1.2	14.0±1.7	26.3±0.6	10.7±1.2	6.7±1.2	28.7±1.2	20.0±1.0	26.7±1.2	9.7±1.5	6.7±1.2	8.7±0.6	12.7±1.2
E. faecalis	7.3±0.6	24.7±0.5	20.3±1.2	28.7±0.6	8.7±0.6	24.7±0.6	7.0±1.0	9.0±0.6	28.7±0.6	14.3±0.6	27.7±0.6	7.3±0.6	8.3±1.2	8.7±0.6	14.7±0.6
S. flexneri	7.3±0.6	22.7±0.5	26.3±0.6	20.7±0.6	8.0±0.0	25.7±1.2	7.7±1.2	8.7±0.6	22.7±0.6	13.3±1.5	19.7±0.6	7.7±1.2	6.7±1.2	8.0±0.0	16.0±1.0
L. monocytogenes	7.3±0.0	21.3±1.6	26.0±1.2	18.7±1.2	7.7±1.5	24.3±0.6	7.3±1.2	7.7±0.6	24.3±1.2	14.3±0.6	12.0±0.0	6.7±1.2	9.0±0.0	8.0±0.0	12.0±0.6
K. pneumoniae	7.0±0.0	20.7±0.6	20.3±0.6	16.7±1.2	11.7±0.6	21.7±1.5	8.0±0.0	7.0±0.0	26.0±1.0	21.3±1.2	14.7±0.6	8.7±1.2	8.3±0.6	7.3±0.6	15.3±0.6
B. subtilis	10.7±0.0	22.7±1.0	25.3±0.6	20.3±0.6	8.7±0.6	27.3±1.2	7.7±1.5	6.7±0.0	18.7±1.2	17.3±1.2	19.3±0.6	7.3±1.2	7.7±1.2	8.3±0.6	22.0±0.6
C. sporogenes	8.0±0.6	21.3±0.6	14.3±0.6	22.3±0.6	8.3±0.6	23.7±1.5	8.7±0.6	6.0±0.0	22.3±0.6	20.0±2.0	10.7±1.2	7.3±1.2	8.0±0.6	7.7±0.6	20.3±0.6

P. aeruginosa: Pseudomonas aeruginosa, S. aureus: Staphylococcus aureus, S. typhi: Salmonella typhi, E. coli: Escherichia coli, E. faecalis: Enterococcus faecalis, S. flexneri: Shigella flexneri, L. monocytogenes: Listeria monocytogenes, K. pneumoniae: Klebsiella pneumoniae, B. subtilis: Bacillus subtilis, C. sporogenes: Clostridium sporogenes

Table 4: The MIC of honey samples against the test bacteria

Organisms	H1 (%)	H2 (%)	H3 (%)	H4 (%)	H5 (%)	H6 (%)	H7 (%)	H8 (%)	H9 (%)	H10 (%)	H11 (%)	H12 (%)	H13 (%)	H14 (%)
P. aeruginosa (LIO)	25.0	12.5	12.5	3.13	12.5	3.13	50	50	6.25	3.13	12.5	50	50	50
S. aureus (NCIB 8588)	25.0	6.25	12.5	12.5	25	6.25	50	50	3.13	6.25	6.25	50	50	50
S. typhi (LIO)	25.0	6.25	6.25	3.13	12.5	6.25	50	50	12.5	3.13	12.5	50	50	50
E. coli (LIO)	12.5	12.5	6.25	6.25	25	3.13	50	50	6.25	6.25	12.5	50	50	50
E. faecalis (NCIB 775)	25.0	12.5	12.5	3.13	25	3.13	50	50	3.13	3.13	3.13	50	50	50
S. flexneri (LIO)	12.5	12.5	6.25	6.25	12.5	3.13	50	50	3.13	6.25	6.25	50	50	50
L. monocytogenes (NCIB)	25.0	6.25	12.5	3.13	25	3.13	50	50	3.13	3.13	12.5	50	50	50
K. pneumoniae (LIO)	25.0	12.5	12.5	6.25	12.5	50.0	50	50	6.25	6.25	12.5	50	50	50
B. subtilis (LIO)	12.5	12.5	6.25	6.25	25	12.5	50	50	6.25	12.5	12.5	50	50	50
C. sporogenes (NCIB)	25.0	6.25	12.5	3.13	12.5	9.43	50	50	12.5	3.13	3.13	50	50	25

P. aeruginosa: Pseudomonas aeruginosa, E. coli: Escherichia coli, E. faecalis: Enterococcus faecalis, S. flexneri: Shigella flexneri, L. monocytogenes: Listeria monocytogenes, K. pneumoniae: Klebsiella pneumoniae, C. sporogenes: Clostridium sporogenes, MIC: Minimum inhibitory concentration

Table 5: The MBC of honey samples against the test bacteria

Organisms	H1 (%)	H2 (%)	H3 (%)	H4 (%)	H5 (%)	H6 (%)	H7 (%)	H8 (%)	H9 (%)	H10 (%)	H11 (%)	H12 (%)	H13 (%)	H14 (%)
P. aeruginosa (LIO)	25.0	12.5	12.5	3.13	12.5	3.13	50	50	6.25	3.13	12.5	50	50	50
S. aureus (NCIB 8588)	25.0	6.25	12.5	12.5	25	6.25	50	50	3.13	6.25	6.25	50	50	50
S. typhi (LIO)	25.0	6.25	6.25	3.13	12.5	6.25	50	50	12.5	3.13	12.5	50	50	50
E. coli (LIO)	12.5	12.5	6.25	6.25	25	3.13	50	50	6.25	6.25	12.5	50	50	50
E. faecalis (NCIB 775)	25.0	12.5	12.5	3.13	25	3.13	50	50	3.13	3.13	3.13	50	50	50
S. flexneri (LIO)	12.5	12.5	6.25	6.25	12.5	3.13	50	50	3.13	6.25	6.25	50	50	50
L. monocytogenes (NCIB)	25.0	6.25	12.5	3.13	25	3.13	50	50	3.13	3.13	12.5	50	50	50
K. pneumoniae (LIO)	25.0	12.5	12.5	6.25	12.5	50.0	50	50	6.25	6.25	12.5	50	50	50
B. subtilis (LIO)	12.5	12.5	6.25	6.25	25	12.5	50	50	6.25	12.5	12.5	50	50	50
C. sporogenes (NCIB)	25.0	6.25	12.5	3.13	12.5	9.43	50	50	12.5	3.13	3.13	50	50	25

P. aeruginosa: Pseudomonas aeruginosa, S. aureus: Staphylococcus aureus, S. typhi: Salmonella typhi, E. coli: Escherichia coli, E. faecalis: Enterococcus faecalis, S. flexneri: Shigella flexneri, L. monocytogenes: Listeria monocytogenes, K. pneumoniae: Klebsiella pneumoniae, B. subtilis: Bacillus subtilis, C. sporogenes: Clostridium sporogenes, MBC: Minimum bactericidal concentration

Table 6: Colony count for the extent and rate of killing of *B. subtilis* exhibited by honey (H9)

Time (min)	Mean colony count (cfu/ml) for control	SD	Mean colony count (cfu/ml) for control at 1×MIC	SD	Mean colony count (cfu/ml) for control at 2×MIC	SD	Mean colony count (cfu/ml) for control at 3×MIC	SD	Log_{10} mean survival of bacterial cells at 1×MIC	Log_{10} mean survival of bacterial cells at 2×MIC	Log_{10} mean survival of bacterial cells at 3×MIC	Percentage of the bacteria killed at 1×MIC	Percentage of the bacteria killed at 2×MIC	Percentage of the bacteria killed at 3×MIC
00	188	1.0	188	1.0	188	1.0	188	1.0	2.27	2.27	2.27	00.00	00.00	00.00
15	180	1.0	140	0.6	131	1.0	65.0	0.6	2.15	2.12	1.81	25.50	30.30	65.40
30	180	0.8	105	0.6	75.0	0.6	20.0	0.6	2.02	1.88	1.30	44.10	60.10	89.30
60	175	1.0	60.0	0.0	65.0	1.5	8.00	1.0	1.78	1.81	0.90	68.00	65.40	95.70
90	170	0.5	40.0	1.0	25.0	0.5	0.00	0.0	1.60	1.40	0.00	78.80	86.70	100.0
120	170	0.6	10.0	1.0	8.00	0.5	0.00	0.0	1.00	0.90	0.00	94.60	95.70	100.0

cfu/ml: colony forming unit/ml, SD: Standard deviation, MIC: Minimum inhibitory concentration, *B. subtilis: Bacillus subtilis*

Table 7: Colony count for the extent and rate of killing of *K. pneumoniae* exhibited by honey (H9)

Time (min)	Mean colony count (cfu/ml) for control	SD	Mean colony count (cfu/ml) for control at 1×MIC	SD	Mean colony count (cfu/ml) for control at 2×MIC	SD	Mean colony count (cfu/ml) for control at 3×MIC	SD	Log_{10} mean survival of bacterial cells at 1×MIC	Log_{10} mean survival of bacterial cells at 2×MIC	Log_{10} mean survival of bacterial cells at 3×MIC	Percentage of the bacteria killed at 1×MIC	Percentage of the bacteria killed at 2×MIC	Percentage of the bacteria killed at 3×MIC
0	158	1.6	158	1.6	158	1.6	158	1.6	2.20	2.20	2.20	0.0	0.0	00.0
15	150	1.0	140	0.6	131	1.0	65	0.6	2.15	2.12	1.81	11.4	17.0	58.9
30	140	1.0	105	0.6	75	0.6	20	0.6	2.02	1.88	1.30	33.5	52.7	87.3
60	125	0.6	60	0.0	65	1.5	8	1.0	1.78	1.81	0.90	62	58.9	94.9
90	120	1.5	40	1.0	25	0.5	0	0.0	1.60	1.40	0.00	74.7	84.2	100
120	121	1.5	10	1.0	8	0.5	0	0.0	1.00	0.90	0.00	93.7	94.9	100

cfu/ml: Colony forming unit/ml, SD: Standard deviation, MIC: Minimum inhibitory concentration, *K. pneumoniae: Klebsiella pneumoniae*

Table 8: Linear model between the pH value and sensitivity

Model	Explanatory variable	Coefficients	Standard error	*t* value	Pr (>|t|)	Adjusted R^2
Sensitivity	Intercept	50.229	4.712	10.66	1.79e-07***	
	pH	−8.629	1.133	−7.614	6.22e-06***	0.8142

Significant codes: 0 ***0.001, **0.01, *0.05, '.' 0.1, ' ' 1

CONCLUSION

In this study, honey possesses antimicrobial properties which compared favorably with the standard antibiotic-streptomycin. Honey has been shown to have a potent broad spectrum antibacterial activity. However, further studies are recommended to assess its practicality in terms of use in the clinical setting especially the super dark amber and bitter honey.

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