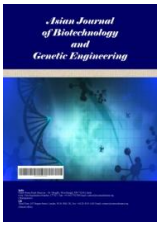

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Antibacterial Effect of Honey and Lime Extract on Selected Pathogens

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Authors' contributions

This work was carried out in collaboration among all authors. Author NRC designed the study, managed the literature searches and wrote the first draft of the manuscript. Author OOO performed the statistical analysis, supervised the entire research process and wrote the protocol, while author NKE was responsible for the molecular analysis, DNA blast and preliminary review of the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

This study was carried out to determine the in vitro antibacterial activities of honey and lime extract on pathogens isolated from stool samples using ciprofloxacin as control. Identification of the isolates was carried out using molecular characterisation, the sequence analysis of the 16S rRNA region of the isolates using GenBank Basic Local Alignment Search Tool showed that the isolates are *Bacillus cereus* (ACCESSION MK011879.1)*, Lysinibacillus xylanilyticus* (ACCESSION MK011878.1) and *Bacillus anthracis* (ACCESSION MK011880.1). The antibacterial susceptibility test was determined using the agar well diffusion method while the Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) were determined using the broth dilution and drop plate methods, respectively. The maximum mean zone diameter of inhibition was observed using lime extracts on *B. anthracis* with a mean zone diameter of inhibition (16 mm), followed by *B. cereus* (10 mm) and *L. xylanilyticus* (6 mm). Honey had antibacterial effect on *B. cereus* only with a mean zone diameter of inhibition of 4 mm. Lime extract had higher inhibitory and bactericidal effects than honey on the isolates with an MIC and MBC values of 20% v/v and 50% v/v, respectively which was observed on

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B. anthracis. Honey showed inhibitory and bactericidal effects on *B. cereus* only with an MIC and MBC values of 70%v/v and 99%v/v respectively. The findings showed that there was significant variation ($P < 0.05$) in the combination and single use of lime extract and honey. This suggests that crude extracts of lime and Honey can be used as alternative antibacterial therapy for the treatment of infections caused by the isolates.

Keywords: Antibacterial; bactericidal; honey; inhibition; lime.

1. INTRODUCTION

There is a general belief in the healing ability of honey and its consequent use in treating skin infections, burn wounds, cough, cold sores, blisters, gastric disorders [1,2]. There is also a common practice of using freshly squeezed lime extract as therapeutic agent in treating abdominal disorders, cholera, eye and skin infections, ulcerations, typhoid infection and malaria [3]. Even though these substances have been used for ages by the traditional medical practitioners, the active ingredients within them, the principle behind their antimicrobial activities as well as the concentration to be administered is not properly understood, leading to the administration of either under dose or overdose. Thus, there is need to conduct a research to really determine the antimicrobial effects of honey and lime extract on pathogen such as *Lysinibacillus xylanilyticus*, *Bacillus anthracis* and *B. cereus* isolated from stool sample in order to provide an alternative antimicrobial therapy to modern antibiotics and to provide a natural solution to current antibiotic resistance.

The aim of this study was to determine the antibacterial effects of honey and lime extracts on clinical isolates, while the objectives of the study were to isolate and identify bacteria from clinical samples; to determine the spectrum of activity of honey and lime extract on the isolates; to compare the antibacterial effect of honey and lime extract on the isolate; to assess the synergistic antibacterial effect of honey when mixed with lime extract on the isolates; to determine phylogenetic relationship of the isolates; to determine the Minimum inhibitory concentration (MIC) and the Minimum bactericidal concentration (MBC) of honey and honey mixed with lime extract only, on the selected isolates.

2. MATERIALS AND METHODS

2.1 Isolation of the Test Organisms

The test organisms (*B. cereus, L. xylanilyticus* and *B. anthracis*) were isolated from stool samples of patients suffering from gastroenteritis at JEM Medical laboratory (in Ebonyi State, South-East Nigeria). The samples were cultured on Nutrient Agar in triplicates and incubated aerobically at 37°C for 24 hours [4].

2.2 Identification of Isolates

Extraction of Genomic DNA: Genomic DNA extraction from fungal and bacterial isolates was performed by using the ZR Fungal/ Bacterial DNA MiniPrep™ Kit (Zymo Research, USA) following the manufacturer instructions.

PCR Amplification: The 16S rDNA gene was amplified from genomic DNA obtained from bacterial cultures by PCR with previously described universal primer F1 (5'-AGAGTTTGATCCTGGCTCAG-3') and R5 (5'-ACGGCTACCTTGTTACGACTT-3') [5]. PCR was performed in a total volume of 50 µl containing 30-50 ng DNA, 100 mM of each primer, 0.05 U/µl Taq DNA polymerase, 4 mM MgCl2, and 0.4 mM of each dNTP. The amplification reaction was performed with a DNA Engine DYAD Peltier thermal cycler (BioRad, USA). The thermal cycling condition used was an initial denaturation at 96°C for 5 min, followed by 30 cycles of denaturation at 96°C for 45 s, annealing at 56°C for 30 s and extension at 72°C for 2 min, followed by a final extension at 72°C for 5 min and a holding period at 4°C for infinite time. The PCR amplicons were analyzed by electrophoresis in 1% (w/v) agarose gel with EtBr (Ethidium Bromide), 1 kb DNA ladders were loaded in 5 µL volumes, while 7 µL of the sample was loaded with 2 µL of loading dye. The gel was allowed to run for 2 h at 60 V. Gel results were visualized with a ChemiDoc™ MP System (Bio-Rad Laboratories, Hercules, CA, USA) to confirm the expected size of the product. The remaining PCR products were purified using NucleoSpin Gel and PCR Clean-up kit (Macherey-Nagel, Germany).

DNA Sequencing: The sequencing of the purified PCR products were done at Inqaba Biotechnical Industrial (Pty) Ltd, Pretoria, South Africa with PRISM™ Ready Reaction Dye

Terminator Cycle Sequencing Kit using the dideoxy chain termination method and electrophoresed with a model ABI PRISM® 3500XL DNA Sequencer (Applied Biosystems, Foster City, CA, USA) by following manufacturer's instructions.

2.3 Sequence Analysis

ChromasLite version 2.33 software was used for the analysis of Chromatograms, (sense and antisense) resulting from sequencing reaction for good quality sequence assurance [6]. The resulting chromatograms were edited using BioEdit Sequence Alignment Editor [7]. After this the resulting consensus 16S rDNA sequences obtained were Blast in the NCBI database with the Basic Alignment Search Tool (BLASTn) for homology in order to identify the probable organism in question [8]. These sequences were deposited in the GenBank.

2.4 Phylogenetic Analysis

The phylogenetic analyses based on the 16S rDNA gene were further used to characterize the organism in order to establish relationships among them. The partial 16S rDNA sequences obtained were utilized in the search of reference nucleotide sequences available in NCBI GenBank database using BlastN algorithm [8]. Mafft version 7.0 was employed in the multiple alignment of nucleotide sequences [9] while trees were drawn based on character based method (Maximum Likelihood) for comparing set of data against set of models of evolution using MEGA 6 [10]. Putative chimeric sequences were identified using the Chimera Buster 1.0 software. Manipulation and tree editing were carried out using TreeView [11].

2.5 Preparation of Different Concentrations of Honey (%v/v)

Increasing concentrations of the honey from 10% to 99% in ml (i.e. 1%= 1 ml of honey in 99 ml of distilled water, 10% = 10 ml of honey diluted in 90 ml of distilled water, 20%= 20 ml of honey diluted in 80 ml of distilled water….. 99% = 99 ml of honey diluted in 1 ml of distilled water) were prepared [12,13].

2.6 Preparation of Different Concentrations of Lime Extracts (% v/v)

Increasing concentrations of the lime extract from 10% to 99% as above were prepared [12,13].

2.7 Preparation of Different Concentrations of Honey Mixed with Lime Extract (%v/v)

Increasing concentrations of the honey mixed with lime extract from 10% to 99% (i.e. 10% = 5 ml of honey mixed with 5 ml of lime extract diluted in 90 ml of distilled water, 20%= 10 ml of honey mixed with 10 ml of lime extract diluted in 80 ml of distilled water….. 99% = 49.5 ml of honey mixed with 49.5 ml of lime extract diluted in 1 ml of distilled water) were prepared [12,13].

2.8 Preparation of the Positive Control

The positive control, Ciprofloxacin (JUHEL Nig.) was prepared by dissolving 250 ug of the ciprofloxacin antibiotic tablet in 2.5 ml of sterile distilled water.

2.9 Standardization of the Isolates

Bacterial inoculum for each of the test organisms was prepared by culture in Mueller-Hinton broth (beef infusion solids, 2.0 g/L; casein hydrolysate, 17.5 g/L; starch, 1.5 g/L; pH at 25°C) for 24 hours at 37°C. The inoculums obtained were adjusted to 1×10^8 CFU/mL mc Farland turbidity standard (0.05 ml of 1% Barium chloride ($BaCl_2$) with 9.95ml of1% sulphuric acid (H_2SO_4)) [14].

2.10 Antimicrobial Susceptibility Test

Muller Hinton agar was prepared according to the manufacturer's (Oxoid ltd) specification and the media was poured into four sterile disposable petri plates and allowed to gel/solidify. A sterile cotton swab was dipped into each of the standardized culture and the excess fluid was drained by the side of the test tube and the drained swab was used to streak uniformly across the surface of the agar. A sterile 6mm diameter cork borer was used to bore five holes/wells, 3 cm apart on the surface of the Muller Hinton agar. A sterile micro pipette was used to introduce 0.5 ml of the antibacterial agents (honey, lime extract, honey mixed with lime extract, the negative control (sterile distilled water) and the positive control (ciprofloxacin) into each of the well/hole. The plates were left on the laboratory bench for one hour and then wrapped in an aluminium foil and incubated (Techmel & Techmel, TT-9052) at a temperature of 37°C for 24 hours, after which the zones of inhibition (in

mm) were read and recorded. The tests were carried out in triplicates [15].

2.11 Minimum Inhibitory Concentration (MIC)

2 ml of Muller Hinton broth was added into 11 appropriately labelled test tubes. 2 ml of the different concentrations (10%-99%) of honey, lime extracts, honey mixed with lime extract were added into test tubes. 1ml of the standardised test organisms was added into each of the tubes. The tubes were shaken and then kept on top of the laboratory bench for one hour and then incubated (Techmel & Techmel, TT-9052) at a temperature of 37ºC for 24 hours and the tubes were assayed for viable growth and the MIC for each antimicrobial agent (in %) were recorded. The tests were carried out in triplicates [15].

2.12 Minimum Bactericidal Concentration (MBC)

The MIC tubes which showed no growth were sub cultured on the surface of a Muller Hinton agar and incubated at a temperature of 37ºC for 24 hours. The lowest concentration of the antimicrobial agents which inhibited or prevented the growth of the isolates was recorded as the MBC value. The tests were carried out in triplicate [15].

2.13 Statistical Analysis

The data generated were subjected to statistical analysis using a one-way ANOVA without interaction. (SPSS, version 22.0.0.0).

3. RESULTS

Identification of the isolates was done using DNA amplification and sequencing.

Neighbour Joining method of phylogenetic tree based on partial 16S rDNA gene sequence, showing the phylogenetic relationships between isolated bacteria and the most closely related strains from the GenBank. Numbers at the nodes indicate the levels of bootstrap support based on 1000 resampled data sets. Only values greater than 50% are shown. The scale bar indicates 0.5 base substitution per site.

Likelihood phylogenetic tree based on partial 16S rDNA gene sequence, showing the phylogenetic relationships between isolated bacteria and the most closely related strains from the GenBank. Numbers at the nodes indicate the levels of bootstrap support based on 1000 resampled data sets. Only values greater than 50% are shown. The scale bar indicates 0.5 nucleotide substitution per site.

UPGMA phylogenetic tree based on partial 16S rDNA gene sequence, showing the phylogenetic relationships between isolated bacteria and the most closely related strains from the GenBank. Numbers at the nodes indicate the levels of bootstrap support based on 1000 resampled data sets. Only values greater than 50% are shown. The scale bar indicates 0.5 nucleotide substitution per site.

3.1 NCBI GenBank Deposit of 3 Bacterial Isolates with their Accession Numbers

1. 550 bp DNA linear BCT. *Lysinibacillus xylanilyticus* strain ECL 16S ribosomal RNA gene, partial sequence. ACCESSION MK011878.1.
2. 355 bp DNA linear BCT. *Bacillus cereus* strain PSP 16S ribosomal RNA gene, partial sequence. ACCESSION MK011879.1.
3. 589 bp DNA linear BCT. *Bacillus anthracis* strain STP 16S ribosomal RNA gene, partial sequence. ACCESSION MK011880.1.

3.2 Results of Antibiotic Susceptibility Test

Crude extracts of lime and honey with ciprofloxacin used as a control were screened for their antibacterial effects on 3 bacteria isolated from clinical samples. The results of the tests are presented in Table 1. The pH of the lime extract was 2.1 while the pH of honey was 5.0. The crude extracts of the lime (*C. aurantifolia*) inhibited the growth of the entire test isolates (Plates 1, 2 and 3). The honey inhibited only the growth of *B. cereus* and showed no inhibition to the rest of the test isolates however, when combined with lime extract it inhibited the growth of *B. anthracis and L. xylanilyticus*. Lime extract had the highest effect and this effect was exerted more on *B. anthracis* with inhibition zone of 16 mm. The mean zone diameters of inhibition recorded using crude extracts of the lime were 10 mm, 6 mm and 16 mm for *B. cereus, L. xylanilyticus* and *B. anthracis* respectively. The maximum mean zone diameter of inhibition observed using lime extract was 16mm which

was shown by *B. anthracis*. While the minimum mean zone diameter of inhibition was 6 mm and it was shown by *L. xylanilyticus*.

The mean zone diameters of inhibition observed using honey were 4 mm, 0 mm and 0 mm for *B. cereus, L. xylanilyticus* and *B. anthracis* respectively. The minimum and maximum mean zone diameter of inhibition observed using honey was 4mm and it was shown by *B. cereus* only. This was the only observed zone of inhibition observed using honey. While the mean zone diameters of inhibition observed using lime extract in combination with honey were 9.5 mm, 6mm and 10 mm for *B. cereus, L. xylanilyticus* and *B. anthracis* respectively. The maximum mean zone diameter of inhibition observed using honey combined with lime extract was observed to be 10 mm which was shown by *B. anthracis*. While the minimum mean zone diameter of inhibition was 6mm and it was shown by *L. xylanilyticus*, The mean zone diameters of inhibition observed using ciprofloxacin (control) was 14.5 mm, 15 mm and 21 mm for *B. cereus, L. xylanilyticus* and *B. anthracis* respectively. The maximum mean zone diameter of inhibition observed using ciprofloxacin was 21 mm which was shown by *L. xylanilyticus*. While the minimum mean zone diameter of inhibition was 14.5 mm and it was shown by *B. cereus.*

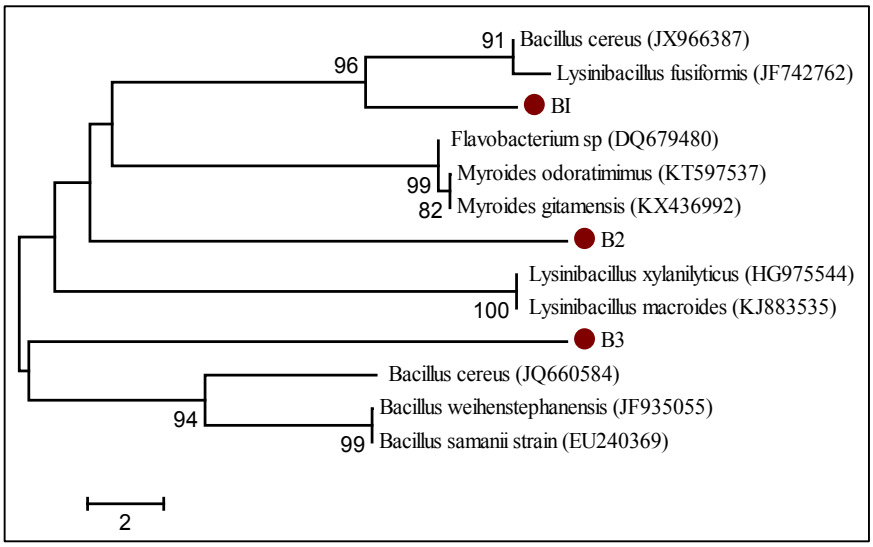

Fig. 1. Identification of the isolates using DNA amplification and sequencing

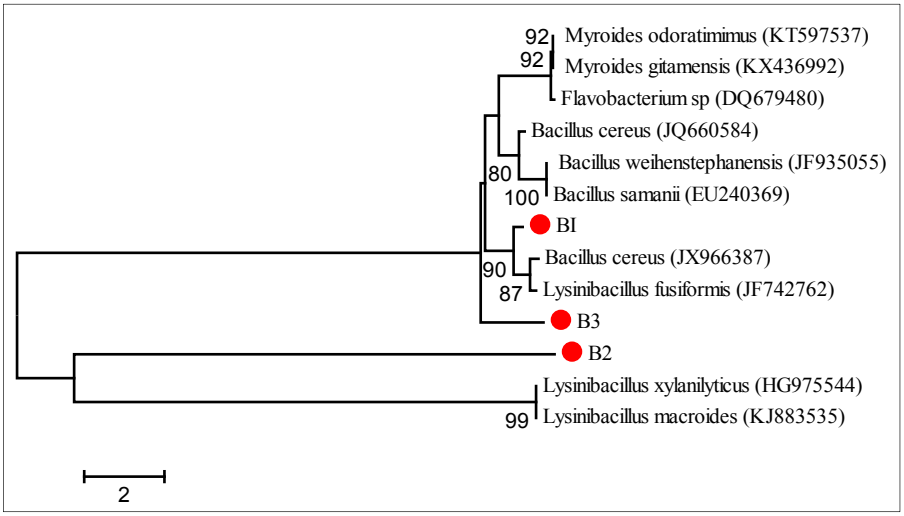

Fig. 2. Phylogenetic tree based on partial 16S rDNA gene sequence

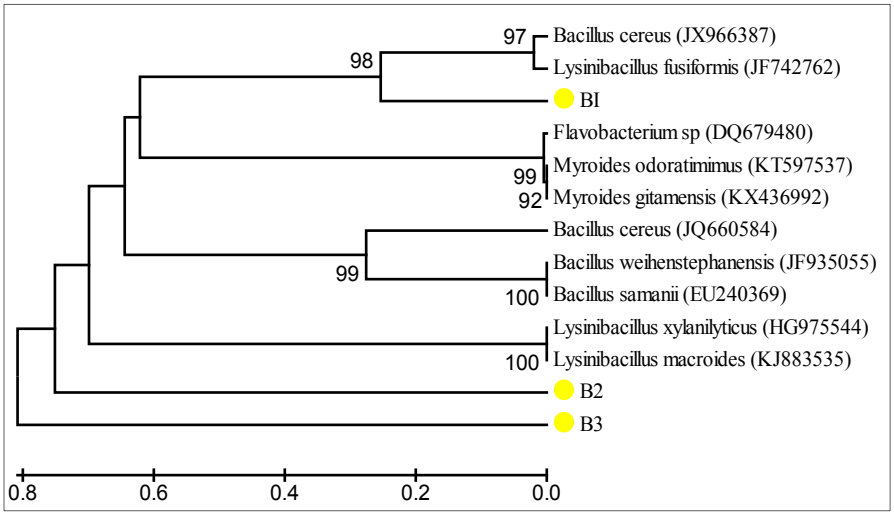

Fig. 3. UPGMA phylogenetic tree based on partial 16S rDNA gene sequence

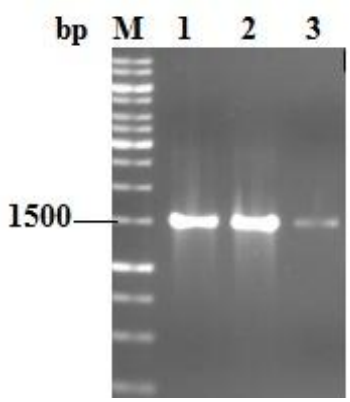

Fig. 4. Gel-results

3.3 Result of Minimum Inhibitory Concentration, MIC (%v/v)

The minimum inhibitory concentration (MIC) values of honey mixed with lime extract were 30%, 60% and 50% for *B. cereus, L. xylanilyticus* and *B. anthracis* respectively (Table 2). The minimum inhibitory concentration (MIC) value for the honey was 70% and it was observed on *B. cereus*. The growth of *L. xylanilyticus* and *B. anthracis* were not inhibited by the honey as they grew and produced visible turbidity within the MIC tubes. The lowest MIC value (20%) was observed in lime and it was shown by *B. anthracis* and *L. xylanilyticus*. The highest MIC value was 70% and it was observed on *B. cereus*. The MIC values of lime were 30%, 20% and 20% which was observed on *B. cereus, L. xylanilyticus* and *B. anthracis* respectively. However, the highest MIC value was 70% which was observed on *B. cereus* as on honey.

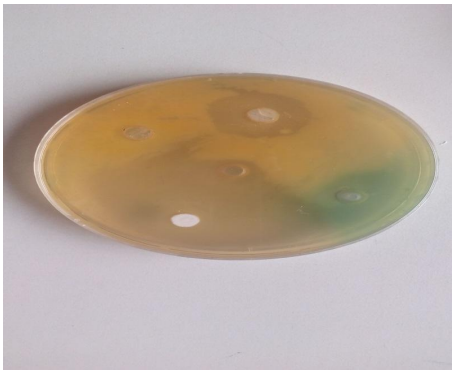

Plate 1. Culture plate of *B. anthracis* showing clear zones of inhibition

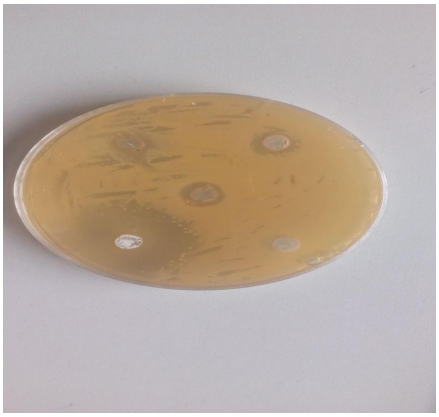

Plate 2. Culture plate of *B. cereus* showing clear zones of inhibition

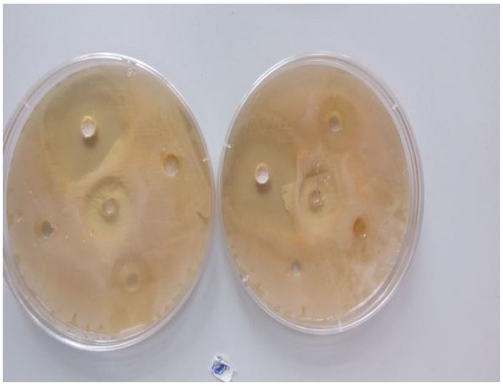

Plate 3. Culture plates of *L. xylanilyticus* showing clear zones of inhibition

3.4 Result of Minimum Bactericidal Concentration, MBC (%v/v)

The minimum bactericidal concentrations (MBC) values for lime extract were 50%, 60% and 50% which was observed in *B. cereus, L. xylanilyticus* and *B. anthracis* (Table 3). The minimum bactericidal concentration (MBC) value for honey was 90% which was shown by *B. cereus*. The honey had no bactericidal effect on *L. xylanilyticus* and *B. anthracis*. The minimum bactericidal concentration (MBC) values for honey mixed with lime extract were 60%, 80% and 80% which was shown by *B. cereus, L. xylanilyticus* and *B. anthracis* respectively. The highest MBC value was 99% which was shown by *B. cereus* on honey. The lowest minimum bactericidal concentration (MBC) value observed was 50% which was shown by *B. cereus* and *B. anthracis* on lime extract.

3.5 Statistical Interpretation of the Result of Antimicrobial Sensitivity Testing

The statistical computation carried out on the zones of inhibition of honey, lime, honey mixed with lime extract, ciprofloxacin and sterile distilled water on the test isolates (*B. cereus, L. xylanilyticus* and *B. anthracis*) showed the following. The mean±S.D of zone of inhibition of *B. cereus* was greatest using ciprofloxacin (positive control) and lowest using sterile distilled water (negative control). The mean of zone of inhibition of *B. cereus* is significantly higher in both Lime and honey mixed with lime extracts when compared with the zone of inhibition obtained using Honey ($p<0.05$, in each case) while the mean value of zone of inhibition of *B. cereus* was higher when treated with Lime compared to the zone of inhibition of extract of honey mixed with lime, but was not statistically significant ($p>0.05$). Similarly, the mean of zone of inhibition of *B. cereus* when treated with ciprofloxacin was higher compared to the zone of inhibition of *B. cereus* treated with lime, but was not statistically significant ($p>0.05$). The mean of zone of inhibition of *B. cereus* is significantly higher when treated with the ciprofloxacin (positive control) compared to the mean of zone of inhibition of *B. cereus* when treated with honey and honey mixed with lime ($p<0.05$, in each case). Moreover, the mean of zone of inhibition of *B. cereus* was significantly higher when treated with extracts of Lime, honey mixed with lime and ciprofloxacin compared to the corresponding value obtained when *B. cereus* was treated with sterile distilled water (negative control) ($p<0.05$). The mean of zone of inhibition of *B. cereus* was higher in treatment using honey compared to the mean of zone of inhibition obtained when *B. cereus* was treated with sterile distilled water (negative control) but was not statistically significant ($p>0.05$).

The mean±S.D of zone of inhibition of *L. xylanilyticus* was greatest when treated with ciprofloxacin and lowest when treated with sterile distilled water. The mean of zone of inhibition of *L. xylanilyticus* obtained was significantly higher when treated with lime and honey mixed with lime compared to the corresponding values obtained when *L. xylanilyticus* was treated with honey ($p<0.05$, in each case). The mean zone of inhibition of *L. xylanilyticus* treated with Lime was the same compared with the mean zone of inhibition of *L. xylanilyticus* when treated with honey mixed with lime and was not statistically significant ($p>0.05$). The mean zone of inhibition of *L. xylanilyticus* was significantly higher when treated with Ciprofloxacin compared to the corresponding values obtained when *L. xylanilyticus* were treated with Honey, Lime and honey mixed with lime ($p<0.05$, in each case). The mean zone of inhibition of *L. xylanilyticus* when treated with Lime, honey mixed with lime and Ciprofloxacin was significantly higher than the corresponding values obtained when *L. xylanilyticus* was treated with sterile distilled water ($p<0.05$, in each case).

The mean±S.D zone of inhibition of *B. anthracis* was greatest when treated with ciprofloxacin and lowest when treated with sterile distilled water. The mean of zone of inhibition of *B. anthracis* obtained was significantly higher when treated with Lime and honey mixed with lime compared

to the corresponding values obtained when *B. anthracis* was treated with honey (p<0.05, in each case). The mean of zone of inhibition of *B. anthracis* was significantly higher when treated with ciprofloxacin compared to the corresponding values obtained when *B. anthracis* was treated with Honey, Lime and honey mixed with lime (p<0.05, in each case). The mean of zone of inhibition of *B. anthracis* was significantly higher when treated with Lime than the mean of zone of inhibition of *B. anthracis* obtained when treated with Honey (p<0.05). The mean of zone of inhibition of *B. anthracis* was significantly higher when treated with Lime, honey mixed with lime and ciprofloxacin than the corresponding values obtained when *B. anthracis* was treated with sterile distilled water (p<0.05, in each case). The mean of zone of inhibition of *B. anthracis* when treated with Honey was the same as the mean of zone of inhibition of *B. anthracis* when treated with sterile distilled water but was not statistically significant (p>0.05).

4. DISCUSSION

In this study, honey and lime were tested singly and in combination with each other for their antibacterial effects on the selected test organisms. The honey was found to have bacteriostatic and bactericidal effects on the *B. cereus*. However, when combined with lime extract the honey exhibited bacteriostatic and bactericidal effects on the entire test isolates. This antibacterial effect could probably be because of the effects of the lime. Although microbial resistance to honey is yet to be reported and documented, [16] such report disagrees with this research work as honey inhibited only the growth of *B. cereus.* This contrast could be due to the differences in the species of honey used in these studies, or to the geographical differences and the botanical sources of the two honeys used in the investigations [17]. The test isolates (with the exception of *B. cereus)* were resistant to honey, and this could be the result of the high pH of the honey used in this study which is 5.0 compared to the conventional pH of honey which ranges from 3.2-4.5 [18]. The bacteriostatic and bactericidal effects exerted on the test bacteria by the lime may be the result of the low pH (2.1) of lime extract used for this study. Result from this study showed that the test bacteria were more sensitive to the lime extract than honey, and this difference could be as a result of the marked difference between the pH of the lime extract (2.1) and the pH of honey (5.0).

The test bacteria which showed a marked resistance to honey when used singly were susceptible to honey when it was combined with the lime extract, and this susceptibility may be as a result of a more antibacterial activity produced by the combined effects of both Lime and honey. This work is in contrast with the research work carried out by Mandal and Mandal, [19] which

Table 1. Mean zone diameter of inhibition (mm) in triplicates

Isolates	Honey	Lime	Honey +lime	PC	NC
B. cereus	4	10	9.5	14.6	0
L .xylanilyticus	0	6	6	15	0
B. anthracis	0	16	10	21	0

PC= positive control (ciprofloxacin); NC= negative control (sterile distilled water)

Table 2. Minimum inhibitory concentration, MIC (% v/v) in triplicates

Isolates	Honey	Lime	Honey + lime
B. cereus	70	30	30
L. xylanilyticus	-	20	60
B. anthracis	-	20	50

- No inhibition

Table 3. Minimum bactericidal concentration, MBC (% v/v) in triplicates

Isolates	Honey	Lime	Honey + lime
B. cereus	99	50	60
L. xylanilyticus	-	60	80
B. anthracis	-	50	80

- Not bactericidal

found the mean zone diameter of inhibition of some Gram-positive organisms including *B. cereus* to be between 6.74 mm-37.94 mm. However, this work is in tandem with an earlier research carried out by Hayam and Dalia, [20] which compared the antimicrobial activities of some Egyptian honey samples against various Gram-negative and Gram-positive bacteria including *B. cereus* and found out that at 75% dilution, the bacteria survived up to 40%. This research is in complete accord with an earlier research by Gbadago, [21] in which crude extracts of lime alongside other plant extracts were tested on *B. cereus, Shigella* and *Salmonella* whereby the MIC values of lime extracts on *B. cereus* were lower than other organisms.

There were remarkable differences between the MIC of *L. xylanilyticus* and *B. cereus* on the lime extract in comparison with lime extract combined with honey and this effect could be from the lime extract considering the fact that *B. cereus* and *L. xylanilyticus* were resistant to the honey. The growth of the entire test bacteria was inhibited by the lime extracts and this could be the reason why lime extract has been applied and used successfully in the prevention and treatment of skin and gastrointestinal pathogens [2]. It was also observed during the study that the ciprofloxacin antibiotic (positive control) possessed higher antibacterial effect in comparison to the crude extracts of lime and honey singly and combined. Honey appears not to have an appreciable level of antibacterial effects on the test organisms in comparison with the lime extracts. The MIC and MBC values of honey against *B. cereus* were 70%v/v and 99%v/v respectively. These values contradict an earlier research carried out by Kwakman *et al* [13] where the MIC and MBC values of Revamil Medical grade Honey on methicillin resistant *B. cereus* were 20%v/v and 40%v/v respectively. The positive control drug, ciprofloxacin inhibited the growth of the entire test bacteria, and this is because ciprofloxacin is a fluoroquinolone, which inhibits the synthesis of nucleic acids by inhibiting the enzymes involved in DNA replication, thereby disrupting normal essential cellular processes [22].

The antimicrobial effects of lime extract singly and in combination with other herbs such as ginger or garlic have been investigated by scientists and it has been found that lime extract possess higher antimicrobial activity and effects than the others [12]. This finding is also in agreement with our research where lime extract and honey were tested on selected isolates and it was discovered that the lime had more antimicrobial effects than the honey. There is no report or record on antibacterial effects of honey on *B. anthracis* and *L. xylanilyticus*, making this study possible the very first which assayed antibacterial activities of honey on *B. anthracis* and *L. xylanilyticus*. There was no significant difference in the mean zone diameter of inhibition observed when lime was used or when Lime combined with honey were used and when ciprofloxacin was used. This suggests that either lime, honey combined with lime or ciprofloxacin ($p<0.05$ in each case) can be used singly or in combination with each other for the treatment of infections caused by *B. cereus*. The result therefore suggest that mixtures of honey and lime extract can be used as alternative antibacterial therapy to ciprofloxacin antibiotic for the treatment and prevention of bacterial infections particularly those arising from the test isolates.

Having found lime to be antibacterial, the incorporation of lime into soaps, creams, toothpastes, lotions and other toiletries in order to prevent and protect the skin from infections is highly recommended. It seems like the source of honey affects antimicrobial activity, and therefore it is therefore important to choose a honey that has been assayed for antimicrobial effects in the laboratory before recommending such honey for use as an antimicrobial agent.

5. CONCLUSION

Having found lime to be antibacterial, the incorporation of lime into soaps, creams, toothpastes, lotions and other toiletries in order to prevent and protect the skin from infections is highly recommended. It seems honey from certain trees and plants have higher antimicrobial activity than honey from other trees but there are few evidences to support this hypothesis. It is therefore important to choose a honey that has been assayed for antimicrobial effects in the laboratory before recommending such honey for use as an antimicrobial agent. Having found lime to be antibacterial, Incorporation of lime into soaps, creams, toothpastes, lotions and other toiletries in order to prevent and protect the skin from infections is recommended. It seems honey from certain trees and plants have higher antimicrobial activity than honey from other trees but there are few evidences to support this

hypothesis. It is therefore important to choose a honey that has been assayed for antimicrobial effects in the laboratory before recommending such honey for use as an antimicrobial agent.

CONSENT AND ETHICAL APPROVAL

As per international standard or university standard guideline patients' consent and ethical approval has been collected and preserved by the authors.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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