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Comparative Antibacterial and Antifungal Activities of Manuka, Obudu and Ngaundere Honey Against Common Wound Pathogens

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Abstract **Original Research Article**

Background: The rise of antimicrobial resistance has necessitated the search for alternative agents for wound management. Honey has demonstrated broad-spectrum antimicrobial and wound healing properties. Manuka honey is the clinical gold standard, but African honeys remain largely unevaluated.

Aim: This study evaluated the antibacterial and antifungal activities of honey from Obudu, Nigeria, Ngaoundere, Cameroon, and compared them with Manuka honey against clinical isolates of common wound-infecting microbes *in vitro*.

Methods: The Bauer *et al*. Agar well diffusion and Broth dilution methods determined the zone of inhibition (ZOI), minimum inhibitory concentration (MIC), and minimum bactericidal concentration/ minimum fungicidal concentration (MBC/MFC). Thirteen wound-infecting bacterial and fungal isolates were used, including methicillin-resistant *Staphylococcus aureus* (MRSA), vancomycin-resistant enterococci (VRE), *Staphylococcus aureus, Corynebacterium ulcerans, Streptococcus pyogenes, Escherichia coli, Proteus mirabilis, Proteus vulgaris, Pseudomonas aeruginosa, Candida albicans, Candida krusei, Candida stellatoides*, and *Candida tropicalis*.

Results: Isolates were susceptible to all honey treatments at ≤ 48 mg/ml, though ZOI, MIC, and MBC/MFC values differed,and some differences were statistically significant. Obudu honey produced the highest ZOI across all organisms. Ngaundere honey had the lowest MIC for *Staphylococcus aureus, Corynebacterium ulcerans, Proteus vulgaris Pseudomonas and Candida albicans*, while Obudu honey had the lowest MIC for MRSA, *S. aureus, P. mirabilis, P. vulgaris* and *C. krusei*. Manuka honey had the lowest MIC for nine organisms, including all *Candida* species. The MBC/MFC for all honeys was ≤ 48 mg/mL, ranging from 3 mg/mL (MRSA, *C. krusie*, *C. stellatoidea*, and *C. tropicalis*) to 48 mg/mL (VRE, *S. pyogenes*, *E. coli*, and *P. aeruginosa*) for Obudu honey, while Manuka honey's MBC/MFC ranged from 4 and 24 mg/mL. Manuka honey was more effective against the fungal agents evaluated.

Conclusion: Obudu and Ngaoundere honey showed antimicrobial activity comparable to Manuka honey and is suitable for treating wound-infecting microbes and for manufacture honey-embedded wound dressings.

Keywords: Comparative antibacterial activity, Manuka honey, Obudu honey, Ngaoundere honey, wound-infecting microorganisms.

INTRODUCTION

The emergence of multidrug-resistant bacteria has necessitated the exploration of alternative treatments, including natural products like honey, which exhibit potent antibacterial properties against a range of pathogens, including methicillin-resistant Staphylococcus aureus (MRSA) and Pseudomonas aeruginosa (Mandal, M.; Mandal, S. 2011). The ability of honey to enhance wound healing while combating infection makes it a valuable adjunct in modern medical practices, particularly in managing chronic and acute wounds (Montenegro, G.; Mejías, E.). Honey's unique properties, particularly its antimicrobial and wound-healing capabilities, have been the subject of extensive research. It is a natural product with diverse types. There are different types of honeys, each characterized by its unique floral origin and distinct metabolic composition. Manuka honey, derived from the *Leptospermum scoparium* plant, has gained prominence due to its high Unique Manuka Factor (UMF), which correlates with its antibacterial potency (Gill, R.; Poojar, B.; Bairy, L.; Praveen, K.). Studies have demonstrated that Manuka honey inhibits bacterial growth and synergizes with conventional antibiotics, enhancing their effectiveness against resistant strains [,Montenegro, G.; Mejías, E., Gill, R.; Poojar, B.; Bairy, L.; Praveen, K.)..This honey's ability to combat infections has led to its widespread recognition and use in medical applications, especially in wound care and hence has been in cooperated in many antimicrobial and wound healing products. Obudu honey from Obudu, Cross River State, Nigeria, which lies within latitude 40°41'South and 60°30' North and between longitude 8° and 9°00' East of the equator. .The mean annual temperature varied from 74 to 79%. The climate of the study areas could therefore be described as sub-humid (Adekambi *et al.,* 2019). Ngaoundere honey was harvested from Ngaoundere in the Adamawa region of Cameroon Studies indicated the presence of fourteen phytochemical constituents.(Awasum *et al.,* 2015). On comparison of the mass spectra of the constituents with the NIST library 2005, the fourteen phytoconstituents were characterised and identified (Awasum *et al*., 2015). Among the fourteen (14) chemical compounds identified in Ngaoundere honey were 2, 4-Dimethyl-1-pentanol, 3,5-Dihydroxy-6-methyl-2,3-dihydro-4H-pyran-4-one, 2-Furancarboxaldehyde 5-hydroxymethyl, and 2-Butoxyethyl acetate, of which 2-Furancarboxaldehyde, 5-hydroxymethyl was the most abundant, which could be bioactive (Awasum *et al*., 2015). Ngaoundere honey has been reported to have some antibacterial activity against some bacterial organisms of interest. Reports have shown it had MICs of 6.13 and MBC of 49.02 for *S. aureus*, 12.25 and 49.02 for *P. aeruginosa*, 24.51 and 49.02 for *K. pneumoniae*, and 12.25 and 49.02 for *E. coli.* (Teke and Ngienyikeh, 2016).

MATERIALS AND METHODS

Antimicrobial studies were done in the Department of Veterinary Microbiology laboratories of Ahmadu Bello University zaria, Pure polyfloral (wildflower) honey was obtained from Obudu, Cross River State, Nigeria, which lies within latitude 40°41'South and 60°30' North and between longitude 8° and 9°00' East of the equator, Nguaoundere honey from Cameroon was purchased from local bee farmers in Ngaoundere and. Wedderspoon 100% raw Manuka honey with KFactor 12 obtained from USA was used for the study.

Sensitivity test

The diffusion method of Bauer and Kirby (1966) was used to determine the antimicrobial and antifungal activities of the test honeys against the bacterial pathogens that infect wounds listed above. 1 ml of each of the honeys was weighed, which was 1.2g, and dissolved in 10 milliliters of distilled water to obtain a concentration of 12mg/ml that was used to test the sensitivites of the honeys.

Determination of zone of inhibition.

The diffusion method of Bauer *et al.* (1966) was

used to determine this. Muller-Hinton agar was used as the growth medium and was prepared according to the manufacturer's instructions. While Sabouraud Dextrose Agar (SDA) was used for the fungi .The medium was sterilized in an autoclave at 121 ^{0}C for 15 minutes and poured then allowed to cool, 20 millilitres of the sterilized medium was poured on to sterile petri dishes and allowed to solidify and then seeded with 0.1ml of the test microbes and spread evenly over the surface of the medium by the use of a sterile swab. A standard cork borer of 6 mm in diameter was used to punch uniform wells on the surface of the inoculated medium. 0.1ml of the honey concentration of 12mg/ml was then introduced into each well of the inoculated medium and incubated at 37 °C for 24 hours, and zones of inhibition were measured to the nearest millimetre with a transparent ruler and pictures taken.

Determination of Minimum Inhibitory Concentration (MIC)

The MIC was carried out using the broth dilution method. Mueller-Hinton broth and SDA for fungi were prepared according to the manufacturer's instructions. 10 millilitres each of the medium was dispensed into test tubes and sterilised in an autoclave at 121 ^{0}C for 15 minutes and allowed to cool. McFarland's turbidity standard scale 0.5 was prepared to give a turbid solution. Ten millilitres of normal saline was dispensed into a sterile test tube and the test microbes were inoculated and incubated at 37^{0}C for six hours. Dilution of the micro-organisms was done continuously using the normal saline until the turbidity matched that of the McFarland's scale by visual comparison. At this point, the organisms were at a concentration of $1.5x10^8$ cfu/ml. One millilitre of each honey sample was then added to four millilitres of the nutrient broth to get a concentration of 20 mg/ml of the honey in the broth. Two-fold serial dilution of the honey in the broth was made to obtain concentrations of 12 mg/ml, 6 mg/ml, 3 mg/ml, 1.5 mg/ml and 0.75 mg/ml. 0.1 ml of the microorganisms was inoculated into the different dilutions, and the tubes incubated at 37°C for 24 hours. The broth was then observed for turbidity, which indicated growth. The lowest concentration of the honey which showed a clear solution (no turbidity) represents the MIC.

Determination of Minimum bactericidal concentration/ Minimum fungicidal concentration. (MBC/MFC)

This was carried out to check if the honey would kill the test organisms or if only their growth would be inhibited as described by Prescott *et al.*, 2008. Muller-Hinton agar was prepared and sterilized at 121 ^{0}C for 15 minutes, poured into sterile petri dishes and allowed to cool and solidify. The content of the MIC in the serial dilution was then subcultured onto the prepared medium and incubated at 37°C for 24 hours for bacteria and 48 hours for fungi after which the plate was observed for colony growth. The MBC/MFC are the plates with the lowest concentration of the honey without colony growth.

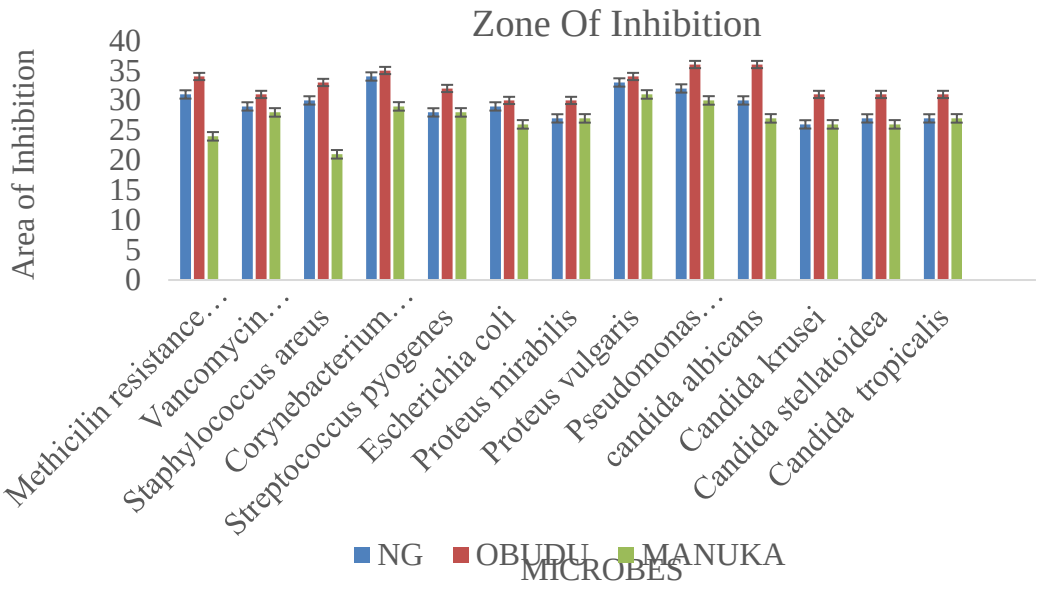
3.5 Statistical analysis. Data were expressed as mean ± standard deviation. Differences in zones of inhibition were analyzed using One-way ANOVA followed by Tukey's post-hoc test. $P<0.05$ was considered statistically significant.

RESULTS

Zone of inhibition.

MRSA: Zone of inhibition (ZOI) was 31 ± 2.3 for NG, 34 ± 2.31 for OB and 24 ± 2.31for MH. *Vancomycin-resistant enterococci*: ZOI for this organism was found to be 29 ± 2.89, 31 ± 2.89 and 28 ± 2.89 for NG, OB and MH, respectively. There was a statistically significant difference in the various ZOIs with $p \leq 0.05$, Staph. aureus: The ZOI for this organism was found to be 30 ± 1.73 for NG, while that of OB was 33 ± 1.73 and for MH it was 21 ± 1.73. Corynebacterium ulceron: Had a ZOI of 34 ± 2.31 for NG, 35 ± 2.31 for OB and 29 ± 2.31 for MH. Streptococcus pyogenes: This organism was more susceptible to OB with a ZOI of 32 ± 2.89, while both NG and MH had ZOIs of 28 each. E. coli: ZOI for NG was 29, while that of OB was 30 and MH was 26, respectively. Proteus mirabilis: This had a ZOI of 27 when it was tested using NG and 30

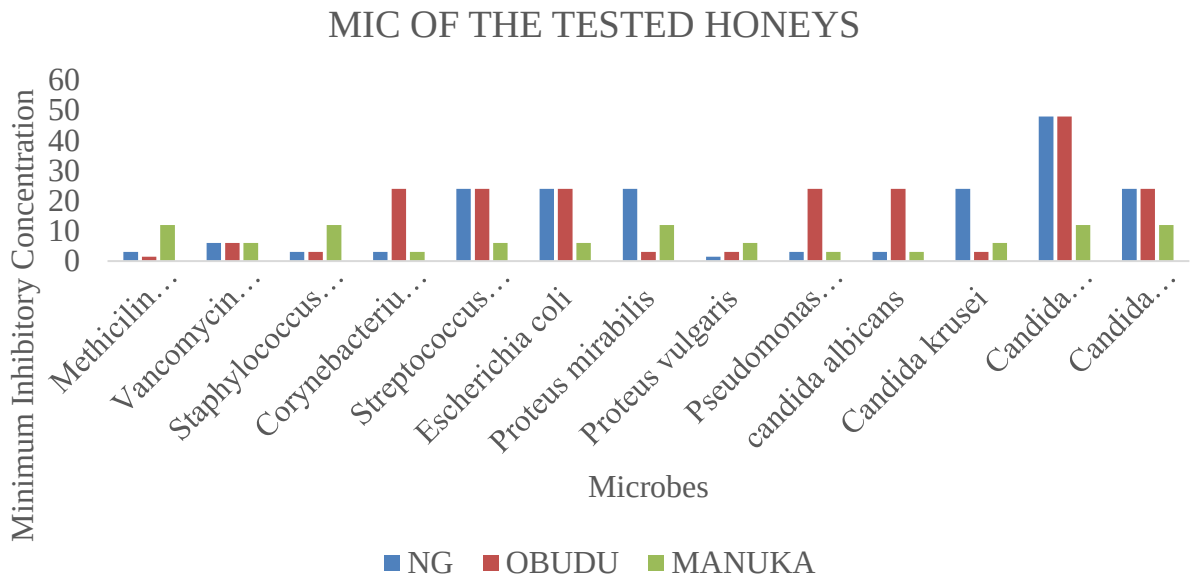
with OB and 27 ± 2.9 when it was tested using MH. Proteus vulgaris: ZOI values gotten for NG, OB and MH were 33 ± 1.73, 34 ± 1.73 and 31 ± 1.73, respectively. There was a significant difference between them. *Pseudomonas aeruginosa*: OB had the highest ZOI with a value of 36 ± 1.16, with NG and MH having 32 and 30 respectively. *Candida krusei*: the ZOI values in this case was 26, 31 and 26 for NG, OB and MH respectively. *Candida stellatoidea*: OB had the highest value for ZOI of 31 ± 1.16, followed by NG with 27 ± 1.16 and MH at 26 ± 1.16.,*Candida tropicalis:* ZOI values for NG was 27, for OB was 31 and for MH was 27 as shown in the figure below.



Results showing comparison of the Zone of Inhibition produced by the Nguaoundere (NG) Obudu (OB) and Manuka (MH)

Minimum inhibitory concentration

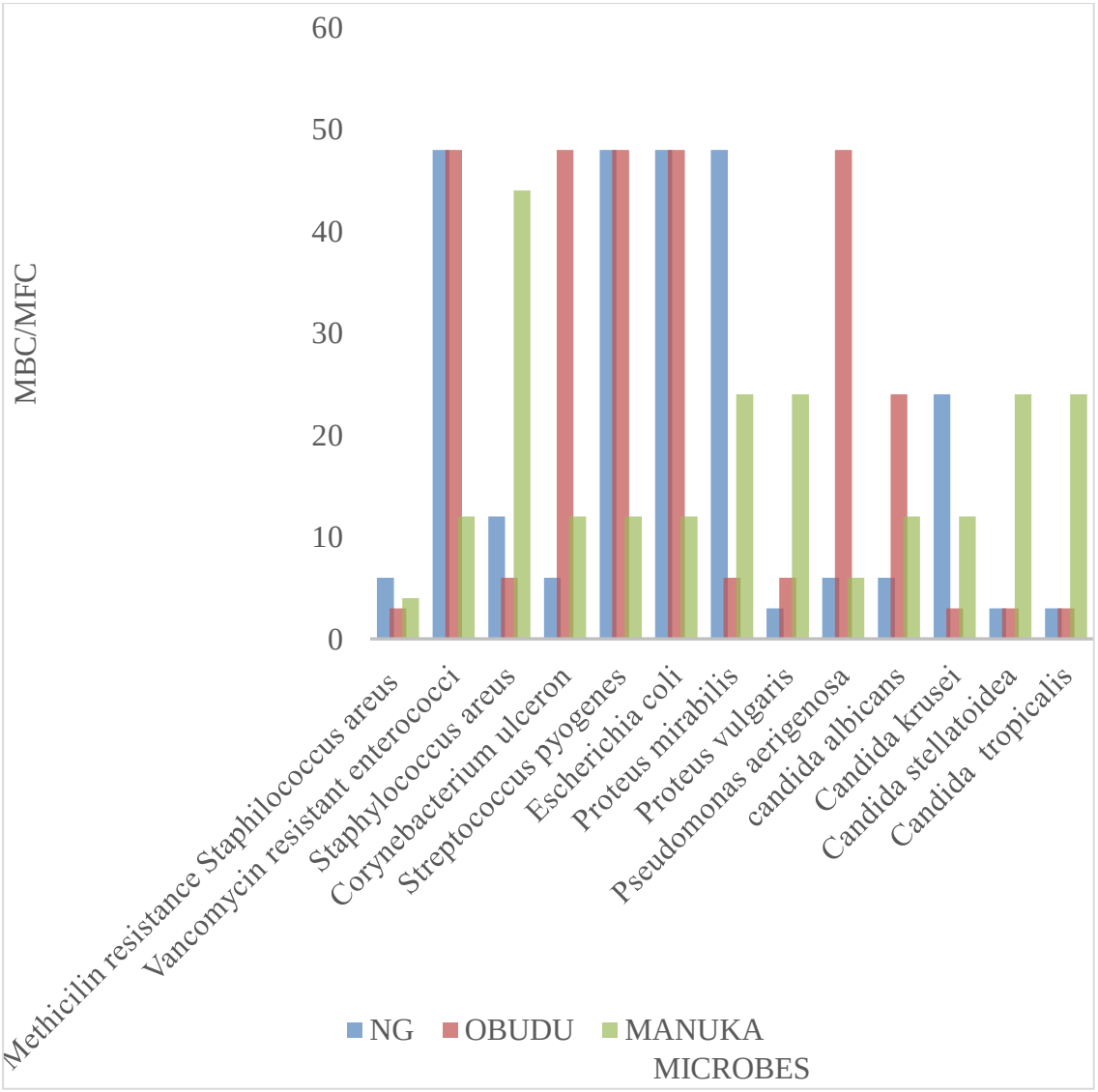
From the results it could be seen that 6 of the microbes had the best results in Ngaundere honey: *Methicillin-resistant Staphylococcus aureus, Staph aureus, Corynebacterium ulcerans, Pseudomonas* and *C. albicans* all had MICs of 3 and *Proteus vulgaris* had 1.5. Obudu had 1.5 for MRSA and 3 *for Proteus mirabilis, Proteus vulgaris* and *Candida krusei.* Manuka did best in 7 of the organisms evaluated, as shown in the figure below.



Results showing the comparison of the Minimum Inhibitory Concentration of the organisms produced by the Ngaoundere (NG), Obudu (OB) and Manuka (MH).

MINIMUM BACTERIOCIDAL/ FUNGICIDAL CONCENTRATION.

Ngaoudere had 6 of the organisms at best results among the 3 honeys. *C. ulcerans*, *P, vulgaris*, had mbc/fbc of 6 both in *P. aeruginosa* and *C. albicans* an also an mbc/fbc of 3 in *C, stellatoidea* and C. *tropicalis*. Obudu honey was also best in 6 microbes which included *MSRA* , *Staph aureus, E. coli* had mbc/mfc of 3, 6, and 6 respectively and then 3 in *C. krusie, C. stellatoidea* and *C. tropicalis*. Manuka honey did best in 4 microbes with mbc/fmc of 12 for *Vancomycin Resistant Enterococci, S. pyogenes* and *E. coli* and *Proteous vulgaris* as shown in the figure below;



DISCUSSION

The antimicrobial activity of the tested honeys revealed varying effects against specific bacterial organisms at different concentrations. All bacterial and fungal agents tested were susceptible to honey at concentrations below 48%. Obudu honey demonstrated the largest zone of inhibition among all the microbes tested. This indicates that Obudu honey can be applied to wounds to prevent infections by microbes due to its broad spectrum of activity and a strong concentration of active ingredients. The *zone of inhibition (ZOI) for Methicillin-resistant Staphylococcus aureus (MRSA)* significantly differed among Obudu, Ngaoundere, and Manuka honeys. Grego *et al.*, (2016) reported the ZOI for MRSA in Manuka honey to be 25mm, which is significantly lower than our study. The ZOI for vancomycin-resistant enterococci was significant when comparing Obudu with Ngaoundere and Manuka honeys in the various ZOIs. For *Staphylococcus aureus*, the ZOI for these organisms was higher in Obudu than in Ngaoundere and Manuka honeys. Obudu honey was significantly different to both Ngaoundere and Manuka honey. Tom *et al*. (2008) reported 25mm for Manuka honey.

Corynebacterium ulceron: the ZOI for Obudu and Ngaoundere were not significantly but were different between them and Manuka honey. Maddocks *et al*. (2013) have reported a zone of 15-20mm for Manuka honey, while Roberts *et*

al. (2015) have reported 18-22mm. Obudu honey had a statistically significant higher ZOI in *Streptococcus pyogenes, Proteus mirabilis, Proteus vulgaris,* and *Candida stellatoidea.* Grego *et al. (*2016) reported a ZOI of 13 mm. For *Pseudomonas aeruginosa*, Obudu honey had the highest ZOI, but with no significant difference. Grego *et al*. (2016) reported a ZOI of 14 for Manuka honey, which was much lower than what we got. *Candida albicans, Candida krusei*, and *Candida tropicalis* had no significant difference among the 3 honeys. This suggests that Obudu honey has a higher antimicrobial activity and greater sensitivity and might have a better therapeutic outcome. This can be attributed to its having the highest concentrations of Flavonoids, Saponins, oxalates, and its low pH value as seen in my unpublished works.

Results for Minimum inhibitory concentration (MIC) and Minimum bacteriocidal/ minimum fungicidal concentration (MBC/MFC) showed that all the organisms tested were susceptible to the honeys at different concentrations below 24 mg/ml and were both bacteriostatic and bacteriocidal, and fungicidal at various concentrations. There was no resistance to any of the organisms tested; this implies that any of the honeys can be applied to wounds in less than 48% concentration, and any of the organisms tested here shall be eliminated. Even as they were all susceptible, they showed their strength at different concentrations at different levels, except for *Vancomycin-resistant enterococci,* which had a MIC of 6 mg/ml for all the honey samples tested. Ngaoundere had the lowest concentrations of MIC in *Staphylococcus aureus and Corynebacterium ulcerans,* which was significant at P<0.05 *Proteus vulgaris, and Pseudomonas aeruginosa,* also had the smallest MIC while Molan (2012) reported as having a MIC of 10.8 mg/ml and 39mg/ml for Manuka honey and *Candida albicans*. Boatey and Daman (2005) had an MIC of 12.5 mg/ml from their research and 11.25 mg/ml as reported by Grego *et al. (*2016). Obudu honey had the lowest concentrations of MIC in *Methicillin-resistant Staphylococcus aureus,* which was significant P.>0.05 from Ngaoundere and Manuka honey. The results of Tom *et al. (*2009) reported an MIC of 8.75mg/ml, and Shabock *et al. (*2013) reported 12.5 mg/ml, both for MRSA. Obudu honey also had the smallest MIC in *Staphylococcus aureus, Proteus mirabilis,* and *Proteus vulgaris,* the three of which were also significant. Tze Tan *et al.,* (2009) reported an MIC of 11.25 mg/ml for *Candida krusie* working with Manuka honey, while Manaock *et al.* (2012) reported an MIC of 20 mg/ml using Manuka honey against this organism. Manuka honey showed a more potent action against *E. coli*, and *Streptococcus pyogenes,* which were significant and *Corynebacterium ulcerans,* which was not significant. However, MIC of 12.5 mg/ml has also been reported (Greg *et al.,* 2016). Molan (2012) Reported MIC of 6.4 mg/ml for *Corynebacterium ulcerans*.

In addition, more activity with other Manuka honey was evident with other species of Candida, including C. *krusei, C. stellatoidea, and C. tropicalis*. Obudu had equal MIC with Ngaoundere in C. *stellatoidea* and C. *tropicalis,* but the lowest in C. *krusei.* The MBC/MFC was significant from others at $P < 0.05$. Ngaoundere honey had the lowest MBC/MFC in *Corynebacterium ulcerans, Proteus vulgaris, Pseudomonas aeruginosa, Candida albicans, Candida stellatoides and Candida tropicalis. Pseudomonas aeruginosa* and *Candida tropicalis* were significant. It can be seen that the Ngaoundere honey is effective in the control of Gram-positive, Gram-negative, and fungal agents. Grego *et al.* (2016) reported MBC for *Proteus vulgaris* to be 25.5 mg/ml for Manuka honey, but it was lower in our study. This could be due to the time of harvest of the honey used or the vegetation and region it was harvested. Obudu honey had the lowest MBC/MFC in *MRSA, Staph aureus, Proteus mirabilis, Candida krusei, Candida stellatoidea,* and *Candida tropicalis*. All the Candida species were significantly different from the Ngaoundere and Manuka honeys at P< 0.05. Grego *et al*. (2016) reported MBC OF 12.5 mg/ml for *Staph aureus,* which was different from the result obtained in our studies. The pH and moisture content, of the Obudu honey which do not favour bacterial growth, high oxalate, phytate, and saponins, as well as alkaloids and flavonoids, suggest its ability to exert the effects. All these components help enhance the antibacterial properties of

honey. Manuka honey had a significant MBC/MFC in *Vancomycin-resistant enterococci, Streptococci, E. coli,* and *Pseudomonas aeruginosa.* We can see that all organisms tested were susceptible to all honey tested, and Obudu and Ngaoundere honeys competed favourably with Manuka honey. It is concluded that Obudu and Ngaoundere honeys can compete favourably with Manuka honey despite the huge difference in their prices and should be used in the control of microbial agents on wounds and in the preparation of antimicrobial and wound healing dressings.

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