British Journal of Medicine & Medical Research
8(7): 564-569, 2015, Article no.BJMMR.2015.481
ISSN: 2231-0614

SCIENCEDOMAIN *international*
www.sciencedomain.org

Antibacterial and Physico-chemical Properties of Local Honey in Guyana

Atiya Diane N'djelekulu[1], Rajini Kurup[2*] and Abdullah Adil Ansari[1]

[1]Faculty of Natural Sciences, University of Guyana, Turkeyen Campus, Guyana.
[2]Faculty of Health Sciences, University of Guyana, Turkeyen Campus, Guyana.

Authors' contributions

This work was carried out in collaboration between all authors. Authors ADND, AAA and RK designed the study, wrote the protocol and wrote the first draft of the manuscript. Authors ADND and AAA managed the literature searches, analyses of the study and performed the physico-chemical analysis of the sample. Authors RK and ADND managed the experimental process and identified antibacterial properties of the sample. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/BJMMR/2015/17063
Editor(s):
(1) Thomas I. Nathaniel, Center for Natural and Health Sciences, Marywood University, PA, USA.
Reviewers:
(1) Anonymous, University of Rome, Italy.
(2) Anonymous, Erciyes University, Turkey.
(3) Anonymous, University of Maryland College Park, USA.
Complete Peer review History: http://www.sciencedomain.org/review-history.php?iid=1119&id=12&aid=9254

Original Research Article

Received 25th February 2015
Accepted 16th April 2015
Published 14th May 2015

ABSTRACT

Aim: The present study evaluates the antimicrobial activity of different samples of honey produced in Guyana and a comparison of their effectiveness with conventional antibiotics.
Methods: A disk diffusion method was used to assess the activity of honey against microbial pathogens. The honeys were tested at different concentrations ranging from 100%, 50%, 25% and 1%.
Results: The study showed that honey H2 was most effective on *Escherichia coli* and *Klebsiella pneumoniae*, while honey H3 was most effective on *Staphylococcus aureus*. Effective concentration for H1, H2 and H3 were 1%, 25% and 25% & 50% respectively.
Conclusions: The study showed that honey has antibacterial activity (bacteriostatic and bactericidal effect), similar to antibiotics, against test organisms and provides alternative therapy against certain bacteria.

Keywords: Honey; antimicrobial agents.

**Corresponding author: Email: kurup_rajini@yahoo.com;*

1. INTRODUCTION

Antibiotic-resistant bacteria are a serious threat to public health including the recent carbapenem-resistant enterobacteriaceae, or CRE, in northeast Illinois [1]. Especially with increase in the number of bacterial resistance worldwide while very few new antibiotics are being developed. Therefore alternative antimicrobial strategies are urgently needed [2]. Western medical field is reinforcing use of honey because of its inhibitory activity against different detrimental and antibiotic-resistant microbes of infected wounds [3]. Since ancient times honey has been renowned for its wound-healing properties. With the advent of antibiotics, clinical application of honey has been neglected in modern Western medicine, although it is still used in many cultures. The overwhelming use of antibiotics has resulted in widespread resistance, therefore alternative antimicrobial strategies are necessary [4].

Honey has demonstrated potent *in vitro* activity against antibiotic-resistant bacteria and it has been successfully applied as treatment of chronic wound infections not responding to antibiotic therapy [5]. No microbial resistance against honey has been observed, making it attractive as a treatment for wound infections [6]. Honey possesses several antimicrobial properties and can act via various mechanisms of action. There are many different types of honey from around the world, made from different floral sources with variable mechanisms of action. The antimicrobial potency and medical applications of honey are tremendous as it has demonstrated inhibitory effects against a number of pathogenic bacteria [3,5,6].

There are several studies done in understanding the antibacterial properties of honey. Hydrogen peroxidein honey is considered important in the regulation of bacteriostatic and bactericidal activities of honey [7,8]. Certain honey is considered most important in the world due to its documented efficacy in the treatment of infections caused by both antibiotic susceptible and antibiotic-resistant pathogens [9,10]. The quality of honey or the secondary metabolites might vary from protected natural areas with respect to the polluted areas [11]. Good quality of honey produced in protected areas has demonstrated high quality of physicochemical parameters and could produce a good antimicrobial effect [12].

Motivated by the above studies regarding benefits of honey into natural antimicrobials, this study investigates the antibacterial potency of Guyanese honeys against some of the hospital isolated microorganisms.

2. METHODS

2.1 Honey Samples Collection

Honey samples were collected from three different locations in Guyana. H1 was purchased from the supermarket in Guyana as a readymade product from USA, where as H2 and H3 were collected from South Ruimveldt Park and Kuru-Kururu, Soesdyke Linden High way respectively. All samples collected were transferred to the laboratory and kept at 4-5°C in a dark space until analysis. Different concentrations of honey (50%, 25% and 1%) were made to check the minimum inhibitory concentration.

2.2 Chemical Analysis of Honey

The samples of honey were tested according to Association of Official Analytical Chemists (AOAC) standard [13] for pH, Moisture content, Electrical conductivity, Acidity; Reducing sugars in honey (Lane-Eynon method), Elements in honey and ash of honey were collected.

2.3 Preparing Bacterial Culture for Inoculation

Microorganisms used in this study were collected from Public hospital in Guyana. The test organisms used were *Escherichia coli, Staphylococcus aureus and Klebsiella sp.* Pure culture of the bacteria were grown on nutrient agar. Few colonies of each bacterial strain were picked using inoculation loop into sterile saline incubating for 4 hours at 37°C and adjusted to 0.5 McFarland standards. The suspension was spread evenly onto the surface of Mueller Hinton agar plates in triplicates using dry sterile cotton swabs. Three discs were removed from dilute honey and were inserted onto each inoculated agar medium. A control disc, Erythromycin, Gentamicin and Ceftriaxone were also added into each plates. The plates were incubated at 37°C for 48 hours under aerobic conditions and examined at 24 hours for zone of inhibition and again at 48 hours (Plate 1).

2.4 Statistical Analysis

All analysis were performed in triplicate and differences between the activities of the honeys as measured by the zones of inhibition were analyzed using one-way analysis of variance (ANOVA) and $P < 0.05$ was considered statistically significant.

3. RESULTS AND DISCUSSION

3.1 Physicochemical Properties of Honey

The physico-chemical properties detected in three different honey samples are given in Table 1. The honey color ranged from dark, to dark amber, to light amber respectively. There were a significant difference in the pH values of the three honey sample (Anova: P≤ 0.001), however H2 was found to be most acidic compared with other two samples. The ash content in honey have been associated with botanical and geographical origins of honey samples, is generally small and depends on nectar composition of predominant plants in their formation [14].

Magnesium was found to be in the highest concentration, followed by potassium, while copper was found to be in the lowest concentration of them all. ANOVA analysis showed a significant difference between the parameters with F calculated (4827.51)> F critical (8.75) at P≤ 0.001.

Antimicrobial assay with H1 sample showed a greater effect in inhibiting *Klebsiella pneumoniae* compared to the other bacteria at higher concentration of honey. This was followed by *Staphylococcus aureus* and *Escherichia coli* respectively. Interestingly, *E. coli* showed a greater zone of inhibition at lowest concentration of 1%. (ANOVA F calculated (1.37) < F Critical (998.50) (Table 2; Fig 1).

Similarly, H2 sample showed a greater effect in inhibiting *Klebsiella pneumoniae* compared to the other bacteria. Unlike H1, H2 however is much more effective at a lower concentration that is 25% and 1% with statistically significance (F calculated (2.67) < F-Critical (998.50) (Table 3; Fig. 2).

Based on the results, H3 showed a greater effect in inhibiting *Klebsiella pneumoniae* compared to the other bacteria. Unlike H1, H2 however is much more effective at a lower concentration. Thus, it can be said H2 works best in inhibiting *Klebsiella pneumoniae* at concentration of 25% and 1% since at both concentrations the zone measured were the same. Hence, the different dilutions of honey had significantly different effect on the size of inhibition zone (F calculated > F Critical) (Table 4; Fig. 3).

The results also confirm that honeys from different regions of Guyana have a wide variability in their antimicrobial activity. It has been shown that the antimicrobial activity of honey may range from concentrations lower than 1% to concentrations of 50% and higher [15]. It was observed that H2 was most effective on *E. coli* and *K. pneumoniae*, while honey H3 was most effective on *S. aureus*. For effective concentration for honey H1, H2 and H3 were 1%, 25% and 25% & 50% respectively. Some species have shown sensitivity at concentrations as low as 1.8% – 11% indicating great antimicrobial quality. However, honey H2 and H3 showed a greater zone of inhibition than conventional antibiotics to *K. pneumoniae*, except at concentration of 50%. Honey H3 showed a greater zone of inhibition than conventional antibiotics to *S. aureus*, except at concentration of 50%.

Table 1. Physico-chemical analysis of three different honey samples (Mean±SD)

Parameters	H1(Mean±SD)	H2 (Mean±SD)	H3 (Mean±SD)
Free acidity	0.20±0.00	0.20±0.00	0.20±0.00
pH	3.91±0.00	3.48±0.00	4.22±0.00
Brix	85.00±4.00	84.00±0.00	85.10±0.00
EC w (ms)	2.10±0.00	2.00±0.00	1.30±0.00
Water %	19.60±0.00	20.01±0.00	20.78±0.00
Sulphate Ash%	45.70±0.00	49.02±0.00	40.15±0.00
Reducing Sugar%	21.80±0.00	29.43±0.00	29.43±0.00
Sucrose %	15.49±0.00	11.98±0.00	14.15±0.00
Mn (ppm)	0.006±0.0008	0.057±0.0571	0.006±0.0009
Mg (ppm)	0.544±0.1139	0.543±0.1143	0.545±0.1127
Cu (ppm)	0.004±0.0011	0.003±0.0005	0.003±0 0001
Zn (ppm)	0.017±0.0076	0.016±0.0069	0.015±0.0095
Fe (ppm)	0.009±0.0008	0.009±0.0006	0.009±0.0005
K (ppm)	0.521±0.1202	0.526±0.1400	0.526±0.1400

NB: H1= Honey sample 1; H2= Honey sample 2 and H3= Honey sample 3, SD= Standard Deviation

Table 2. The effect of H1 concentrations on the tested microorganism

Species	Pure (100%)	50%	25%	1%
E. coli	0.00±0.00	13.00±0.00	0.00±0.00	16.00±0.00
S. aureus	11.00±0.00	12. 00±0.00	11.00±0.00	0.00±0.00
K. pneumoniae	22.00±0.00	21.00±0.00	12.00±0.00	0.00±0.00

**Impact was measured in diameter of zone of inhibition zones (mm) (Mean ± SD)*

Table 3. The effect of honey H2 concentrations on the tested microorganism

Species	Pure	50%	25%	1%
E. coli	10.00±0.00	22.00±0.00	20.00±0.00	10.00±0.00
S. aureus	0.00±0.00	12.00±0.00	10.00±0.00	0.00±0.00
K. pneumoniae	10.00±0.00	0.00±0.00	26.00±0.00	22.00±0.00

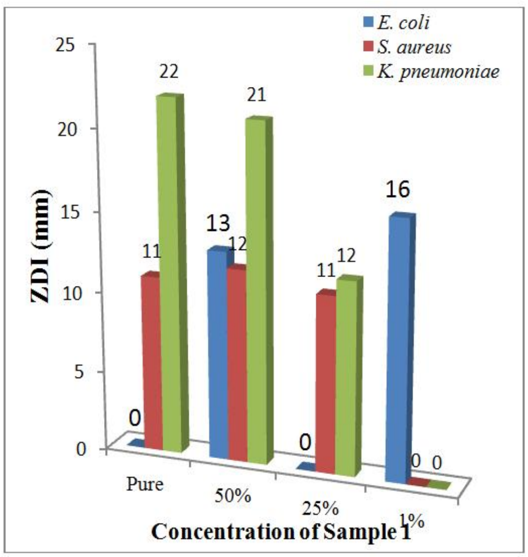

Fig. 1. Antibacterial activity of Sample 1 honey based on the ZDI produced for clinical isolates

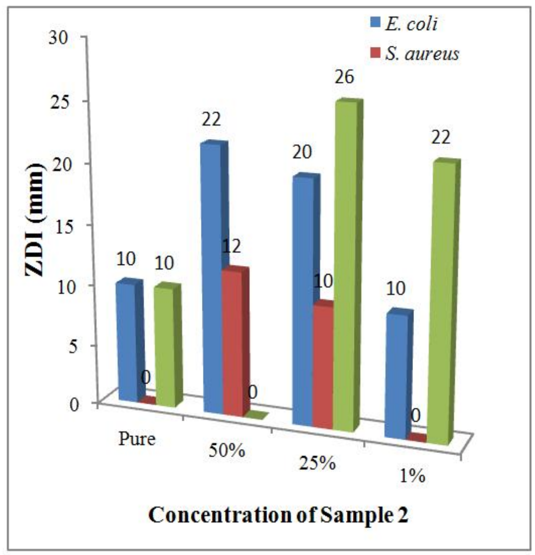

Fig. 2. Antibacterial activity of Sample 2 honey based on the ZDI produced for clinical isolates

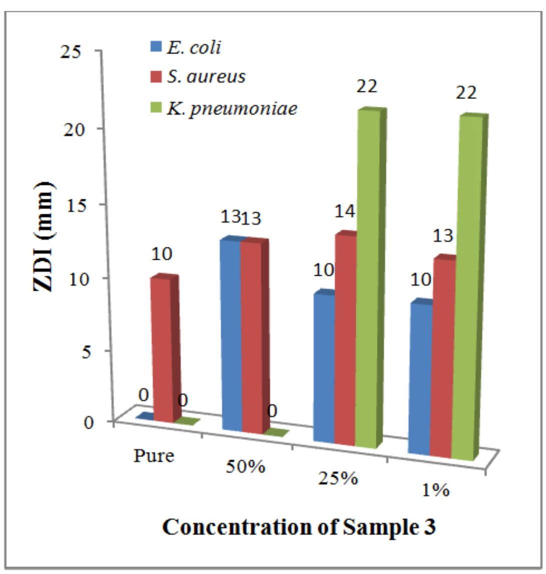

Fig. 3. Antibacterial activity of Sample 3 honey based on the ZDI produced for clinical isolates

Plate 1. Disc diffusion method used in testing antimicrobial property of honey samples

Table 4. The effect of honey H3 concentrations on the tested microorganism

Species	Pure	50%	25%	1%
E. coli	0.00±0.00	13.00±0.00	10.00±0.00	10.00±0.00
S. aureus	10.00±0.00	13.00±0.00	14.00±0.00	13.00±0.00
K. pneumoniae	0.00±0.00	0.00±0.00	22.00±0.00	22.00±0.00

4. CONCLUSION

Based on the results obtained, it is clear that different samples of honey from different regions of Guyana at the concentration range (1%- 50%) works better as an antibacterial agent than some of the conventional antibiotics. It is also evident that different species of bacteria differ in their susceptibility to honey and since pure honey is cheap and easily available and also non-toxic it can be recommended for medical purposes.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. CDC; 2014. Available:http://www.cdc.gov/HAI/organisms/cre/index.html
2. Kwakman PHS, Velde AA, de Boer L, Vandenbroucke-Grauls CMJE, Zaat SAJ. Two major medicinal honeys have different mechanisms of bactericidal activity. PLoS ONE. 2011;6(3).e17709. DOI:10.1371/journal.pone.0017709.
3. Nassar HM, Li M, Gregory RL. Effect of honey on *Streptococcus mutans* growth and biofilm formation. Appl. Environ. Microbiol. 2012;78(2):536-540.
4. Kwakman PH, TeVelde AA, de Boer L, Speijer D, Vandenbroucke-Grauls CM, Zaat SA. How honey kills bacteria. The FASEB Journal. 2010;24(7):2576-2582.
5. Cooper RA, Molan PC, Harding KG. Antibacterial activity of honey against strains of *Staphylococcus aureus* from infected wounds. J. R. Soc. Med. 1999;92(6):283-285.

6. Bizerra FC, Da Silva Jr. PI and Hayashi MAF. Exploring the antibacterial properties of honey and its potential. Front. Microbio. 2012;3:398. DOI:10.3389/fmicb.2012.00398.
7. Fidaleo M, Zuorro A, Lavecchia R. Methylglyoxal: A new weapon against staphylococcal wound infections? Chem. Lett. 2010;39:322-323.
8. Molan PC. Honey: Antimicrobial Actions and role in Disease Management, chapter 9: New Strategies Combacting Bacterial Infection, Eds. Ahmad I, Aqil F, Wiley-VCH. Weinheim; 2009.
9. Cooper RA, Jenkins L, Henriques AF, Duggan RS and Burton NF. Absence of bacterial resistance to medical-grade manuka honey. Eur. J. Clin. Microbiol. Infect. Dis. 2010;29:1237-1241.
10. Otter JA, French GL. Utility of antimicrobial susceptibility-based algorithms for the presumptive identification of genotypically-defined community –associated methicillin-resistant *Staphylococcus aureus* at London teaching hospital. Eur. J. Clin. Microbiol. Infect. Dis. 2011;30(3):459-463.
11. Canini A, Pichichero E, Alesian D, Canuti L, Leonardi D. Nutritional and botanical interest of honey collected from protected natural areas. Plant Biosys - An Int J Dealing with all Aspects of Plant Biology. 2009;143(1).
12. DiMarco G, Canuti L, Impei S, Leonardi D and Canini A. Nutraceutical properties of honey and pollen produced in a natural park. Agri Sc. 2012;3(2):187-200.
13. Horwitz W. Official methods of Analysis of AOAC International. AOAC International, USA. 2000;8(8):423-426.
14. Garcia MC, Perez AC, Harrara A. Pollen analysis and antibacterial activity of Spanish honeys. Food Sci. Tech. Int. 2001; 7:155-158.
15. Basson NJ, Grobler SR. Antimicrobial activity of two South African honeys produced from indigenous *Leucospermum cordifolium* and Erica species on selected micro-organisms. BMC Complement. Altern. Med. 2008;8:41.

Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sciencedomain.org/review-history.php?iid=1119&id=12&aid=9254