Available online at www.elixirpublishers.com (Elixir International Journal)

Bio Sciences

Elixir Bio Sci. 98 (2016) 42476-42481

ISSN: 2229-712X

In vitro antibacterial activity of honey against *Staphylococcus aureus* and *Escherichia coli*

Mguni S[1], Manda J[1], BareJ[1], Mabaya L[2], Makaure J[1] and Makaka Caston[1,*]

[1]Department of Applied Biosciences and Biotechnology, Faculty of Science and Technology, Midlands State University, P. Bag 9055, Gweru, Zimbabwe.

[2]Department of Biochemistry, Faculty of Medicine, Midlands State University, P. Bag 9055, Gweru, Zimbabwe.

ARTICLE INFO

Article history:
Received: 22 June 2016;
Received in revised form:
20 August 2016;
Accepted: 03 September 2016;

Keywords
Disk diffusion method,
Zone of inhibition,
Staphylococcus aureus,
Escherichia coli.

ABSTRACT

The emergence of antibiotic resistant strains of bacteria has made natural products attract more attention in the medical field. Honey is one such natural product and its medicinal importance has been recorded since ancient times. The *in-vitro* antibacterial effectiveness of different types of honey (raw and processed) was tested against two species of bacteria; *Staphylococcus aureus* (Gram-positive) and *Escherichia coli* (Gram-negative), using the disk diffusion method. Disks impregnated with different concentrations of processed and unprocessed honey were applied to Mueller Hinton agar plates inoculated with the two bacterial species, and the diameters of the zones of inhibitions measured after 24 hours of incubation. Both types of honey showed antibacterial activity against the tested organisms, with the zones of inhibition (ZOI) ranging from 7 mm to 25 mm. *S. aureus* was more susceptible (maximum zone of inhibition of 25mm) while *E. coli* was less susceptible *(*maximum inhibition zone of 17mm). Both raw and processed honey significantly ($p < 0.05$) inhibited growth of both species of bacteria at a minimum concentration of 40%. The results of this study point to the potential use of honey as an antibacterial agent and therefore a possible alternative therapy against ailments caused by these two bacterial species.

Introduction

The high incidences of antimicrobial resistance has resulted in increased morbidity, mortality and increased health care costs from treatment failures. Although it is largely agreed that defining the precise public health risk and estimating the increase in costs can be too complex an activity, there is strong certainty that emergence of antibiotic resistance is at the verge of causing a severe global problem (WHO, 2000). The alarming frequency with which bacterial resistance is increasing has not been resolved because of the decrease in the production of new antibiotics (WHO, 2000). The general reduction in development of new antibiotics has been attributed to apparent neglect of the field of antibiotic innovation by several pharmaceuticals (WHO, 2003). This has necessitated the urgent need to develop alternative antimicrobial agents.

Alternatives that can be opted for are traditional medicines. The use of traditional medicines in the treatment of infections has been practiced ever since the origins of mankind. Traditional medicines have sustained a number of generations and innumerable civilizations around the world. Traditional medicines may include plants and plant products. Honey is amongst the oldest of plant products used traditionally for the treatment of several human diseases. In most cultures it was used for both nutritional and medical purposes. The beneficial role of honey is attributed to its antibacterial properties (Molan, 1992). It owes this to a number of properties: its high sugar content confers on it high osmotic effects; high acidity due to its low pH ranging from 3.2 to 4.5; and presence of antibacterial factors such as hydrogen peroxide, anti-oxidants, lysozyme, polyphenols, phenolic acids, flavonoids and bee peptides (Kunkel, 2002). Other components in honey include phytochemicals, lymphocytes and phagocytes (Allen *et al*., 1991).

Staphylococcus aureus are gram positive coccal bacteria that are frequently found in the respiratory tract and on the skin of humans (Ogston, 2003). They are commonly known to cause food poisoning, skin infections such as abscesses and respiratory infections such as sinusitis. Pathogenic strains often promote infections by producing potent protein toxin and expressing cell surface proteins that bind and inactivate antibodies (Ogston, 2003).

Staphylococcus spp are very adaptable and many varieties have become resistant to several antibiotics (Cimolai, 2008). Most strains of *S. aureus* have become resistant to penicillin. Methicillin resistant strains of *S. aureus* are also now common in hospitals and are emerging in many communities (Cimolai, 2008).

Escherichia coli is the most prevalent infecting organism which is found in the intestinal tracts of humans and animals (Barry *et al.*, 2000). Most strains of E. coli are harmless although others can cause bloody diarrhea. Other strains such as a stain called O157:H7 may cause severe anemia or kidney failure which, can lead to death while others cause urinary tract infections (Barry *et al*., 2000).

Prevalent antibiotic usage exerts a selective pressure that promotes the development of antibiotic resistance and so the relationship between increased rates of antimicrobial use and

Tele:
E-mail address: cmakaka@yahoo.com

resistance has been recognized in many bacterial and hospital acquired infections due to *S. aureus* as well as community acquired infections due to *E. coli* (Lalitha, 2004). As resistance develops to the first line drugs, therapy with new, broader spectrum and more expensive antibiotics may be resorted to, but multidrug resistance may subsequently develop. Most *S. aureus* strains have become resistant to several antibiotics which include kanamycin, oxacillin, cloxacillin, gentamicin and streptomycin (Carter *et al*., 2000). Predominant resistance factors may spread rapidly within human and animal populations giving the multidrug-resistant pathogens a way to spread not only locally but also globally, with the most topical pathogens spreading hastily in susceptible hosts.

Some patients tend to develop allergies or side effects when using antibiotics (Bartlett, 2002). Antibiotic side effects and allergies can occur and may interfere with the patient's ability to tolerate specific drugs. A number of frequent side effects and allergies associated with penicillin such as amoxillin, ampicillin and oxacillin may include rash, nausea or vomiting, drug fever and hypersensitivity (Bartlett, 2002). There are also several side effects that are common to most antibiotics, regardless of class or drug and these may include antibiotic-associated diarrhea, yeast infections, serious allergic skin reactions, and complications from intravenous use of antibiotics (Bartlett, 2002).

Besides side effects, access to antibiotics is a major concern in many countries worldwide. Reasons may include accessibility in countries where some populations are isolated or where private pharmacies are distant (Vlieghe, 2012), economic hardships in a large number of nations, and high costs of the most recent antibiotics (Hughes, 2011). Normally, countries with only a few antibiotics available and high mortalities from infections are not protected from antibiotic resistance (Hughes, 2011). Restricting the accessibility of potentially life-saving antibiotics particularly in rural areas where a majority live under the poverty datum line may hinder the progress towards a healthier population.

The use of traditional medicine can be an answer to the possible challenges that are potentially caused by the use of standard antimicrobial agents. In this study honey was selected in a trial to test for bacterial sensitivity on some common bacteria (*E. coli* and *S. aureus*) in which its *in vitro* susceptibility test results can be included in considering honey as an appropriate antimicrobial agent in clinical practices. It is in light of the fact that microbial resistance to antimicrobial agencies poses a very serious threat to public health. The frequencies of antibiotic resistance is increasing, therefore there is an urgent need for other alternative antimicrobial strategies. This situation has led to the re-evaluation of the therapeutic use of traditional and natural remedies in general, hence our move to evaluate honey.

The use of natural remedies is an important practice in treating several infectious diseases. In this study honey was selected as the natural remedy of choice as an antimicrobial agent because of its reported antimicrobial properties and its history as one of the natural remedies which have been used traditionally in its raw state as medicine to treat human ailments. Honey can be used conveniently and efficiently because it does not spoil or rot as no bacteria can survive in it because of its antibacterial properties (Cooper, 2002).

In medicinal practices, honey can be a possible antimicrobial alternative that most common patients can resort to because it can be found in the natural environment. It is therefore less costly unlike many pharmaceuticals, and allergic reactions to it are rare (Kiistala, 1995). In addition it can be a convenient therapy especially to the rural population which does not have access to antibiotics since honey occurs naturally in the environment. As Kwakman *et al.* (2010) assert, the fact that no reports of microbial resistance to honey have been made will make it a drug of choice.

The scope of this research is therefore to evaluate and bring to attention the possible validity of honey in the treatment of bacterial infections so that the increased antimicrobial resistance may be eradicated. The assumption underlining this study is that honey contains antimicrobial properties that are effective and efficient enough to inhibit bacterial growth and therefore kill bacteria. The validity of this research lies upon considering the use and building more trust on honey as an antimicrobial agent in treating infectious diseases in standard medicinal practices. This study thus aimed at determining the efficacy of honey on inhibiting growth of the bacteria *S. aureus* and *E. coli*. It also sought to compare the growth inhibitory effect of raw and processed honey on the two species as well as the effectiveness of different concentrations.

Materials and methods

The study was done at Gweru General Hospital Laboratory in Zimbabwe, using aseptic techniques to maximize sterile conditions. The disk diffusion method was used to test for the susceptibility of *S. aureus* and *E. coli* to honey.

Preparation of the honey concentrations

Liquid forms of raw and processed honey were obtained from local markets in Gweru, Zimbabwe. Raw honey was purchased from Kudzanayi market and processed honey was purchased from OK supermarket. Both types of honey were prepared into concentrations of 20%, 40%, 60%, 80% and 100% by dissolving a volume of honey in 10ml distilled water, determined by the formula below;

$$V = \frac{Percent\ concentration\ (\%) \times 10ml}{100}$$

Where, V = volume of honey, and (%) = desired percentage concentration.

Impregnation of discs

Discs of diameters of approximately 6mm were prepared using Whitman filter paper. The discs were placed in a Petri dish and sterilized by autoclaving. The discs were impregnated with different concentrations of honey by dipping into the appropriate concentrations of raw and processed honey. The discs were then allowed to drip off all the excess honey in the respective sterile Petri dishes.

Acquisition and confirmation of bacterial samples

Samples of *S. aureus* and *E. coli* were obtained and verified from Gweru General Hospital Laboratory, using biochemical and morphological tests. At least five well isolated colonies of both *S. aureus and E. coli* were picked and identified microscopically and biochemically according to the methods used by Holt *et al* (1993). For biochemical tests; gram, catalase, indole, motility, and citrate tests were done to identify the two species of bacteria (cf Holt *et al*., 1993).

Culturing of bacteria and preparation of agar plates

Each strain was then inoculated in Mueller Hinton nutrient broth media and incubated at 35^0C for 24 hours to achieve the standard turbidity {cf to the 0.5 McFarland standards as done by Bassiri (2004)}.

Ten plates of Mueller Hinton media were prepared for inoculation with each strain.

Inoculation of test plates

The plates were inoculated with the bacterial strains cultured from the nutrient broth using the spread plate method. Dried surfaces of ten agar plates were inoculated with *S. aureus* by spreading a sterile cotton swab containing *S. aureus* over the entire sterile agar surface of each plate. This procedure was repeated two to three times for each plate, rotating the plate at approximately 60^0each time to ensure even distribution of inoculum as described by Lalitha (2004). After inoculation, the plates were left open for 5 minutes to allow for any moisture evaporate before applying the honey impregnated disks. The same procedure of inoculation was repeated on inoculating the other ten agar plates with *E. coli* culture.

Deposition of impregnated discs, incubation and measurement of zones of inhibition

Sterile forceps were used to pick the disks from the Petri dish to the surface of the inoculated agar plates. Each disk was pressed down to ensure complete contact with the agar surface. The 20%, 40%, 60%, 80% and 100% concentrations of raw and processed honey impregnated disks were placed into the different plates inoculated with *S. aureus* and *E. coli*, each concentration was placed in its own respective plate. For each strain, 5 plates were treated with different concentrations of raw honey and the other 5 plates were treated with different concentrations of processed honey. Each treatment was replicated five times. The disc replicates were evenly distributed so that they were not in a close proximity with each other. After disk application, all the plates were inverted and incubated at 37^0C for 24 hours.

After 24 hours of incubation, each plate was examined above a black background and illuminated with reflected light (Lalitha, 2004) and the diameters of the zones of inhibition (as judged by a naked eye) were measured and recorded. Faint growth of tiny colonies which could be detected only with a magnifying lens was not considered (Lalitha, 2004). Zones of inhibition were measured to the nearest whole millimetre using a ruler which was held on the back of the inverted plate.

Results

Zones of inhibition

The inhibition zone diameters were measured and recorded for both *S. aureus* and *E. coli* samples. The results indicated the inhibition zone diameters as per treatment with different concentrations of raw and processed honey on both *S. aureus* and *E. coli.* The results of the zones of inhibition by both raw and processed honey on *S. aureus* are shown in Table 1.

Table 1. Inhibition zone diameters for *S. aureus.*

Honey concentrations(%)	Diameter zone of inhibition(mm)					Diameter zone of inhibition(mm)				
	Raw honey					Processed honey				
	R1	R2	R3	R4	R5	R1	R2	R3	R4	R5
20	9	10	8	10	10	6	6	8	7	7
40	12	14	13	14	15	8	10	10	11	11
60	17	16	19	19	18	12	11	12	12	12
80	21	20	20	22	22	13	14	13	14	14
100	23	24	25	25	25	15	15	15	16	14

Key: R - Replicate

As shown in Table 1, both raw and processed honey had some growth inhibitory effect on *S. aureus*. However raw honey showed higher potency as compared to processed honey as indicated by larger inhibition zone diameters ($p < 0.05$). Different honey concentrations also showed different growth inhibitory effects ($p < 0.05$). For both raw and processed honey, the inhibitory effect increased as the concentration increased. There was less inhibition at 20% for raw honey and almost no inhibition for processed honey but the inhibition increased remarkably as the concentration increased for both treatments. Raw and processed honey showed the highest inhibitory effect on *S. aureus* at 100% concentration, although there were remarkable inhibitory effects at 60% and 80%.

Table 2 shows the zones of inhibition for *Escherichia coli* after treatment with different concentrations of both raw and processed honey.

Table 2: Inhibition zone diameters for *Escherichia coli*

Honey concentrations(%)	Diameter zone of inhibition(mm)					Diameter zone of inhibition(mm)				
	Raw honey					Processed honey				
	R1	R2	R3	R4	R5	R1	R2	R3	R4	R5
20	7	8	8	7	8	6	6	6	6	7
40	10	9	10	9	9	7	6	7	7	6
60	11	11	12	12	12	8	8	7	8	8
80	14	14	13	14	15	9	10	10	9	9
100	16	17	16	17	16	11	12	11	11	12

Key: R- Replicate

As shown by the results in Table 2, raw and processed honey had some growth inhibitory effect on *E. coli*. As with *S. aureus*, raw honey showed higher efficacy against *E. coli* than processed honey as indicated by the larger zones of inhibition as compared to the treatments with processed honey ($p < 0.05$). Different honey concentrations also showed significant growth inhibitory effects. For both raw and processed honey, the inhibitory effect increased as the concentrations increased. At low concentrations of 20% and 40%, processed honey showed negligible inhibition effect and so was raw honey at 20% concentration. However, the inhibition level increased for both raw and processed honey as the concentration increased. Both raw and processed honey showed highest inhibitory effect on *E. coli* at 100% concentration although there were some remarkable inhibitory effects at 60% and 80% for both types of honey.

Effects of honey on *S. aureus*

Both raw and processed honey had positive effects in influencing the growth inhibition of *S. aureus* (Fig 1a). However, raw honey had more inhibitory effect over processed honey ($p < 0.05$). At 80% to 100% raw honey concentration, the mean inhibition zone diameter was more than 20mm where as at the same concentration for processed honey the mean inhibition zone diameter was less than 20mm. The inhibitory effect of honey on the bacteria depended on the concentration, as concentration increased the inhibition zone diameter also increased (Fig 1a). There was less inhibition at lower concentrations (20% to 60%) for both raw and processed honey but the inhibition is increased at high honey concentration (80% to 100%) for both raw and processed honey (Fig 1a). There were significant differences in inhibition between successive concentrations of raw honey unlike in processed honey where there were no marked differences between successive concentrations except for first two (Fig 1a).

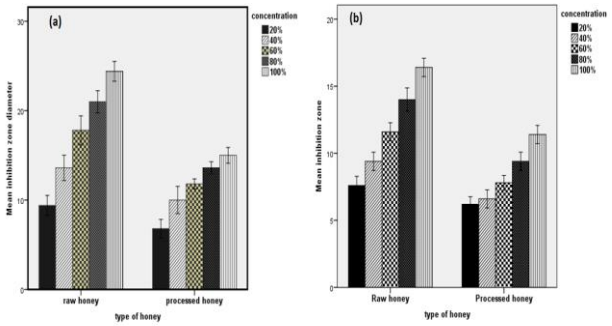

Figure 1. Mean diameters (with 95% CI error bars) of zones of inhibition of raw and processed honey against (a) *S. aureus* and (b) *E. coli.*

Effects of honey on *E. coli*

Figure 1(b) shows that both raw and processed honey had positive effects in influencing the growth inhibition of *E. coli*. It also shows that raw honey had more inhibitory effect over processed honey. At 100% raw honey concentration, the mean inhibition zone diameter was more than 15mm where as at the same concentration for processed honey the mean inhibitory zone diameter was less than 15mm. The inhibitory effect of honey on the bacteria depended on the concentration; as concentration increased the inhibition zone diameter increased. There was less inhibition at low concentration (20% to 60%) for both raw and processed honey but the inhibition is increased at high concentration (80% to 100%) for both raw and processed honey. There were significant differences in inhibition between successive concentrations of raw honey whilst in processed honey significant differences were only marked between the two highest concentrations (Figure 1b).

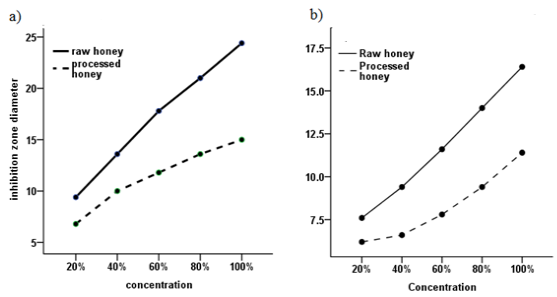

Figure 2. Effects of raw and processed honey on the growth inhibition of (a) *S. aureus* and (b) *E. coli.*

Figure 2 depicts the changes in growth inhibition of raw and processed honey with increase in concentration. For both species of bacteria, raw honey had the greater inhibitory effect as compared to processed honey ($p < 0.05$). As the honey concentration increased, the diameter of inhibition zone also increased for both *S. aureus* and *E. coli.*

Effect of honey and honey concentration on *S. aureus* and *E. coli*

Both species of bacteria were sensitive to treatment with both raw and processed honey, although *S. aureus* was more sensitive (mean zone of inhibition diameter > 20mm) than *E. coli* (mean zone of inhibition diameter < 15) (Fig 1 and 2). Therefore it can be deduced that *S. aureus* was more susceptible to honey than *E. coli*.

The effectiveness of honey in inhibiting bacterial growth increased with increase in concentration. At low concentration there was little or no inhibitory effect (Figures 2a and b). For both *S. aureus* and *E. coli* inhibition was significantly influenced by honey concentration ($p = 0.001$, $\alpha = 0.05$).

Effects of raw and processed honey on the growth inhibition of bacteria

Both raw and processed honey indicated some growth inhibition effect on the two species of bacteria although raw honey had a higher growth inhibitory effect on the bacteria as compared to processed honey. For both *S. aureus and E. coli,* inhibition was dependent on type of honey ($p = 0.001$, $\alpha = 0.05$) with both species being more susceptible to raw honey than to processed honey ($p = 0.001$, $\alpha = 0.05$).

Interaction between the honey concentration and honey type on bacterial growth inhibition

The results indicate that honey type and concentration interact to influence growth inhibition, and this is true for both *S. aureus* and *E. coli* ($p = 0.001$, $\alpha = 0.05$).

Discussion

Inhibitory effect of raw and processed honey

Both *S. aureus* and *E. coli* were sensitive to both types of honey but raw honey had a remarkable inhibitory effect over processed honey as indicated by the higher zones of inhibitions by the former as compared to the latter. This could be attributed to the fact that processed honey may have gone through some processing that renders its antibacterial properties less effective or may have some additives that reduce its potency. During processing, honey goes through ultra-filtration that involves adding water to it and filtering it under high pressure and then removing the water. This process of adding water may alter the honey concentration because during the preparation the honey concentrations were made by dissolving honey in water of which processed honey may have already been diluted during processing. Honey processing may also include heating the honey which affect its antimicrobial properties. Bang *et al* (2003) observed that peroxide activity in honey can be destroyed easily by heat.

However, the fact that both raw and processed honey were effective in reducing bacterial growth points to the preservation of some antimicrobial activity regardless of the processing. Some effective antimicrobial constituencies of honey may have withstood the processing. This is supported by a 2012 study by National Honey Board of USA, which analyzed the properties of honey which included antioxidant levels of raw and processed honey. The study showed that honey processing significantly reduced pollen quantity in honey, but did not affect its antibacterial properties leading the researchers to conclude that the antibacterial effects of honey are not affected by commercial processing (FAQ, 2013).

Inhibitory effect of honey concentration

The present study shows that honey concentration had an effect on the inhibition diameter on both *S. aureus* and *E. coli.* The minimum concentration of honey necessary for significant bacterial growth inhibition was at least 20% v/v for both raw and processed honey. It was observed that the significant inhibition effects of bacterial growth for both raw and processed honey ranges from concentration of 20% v/v to 100% v/v, indicating that the two types of honey had sufficient antibacterial potency to inhibit bacterial growth even when diluted for both *S. aureus* and *E. coli*.

In this study the antibacterial effect of honey was concentration-dependent and significant bactericidal effect was observed at a minimum concentration of 20% v/v for *S. aureus* and a minimum of 40% v/v for *E. coli* for both raw and processed honey. This compares favourably with results obtained when Nilgiri honey was used where a minimum concentration for bacteria inhibition was 25% v/v for *S.*

aureus and 40% v/v for *E. coli* (Rajeswari *et al*., 2010). In addition, the inhibition zone diameter increased as the concentration increased, this showed that the higher honey concentration was more effective than lower concentration.. This can be attributed to the increased levels of antibacterial substances as concentration increases. It was observed that significant inhibition zone diameter was recorded at (13 - 25) mm for *S. aureus* and (10 - 16) mm for *E. coli*. These results were within the ranges obtained by Rajeswari *et al* (2010) on the inhibition zone diameter of Nilgiri honey which were 20 - 21 mm for *S. aureus* and 13 -14 mm for *E. coli*.

Unpredictably, the minimum inhibitory effect of honey concentration was in a range of 2% v/v to 4% v/v for *S. aureus* (Cooper, 1999), 5% v/v to 10 v/v for *E. coli* (Allen *et al.*, 2000) and 55% v/v to 90% v/v for *Pseudomonas* (Cooper *et al*., 1999) after treating the bacterial species with Manuka honey. This contrasts with the present study and the conclusion may be that the diversity in the minimum inhibitory concentration (MIC) of honey depends on the species of bacteria and also on the origin of the honey. The results indicate the unpredictability of the antibacterial potency of different honey. The wide ranges of MICs reported for different types of honey against the same species of bacteria demonstrate the differences in antibacterial potency that may be encountered between honeys.

The study also indicates that honey concentration and honey type showed interaction on influencing the growth inhibition zone of bacteria, therefore it can be deduced that diluted or concentrated honey can be used to treat bacterial infections depending on the level of infection. For example, in wound infections, Molan (2001) observed that the amount of honey required on the wound is related to the amount of fluid exuding from the wound diluting it.

According to Molan's (2001) study, both gram positive and gram negative bacteria exhibited sensitivity to honey. Results from this study indicated that gram positive bacteria (*S. aureus*) were more susceptible to honey than gram negative bacteria (*E. coli*). At 100% raw honey concentration, the mean level of growth inhibition for *S. aureus* was in the range of >20mm< 30mm inhibition zone diameter and at 100% concentration, processed honey had a mean level of inhibition in the range of > 10mm < 20mm inhibition zone diameter. At the same concentration, however, the mean level of inhibition for *E. coli* was > 15mm < 20mm diameter zone of inhibition and>10mm < 15mm for raw honey and processed honey, respectively. The results clearly indicate that the gram positive bacteria (*S. aureus*) were more sensitive than the gram negative bacteria (*E. coli*) to both raw and processed honey.

The sensitivity of *S. aureus* to honey may indicate honey as a possible antimicrobial treatment of infections that are caused by *S. aureus*; these may include abscesses, boils, carbuncles, impetigo and wound infections (Molan, 1999). Studies on the successful performance of honey as a dressing on infected wounds show that there is a possibility for the usage of honey in infected wounds (Hughes, 2011) and it can be suggested that honey should be used on wounds of patients susceptible to Methicillin-resistant *S. aureus* (MRSA) and other antibiotic resistant bacteria (Herszge *et al*., 1980). Nevertheless the variance of antibacterial potency of different honey types should be considered as it may contribute to the divergence on results of using honey on wounds infections.

The sensitivity of *E. coli* to honey makes honey a possible therapy of *E. coli* infections which may include diarrhea, urinary infections, wound infections and septicemia (Molan, 1999). Haffejee and Moosce, (1970) cited in Molan, (2001) reported on a clinical study in which they used honey in oral rehydration in children and infants with gastroenteritis. They found that honey shortens the duration of bacterial diarrhea in infants and young children and also observed that honey does not extend non-bacterial diarrhea duration. Therefore honey can safely be used as a substitute for glucose in oral rehydration solutions that contain electrolytes.

The mechanisms of antibacterial action of honey remain speculative. Honey may inhibit bacterial growth due to a number of different mechanisms. High osmolarity, low pH, production of hydrogen peroxide, proteinaceous compounds, or other unidentified components present in the honey may all provide antimicrobial activity (Mundo *et al.*, 2004). Several components may contribute to the non-peroxide activities of honey, such as the presence of methyl syringate and methylglyoxal (Mavric *et al.*, 2008). Besides its antimicrobial properties, honey can clear infections in a number of ways *in vivo*, like boosting the immune system, its anti-inflammatory activity and antioxidant activities as well as via stimulation of cell growth (Al – Jabri, 2005). However there is lack of scientific research and documentation on the medicinal properties of honey in medicinal practices. Further studies on human subjects are required *in vivo* to understand the efficacy of honey in eliminating bacteria as this study only presents the findings of *in vitro* antibacterial activity of honey. Future studies in this direction will pave the way in establishing the medicinal importance of honey against bacterial infections.

We conclude that the results of this study showed that both processed and raw honey had inhibitory effect on both *S. aureus* and *E. coli* bacteria at different concentrations with minimal inhibitory concentrations of 20% v/v for the former and 40% v/v for the latter. However there were marked differences in the effectiveness of raw and processed honey on the two bacterial species. Differences were also observed between high and low concentrated honey with more concentrated honey being more effective than less concentrated honey. Overally, the results give a reasonable indication of the likely usefulness of honey as an alternative for treating bacterial infections for both gram positive and gram negative bacteria. However, further research with larger randomized trials using well defined honey is recommended to fully evaluate the efficacy of honey as an antimicrobial agent.

References

Allen, K. L., Molan, P. C., Reid, G. M. (1991). A survey of the antibacterial activity of some New Zealand honeys. *J. Pham. Pharmaco*; 43 (12).

Bang, L. M, Chapman T. M. and Goa K. L. (2003). Lercanidipine: a review of its efficacy in the management of hypertension. *Drugs*; 63 (22):2449-72.

Bartlett, J. G. (2002). Clinical Practices 'Antibacterial–associated diseases': 346: 334-9.

Bassiri, E. (2004). Antibiotic Sensitivity Testing, Department of Biology: *Biol* 275: page 2.

Barry, C. S., Llop-Tous M. I., Grierson, D. (2000). The regulation of 1-aminocyclopropane-1-carboxylic acid synthase gene expression during the transition from system-1 to system-2 ethylene synthesis in tomato. *Plant Physiology;* 123,979–986.

Carter, A. P., Clemons, W. M., Brodersen, D. E., Morgan-Warren, R. J., Wimberly, B. T. and Ramakrishnan, V. (2000). Functional insights from the structure of the 30S ribosomal subunit and its interactions with antibiotics. *Nature*, 407, 340–348

Cimolai, N. (2008). "MRSA and the environment: implications for comprehensive control measures". *Eur. J. Clin. Microbiol. Infect. Dis.*27 (7):481-93.

Cooper, R. (1999). The use of honey as an antiseptic in managing *Pseudomonas* infections. *J. Wound Care.* 8: 161–164

.Cooper, R. A., Halas, E. Molan, P. C. (2002). The efficiency of honey in inhibiting strains from infected burns. *Core Rehabiol*; 23: 366-70.

Holt, J. S., Powles, S. B., Holtum, J. A. M. (1993). Mechanisms and agro-nomic aspects of herbicide resistance. *Annu Rev Plant Physiol Plant Mo1 Biol;* 44: 203-229

Hughes, J. M. (2011). Preserving the lifesaving power of antimicrobial agents. 305 (10):1027-28

Kiistala, R., Hannuksela, M., Makinen-kiljunen, S., Niinim, A. and Haahtela, T. (1995). Honey allergy is rare in patients sensitive to pollen, Allegy; 50(10): 844-7.

Kunkel, D. (2002). Bacteriology: Dis 22(6):137-148.. doi: 10.1096/fj.09-150789. Epub 2010 Mar 12.

Kwakman, P. H., teVelde, A. A., de Boer, L., Speijer, D., Vandenbroucke-Grauls C. M., Zaat, S. A. (2010). How honey kills bacteria. *FASEB* J. 24(7):2576-82

Lalitha, M. K. (2004). Manual of antimicrobial Susceptibility Testing, Department of Microbiology; Christian Medical College; page 7.

Mavric, E., Wittmann, S., Barth, G., Henle, T. (2008). Identification and quantification of methylglyoxal as the dominant antibacterial constituent of manuka (*Leptospermum scoparium*) honeys from New Zealand. Mol Nutr Foods Res; 52:483–9.

Molan P. C. (2001). Why honey is effective as a medicine 2. The scientific explanation of its effects. In: Munn P, Jones R, editors. Honey and Healing. UK: International Bee Research Association.

Molan, P. C. (1992). The antimicrobial activity of honey 1. The nature of antibacterial activity. *Bee world*; 73 (1): 5 -28.

Mundo M. A., Padilla-Zakour, O. I., Worobo, R. W. (2004). Growth inhibition of foodborne pathogens and food spoilage organisms by selected raw honeys. *Int J Food Microbiol*; 97:1–8.

Ogston, A. (2003). "On Abscesses". Classics in Infectious Diseases".*Rev Infect Dis* 6 (1):122-28.

Rajeswari T., Venugopal, A., Viswanathan, C., Kishmu, L., Venil, C. K., Sasikumar, J. M. (2010). Antibacterial activity of honey against *Staphylococcus aureus* from infected wounds. *Pharmacologyonline*.1:537–541.

Vlieghe, E. (2012). The First Global Forum on Bacterial Infections. *Anti Inf*. 10 (12):145-48.

World Health Organisation, (2003). Antimicrobial resistance: a global threat. Essential drugs monitor.