academicJournals

Vol. 13(4), pp. 616-625, 22 January, 2014
DOI: 10.5897/AJB11.892
ISSN 1684-5315 ©2014 Academic Journals
http://www.academicjournals.org/AJB

African Journal of Biotechnology

Full Length Research Paper

Antibacterial activity of honey and medicinal plant extracts against Gram negative microorganisms

Ramalivhana JN[1], Obi CL[2*], Samie A[3], Iweriebor BC[3], Uaboi-Egbenni P[3], Idiaghe J.E.[4] and Momba M. N. B[5]

[1]College of Agriculture and Environmental Sciences, School of Agriculture and Life Sciences, University of South Africa.
[2]Academic and Research Division, Walter Sisulu University, Nelson Mandela Drive, Eastern Cape, South Africa.
[3]Department of Microbiology, University of Venda, South Africa.
[4]College of Education, Agbor, Delta State Nigeria.
[5]Department of Environmental, Water and Earth Sciences, Tshwane University of Technology, Pretoria, South Africa.

Accepted 19 July, 2013

There has been a steady rise in antibiotic resistance of bacteria and this urgently calls for the discovery of alternative therapeutic agents. Honey possesses therapeutic potentials which includes antimicrobial activity. Although the antimicrobial activity of honey has been effectively established against an extensive spectrum of microorganisms, it differs depending on the type of honey. To date, not much extensive studies of the antibacterial properties of South African honeys on enteric microorganisms have been conducted. The objective of this study was to compare the antibacterial activity of extracts of six different honeys with those of medical plants commonly used in South Africa. Using a broth dilution method, the antibacterial activity extracts of six South African honeys and medicinal plants against six enteric microorganisms viz- *Enterobacter cloacae*, *Escheriachia coli*, *Klebsiella pneumoniae*, *Citrobacter freundii* isolated from geophagia samples and *Aeromonas hydrophila* and *plesiomonas shigelloides* isolated both from stool and water samples using agar well diffusion method was done. Different concentrations of honey and plant extracts were tested against each type of microorganism. Briefly, two-fold dilutions of honey solutions were tested to determine the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) against each type of microorganism. Extracts from both South African honeys and medicinal plants showed zones of inhibition that ranged from 6.94 to 37.94 mm. The most susceptible bacteria were *Escheriachia coli, Aeromonas hydrophila* and P*lesiomonas shigelloides*. MIC and MBC values of extracts were found in the range of 0.625 to 5.000 mg/ml. Extracts of honey showed good antibacterial activity against most organisms than the standard antibiotics such as Ampicillin and Gentamycin. Honey extracts showed antibacterial activity against most microorganisms which were showing some degree of resistance to commercial antibiotics. Extracts from South African honeys and medicinal plants exhibited variable activities against different microorganisms. This result suggests that the honeys could potentially be used as an alternative therapeutic agent against certain microorganisms.

Key words: Agar well diffusion assay, honey, minimum inhibitory concentration, minimum bactericidal concentration.

INTRODUCTION

Traditional medical practise has assumed exalted status in various communities around the world (Mathabe et al., 2006). People living in rural areas prefer using traditional medicines for the treatment of various diseases and disorders to orthodox medicines (ref). According to the World Health Organization (1999), an estimated 80% of

people living in developing countries rely on harvested wild plants for their primary health care. Several reports on the antibacterial activities of medicinal plants against pathogenic organisms abound in literatures (Obi et al., 2003; Samie et al., 2009, 2010; Eloff et al., 2005; Kaushik et al., 2009; Yasutan et al., 2009). Furthermore, plant extracts and other natural substances have been in use as per the Indian system of medicine (Ayurveda) for the treatment of diseases requiring antimicrobial drugs. One of the popular natural antimicrobial substances described in Ayurveda as a potent medicine for several uses was honey.

Honey has been used for its medicinal properties to treat a wide variety of ailments since ancient times. In particular, it has been used in wound dressings (Molan and Cooper, 2000; Kingsley, 2001). Honey in general has high sugar content but a low water content and acidity, which prevent microbial growth (Farouk et al., 1988; Tan et al., 2009). Most types of honey generate hydrogen peroxide when diluted due to the activation of the enzyme glucose oxidase, which oxidizes glucose to gluconic acid and hydrogen peroxide (Bogdanov, 1984; Bang et al., 2003). Hydrogen peroxide is the major contributor to the antimicrobial activity of honey, and the different concentrations of this compound in different honeys result in their varying antimicrobial effects (Molan, 1992).

The bactericidal action could also be ascribed to the normal acidity of honey, its high sugar content, nitrogennous or other compounds (Radwan et al., 1974; Adeleke, 2006; Basualdo et al., 2007; Namias, 2003). Honey can inhibit the growth of a wide range of bacteria, fungi, protozoa and viruses (Molan, 1992; Blair et al., 2005). Microorganisms such as *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Escherichia coli* frequently are isolated from skin wounds (Tan et al., 2009). There are many reports of honey being very effective as an adjunct in the treatment of wounds, burns, skin ulcers and as an anti-inflammatory agent (Lusby, 2002). Honey also contains various constituents such as water, carbohydrates, proteins, vitamins, amino acid, energy and minerals (Abhishek, 2010). It is also known to cure anaemia and improves calcium fixation in infants (Heerng, 1998) and also reduces and cures eye cataracts and conjunctivitis (Ilechie et al., 2012). The bactericidal effects of honey are reportedly dependent on concentrations of honey used and the nature of the bacteria (Adeleke, 2006). The aim of this study therefore was to ascertain the antibacterial activities of honey in comparison to commercial antibiotics and known medicinal plants extracts.

MATERIALS AND METHODS

Collection of honey samples

Raw honey samples were collected from rural areas in the Limpopo Province of South Africa.

Bacterial isolates

The following bacterial isolates: *Enterobacter cloacae, Escherichia coli, Klebsiella pneumoniae and Citrobacter freundii* were isolated from geophagic samples while A*eromonas hydrophila* and P*lesiomonas shigelloides* were isolated from water and stool samples. The isolates were identified using a range of biochemical and morphological techniques, and the Microscan walk away automated bacterial identification instrument (McDonnell Douglas Health System Company). The isolates were stored on Protect Bacterial Preserver Beads (LabSupply Pierce) at −70°C.

Plant materials used in the study

The following medicinal plants: *Carissa edulis, Erythrina lysistemon*, *Momordica* balsamina, *Psidium guajava* and *Ficus syscomorus* were collected based on the information received from herbalists and their consorts on the basis of their effectiveness against microbial diseases. Plant parts used were leaves, bark, roots, ripe fruits, unripe fruits and twig tips. The plants were collected from their natural environment and stored in the dark at room temperature until they were dry.

Extraction of honey

Extraction of honey was performed by using methanol; 10 g of honey was placed in a centrifuge with 25 ml of solvent and then mixed well by vortexing and shaking with hands for about 30 min. This was centrifuged at 3000 rpm for 20 min at 25°C. Supernatant was collected from each centrifuged tube in a round bottom flask by filtration. The resulting supernatant was dried under nitrogen gas at a temperature of 50°C. All extracts were put in DMSO at a concentration of 100 mg/ml as the extract of honey. All the extracts dissolved in DMSO were collected in sterilized glass tubes and used within 24 h for the evaluation of bacteriostatic and bactericidal activity.

Extraction of medicinal plants

Collected plant materials were ground into fine powder using a local traditional grounding system (Musi and Mutuli, Tshivenda). Extractions were done as previously reported by Samie et al. (2010). About, 50 g of the ground materials of each plant was extracted in 500 ml methanol under continuous shaking for 24 h. The extract was filtered through a 22 µm paper filter. The filtrate was evaporated to dryness using a rotatory evaporator at 40°C. The residues in the form of powder materials were preserved in sterile glass bottles at room temperate until further use.

Antibacterial activity test

Bacterial suspensions were done as described by Ramalivhana and Obi (2010). Agar diffusion and micro-dilution methods were used to determine the antibacterial activity of the medicinal plant extracts against bacterial isolates. Brain heart infusion broth (BHIB) was used for the preparation of bacterial cultures. The determination of the minimum inhibitory concentrations (MICs) was done as recommended by the manufacturer's. Brain heart infusion agar

*Corresponding author. E-mail: c355251@yahoo.com.

(BHIA) was used to determine the activity of the plant extracts against bacterial organisms, this was prepared according to the manufacturer's instruction.

Agar diffusion assay

Bacterial isolates were prepared to match 0.5 McFarland standards. Using the micropipette, 100 µl of organisms (BHIB or SDB) was spread over the surface of an agar plate. This procedure was the same for all test organisms. Using a sterile glass pipette, five holes were punched in each of the culture plates. One of the holes was punched in the center of the plate where 10 µl of Gentamicin was added as positive control; 10 µl of DMSO was added as a negative control in the other hole; 10 and 15 µl of the plant extracts were put in the remaining two holes. The culture plates were then incubated at 37°C for 24 h. The clear zone of inhibition around the plant extract was measured in mm. The experiments were done in triplicate.

Microdilution assay

The microdilution method was used to determine the minimum inhibitory concentrations (MICs) of the plant extracts using 96 well microtitration plates as previously described by Samie et al. (2005). One hundred and eighty-five microliter (185 µl) of the broth was added into each well in the first row of microtitration plate and 100 µl to the rest of the wells from the second row downwards.Fifteen microliter (15 µl) of the plant extracts was then added into each well on the first row (row A), starting with the positive control (Gentamicin for bacteria and Floconazole for yeast, all the antibiotics were from MAST), followed by the negative control (the 20% DMSO used to dissolve the plant extracts) and the plant extracts in the rest of the wells on that row. A twofold serial dilution was done by mixing the contents in each well of the first row and transferring 100 µl to the second well of the same column and the same was done up to the last well of the same column and the last 100 µl from the last well was discarded. Then 100 µl of yeast suspensions was added.

The results were observed after 24 h incubation at 37°C, followed by the addition of 40 µl of a 0.2% Iodo Nitro Tetrazolium (INT) solution after a further incubation of 4 h at 37°C.

Determination of minimum inhibitory concentration (MIC)

Prior to testing, each isolate was cultured from preserver beads by inoculating two beads into 9 mL of TSB and incubating for ~16 h at 37°C. Cultures obtained were diluted with TSB to obtain 2 to 3 × 10^7 cfu/mL, the minimum to produce confluent growth at inoculation positions. The minimum inhibitory concentration (MIC) of active extract was evaluated by tube dilution method. The MICs of all the extracts were determined by dilution of the extract to various concentrations (5.000 to 0.150 mg/mL). Decreasing concentrations of methanol extracts were prepared in serial twofold dilutions using Muellar Hinton Broth (MHM). Controls were included. After an overnight incubation at 37°C, the tubes were examined for turbidity indicating the growth of the microorganisms. The lowest solution of the extract that inhibited the growth of the microorganism as detected by the lack of visual turbidity (matching the negative growth control) was designated the minimum inhibitory concentration.

Determination of minimum bactericidal concentration (MBC)

The bactericidal activities of the extracts (both honey and plant extracts) were tested as follows: the number of the bacteria in the initial microorganism suspension was counted by the surface plate method. After ascertaining the MIC, the number of bacteria was counted in each of the tubes of broth that showed no visible turbidity after overnight incubation, and was compared with the number of bacteria in the initial microorganism suspension. According to NCCLS (1997), the lowest concentration of the extract solution that allowed less than 0.1% of the original inoculum to survive was taken to be the minimum bactericidal concentration.

Antibiotic susceptibility testing

The susceptibility of isolates to antimicrobial agents was examined by an agar diffusion method using paper disks containing the following antibiotic concentrations: Amikacin (30 µg), Ampicillin (10 µg), Gentamicin (10 µg), Cefotaxime (30 µg) and Ciprofloxacin (30 µg). Disks were purchased from Oxoid. Antimicrobial activities were interpreted according to the National Committee for Clinical Laboratory Standards (NCCLS, 1997). *E. coli* ATCC 25922 and *P. aeruginosa* ATCC 27853 were used as quality control strains.

RESULTS

In this study, we compared the MIC values of six honey extracts in comparison with the plants extracts and some commonly used antibiotics. The MBC values of honey extracts also were compared. Under visual inspection, the zones of inhibition for honey extracts ranged from 7.0 to 28.0 mm, while those for plants ranged between 7.0 mm to 29.5 mm and antibiotics from 14.5 mm to 36.0 mm (Table 1). Honey B had the highest *in vitro* activity of 28.0 mm when compared with other honey extracts against *A. hydrophila*. This was followed by extracts of honey E and F against *C. frundii* with 19.0 mm and 19.5 mm zones of inhibition respectively. All the test isolates showed some degree of sensitivity to the different honey extracts as the zones of inhibition ranged from 7.0 mm in honey D extract against *E. coli* to 28.0 mm of honey B extract against *H. hydrophila*.

On the action of honey B extract against the test isolates, the highest antibacterial activity was observed against *A. hydrophila* with 18.5 mm zone of inhibition and lowest activity of 8.5 mm against *E. cloacae* (Figure 1). Similarly, Honey B extract demon-strated the highest antimicrobial activity against *A. hydrophila* with 28.0 mm zone of inhibition and lowest activity of 8.0 mm zone of inhibition against *E. Cloacae* (Figure 2). Honey C extract showed the least activity as the highest zone of inhibition of 10.0 mm was observed against three of the test isolates; *K. pneumonia, A. hydrophila* and *P. shigelloides* and the lowest activity of 8.5 mm was against *E. coli* (Figure 3). The lowest activity of 7.0 mm was demonstrated by honey D extract against *E. coli* while the highest activity of this extract was observed on *A.hydrophila* with a zone of inhibition of 15.5 mm (Figure 4). Both extracts from honey E and F had the highest antibacterial activities of 19.0 and 19.5 mm zones of inhibition respectively against *C. frundii* while the lowest activity of Honey E was observed against *E. cloacae* and

Table 1. *In vitro* antibacterial activities of honey, antibiotics and medicinal plants against Gram negative bacteria.

Parameter	Zone of inhibition (mm diameter)					
	Enterobacter cloacae	***Escherichia coli***	***Klebsiella pneumonia***	***Citrobacter freundii***	***Aeromonas hydrophila***	***Plesiomonas shigelloides***
Honey A	8.5	10.0	9.0	15.5	18.5	10.0
Honey B	8.0	11.0	8.5	12.0	28.0	15.5
Honey C	9.0	8.5	10.0	9.0	10.0	10.0
Honey D	8.5	7.0	11.5	8.5	15.5	8.5
Honey E	9.0	9.5	12.0	19.0	10.0	9.0
Honey F	9.5	9.0	10.0	19.5	9.5	9.5
Antibiotics						
Amikacin (30 µg)	26.0	22.0	16.5	33.0	35.0	36.0
Ampicillin (10 µg)	15.5	8.5	28.0	15.5	28.0	27.0
Gentamicin (10 µg)	25.0	28.0	22.0	33.0	15.5	35.0
Cefotaxime (30µg)	15.0	33.0	19.5	22.0	15.5	19.0
Ciprofloxacin (5µg)	14.5	19.5	17.0	22.5	19.0	15.0
Medicinal plants						
Carissa edulis	19.0	19.5	12.0	19.0	20.0	19.0
Erythrina lysistemon	18.5	10.0	19.0	15.5	18.5	20.0
Momordica balsamina	15.0	21.0	15.5	22.0	23.0	15.5
Psidium guajava	19.5	25.0	20.0	29.5	19.5	20.5
Ficus syscomorus	8.5	7.0	11.5	8.5	15.5	8.5
DMSO	0	0	0	0	0	0

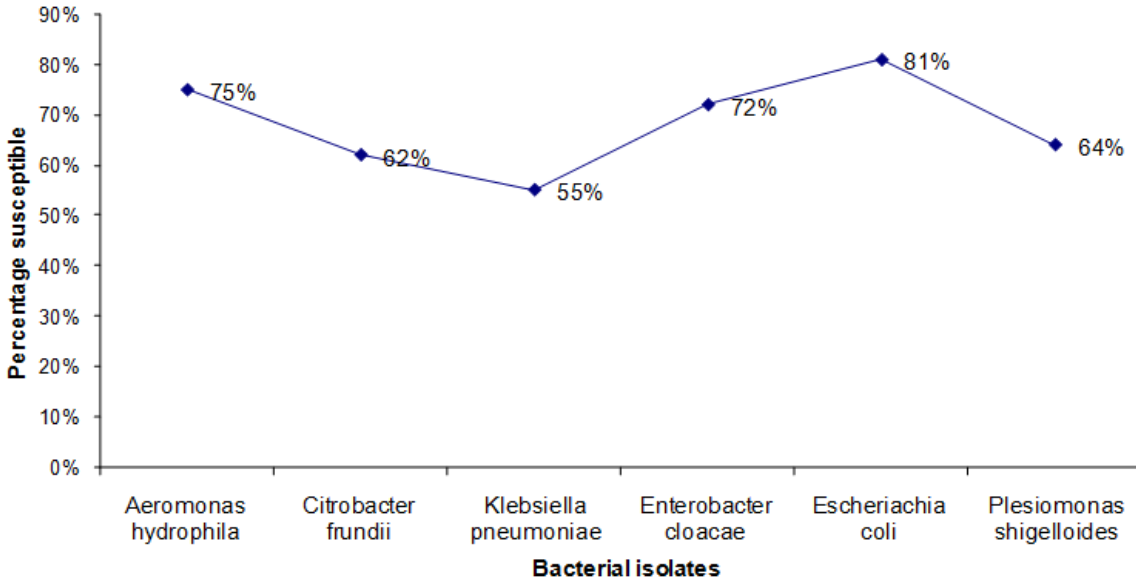

Figure 1. Antibacterial activity of Honey A against Gram negative bacteria. The highest activity was against *E. coli* and moderate activity against *K. pneumonia*.

P. shigelloides with the lowest activity of honey F extract was against *E. coli* (Figures 5 and 6). Comparing the MICs of the honey extracts to those of the plants against the bacterial isolates, the MIC of all the honey extracts against *Aeromonas hydrophilia* was 1.25 mg/ml while for the plant extracts, 0.625 mg/ml was observed for *Dodonea angeostifola* and *E. lysistemon* respectively on the same isolate. For *C. frundii*, 0.625 mg/ml of extracts of honey A and E was the lowest MIC while on the same isolate the lowest MIC of 0.3125 wasnoted on extracts

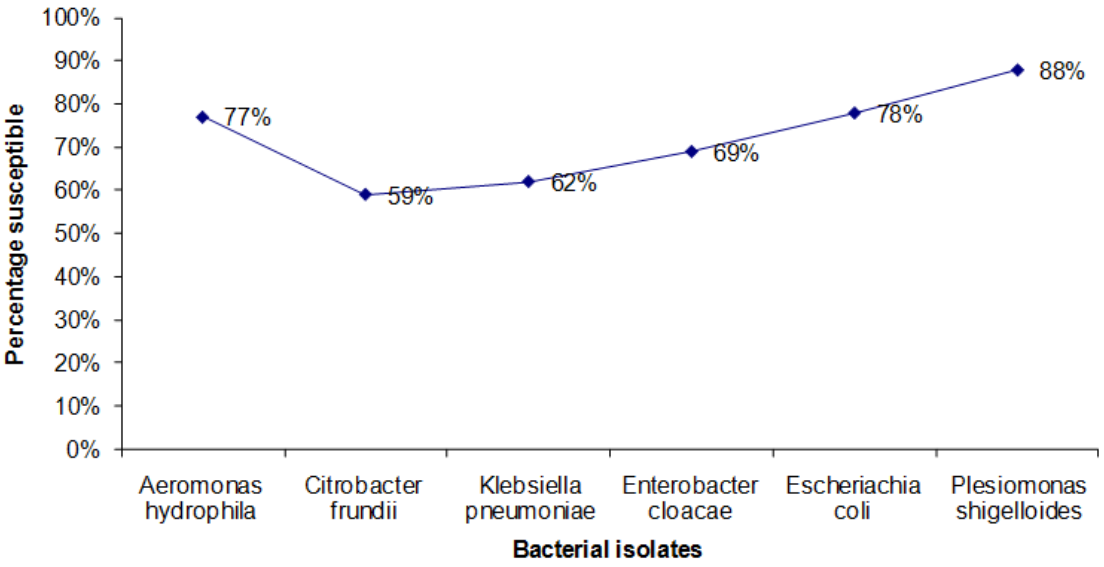

Figure 2. Antibacterial activity of Honey B against Gram negative bacteria. Activity was greatest on *P. shigelloides* and lowest in *C. frundii.*

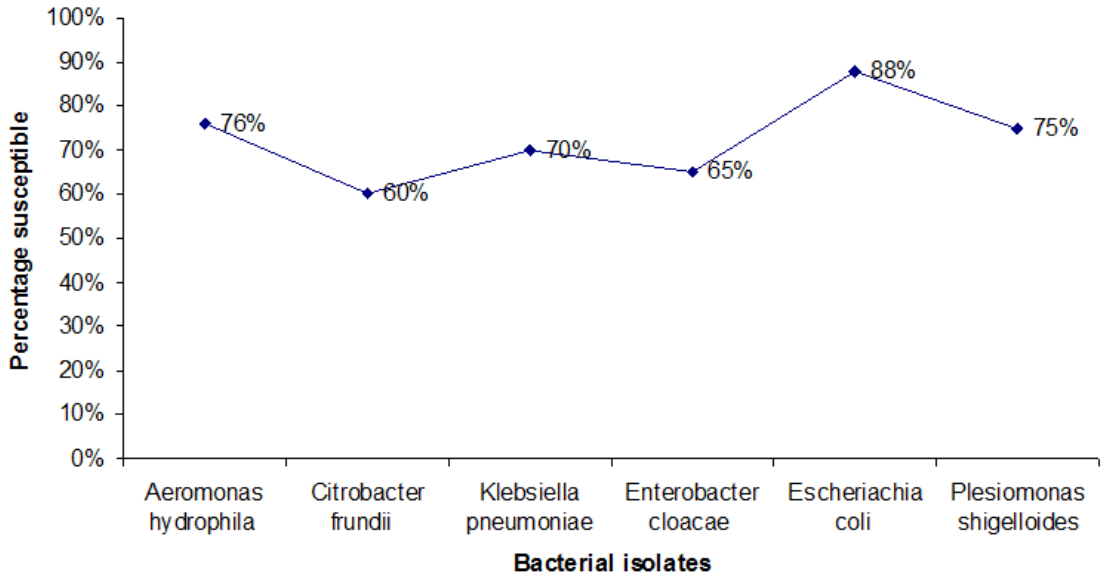

Figure 3. Antibacterial activity of Honey C against Gram negative bacteria. *E. coli* exhibited the greatest sensitivity while *C. frundii* showed moderate sensitivity.

from *D. angeostifolia* and *F. sycomorus* respectively. The MIC of 0.625 mg/ml of honey C was the most effective against *K. pneumonia* while the MIC of 0.3125 mg/ml of *P. guajava* was the lowest of all the plant extracts. The lowest MIC of 0.625 mg/ml of the honey extracts against *E. cloacae* was that from honey C while those of the plant extracts of 0.625 were those of *P. guajava* and *M. balsamina* respectively on the same bacteria isolate. For *E. coli*, the lowest MIC 0.625 was from honey E while for the plant extracts, 0.3125 mg/ml *P. guajava* and *E. lysistemon* were noticed. Similarly, the lowest MIC of 0.625 mg/ml of honey B was noticed against *P. shigelloides* with a similar MIC for extracts of *P. guajava* and *E.lysistemon* as shown in Table 2.

Comparison of the minimum bactericidal concentrations of the both the honey and plant extracts against the test isolates showed that the bactericidal activities of the honey C and E along with those of *Dodonea angeostifolia*

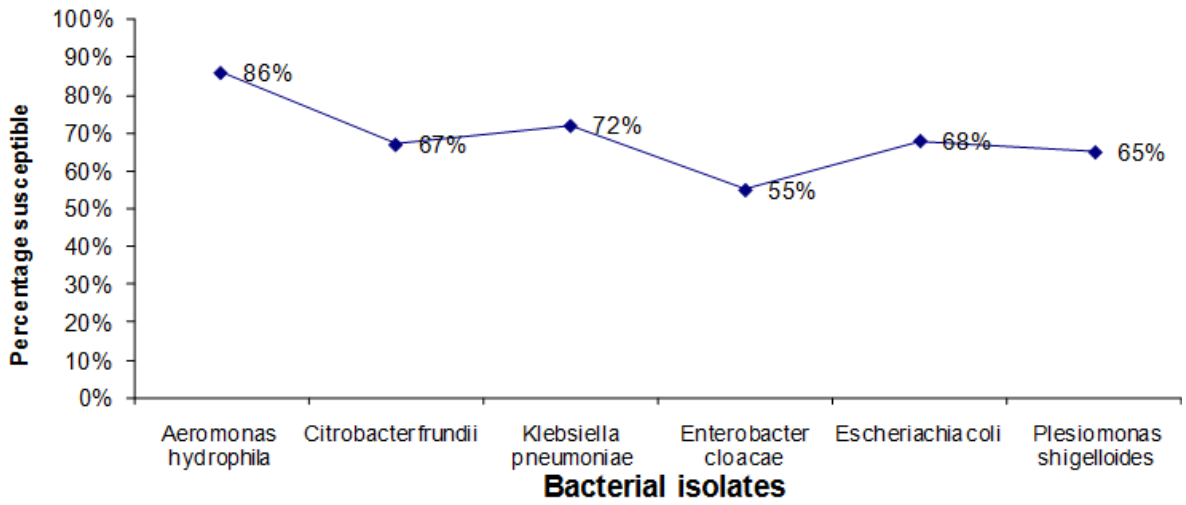

Figure 4. Antibacterial activity of honey D against gram negative bacteria. Honey D extract demonstrated a very high activity of 86% susceptibility against *A. hydrophila* with moderate effect of 55% on *E. Cloacae*.

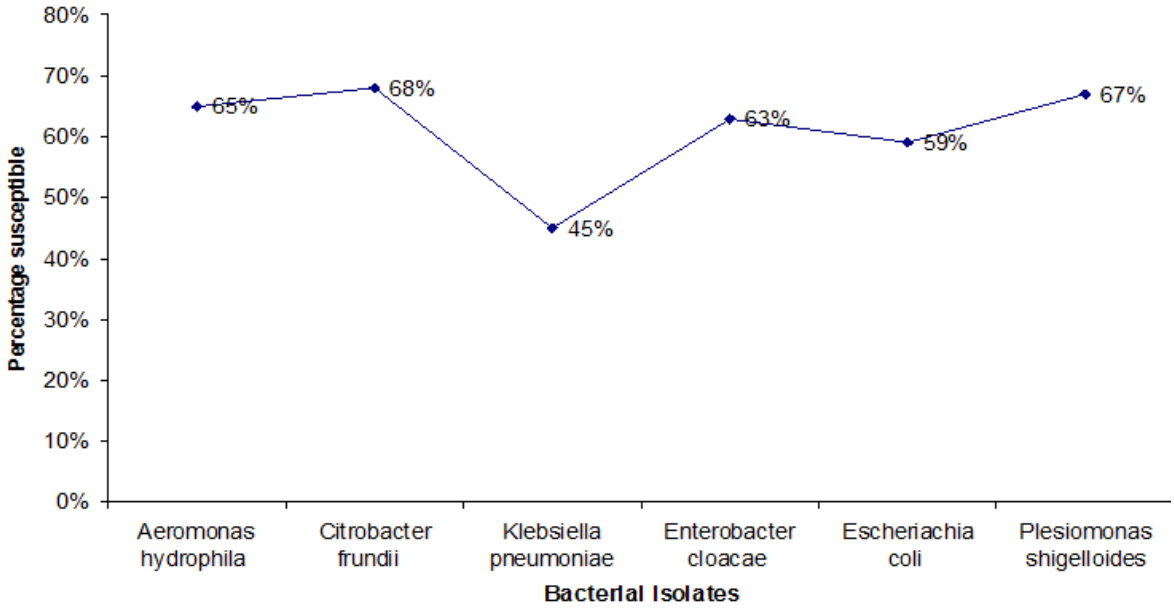

Figure 5. Antibacterial activity of Honey E against Gram negative bacteria. The highest antibacterial effect was on *C. frundii* and with the lowest activity of 45% was on *K. Pneumonia*.

and *Erythrina lysistemon* had the lowest concentration of 0.625, respectively against *Aeromonas hydrophila* while honey C and D had the lowest MBC of 0.3125 each on *Citrobacter frundii* and *Psidium guajava* and *E.lysistemon* demonstrated the lowest MBC of 0.625 on the same organism (Figure 7). For *K. pneumonia*, honey A and F extracts showed the lowest MBC of 0.3125 while *P. guajava* and *M. balsamina* demonstrated the lowest MBC of 0.625 respectively against the isolate. Honey A, B and plant extracts of *P. guajava* and *M. balsamina* each exhibited the lowest MBC of 0.625 against *E. cloacae*. The MBC of extracts of honey A, *P. guajava, M. balsamina* and *E. lysistemon* against *E. coli* were 0.625 each while on *P. shigelloides*, extracts of honey C,E, *D. angeostifolia* and *E. lysistemon* demonstrated the lowest MBC of 0.625 as shown in Table 3.

The lowest MIC value of 0.312 mg/ml was observed against *K. pneumoniae, E. coli* and *C. freundii* using *P. guajava, F. sycomorus*, *D. angeostifolia* and *E. lysistemon* extracts respectively.

Table 2. MIC values of different extracts of Honey and medicinal plants.

Zone diameter of inhibition (in mm) including the diameter of well (6 mm) Isolates	Honey A	Honey B	Honey C	Honey D	Honey E	Honey F	*Psidium guajava*	*Momordica balsamina*	*Dodonea angeostifolia*	*Ficus sycomorus*	*Erythrina lysistemon*
	MIC(mg/ml)										
Aeromonas hydrophila (n= 200)	1.25	1.25	1.25	1.25	1.25	1.25	2.5	1.25	0.625	1.25	0.625
Citrobacter freundii (n=49)	0.625	1.25	1.25	1.25	0.625	1.25	0.625	0.625	0.3125	0.3125	2.5
Klebsiella pneumoniae(n=55)	1.25	0.625	1.25	1.25	1.25	1.25	0.3125	2.5	2.5	5	1.25
Enterobacter cloacae (n=46)	1.25	1.25	0.625	1.25	1.25	1.25	0.625	0.625	2.5	2.5	1.25
Escheriachia coli (n=88)	1.25	1.25	1.25	1.25	0.625	1.25	0.3125	1.25	1.25	1.25	0.3125
Plesiomonas shigelloides (n=89)	1.25	0.625	1.25	1.25	1.25	1.25	0.625	2.5	1.25	2.5	0.625

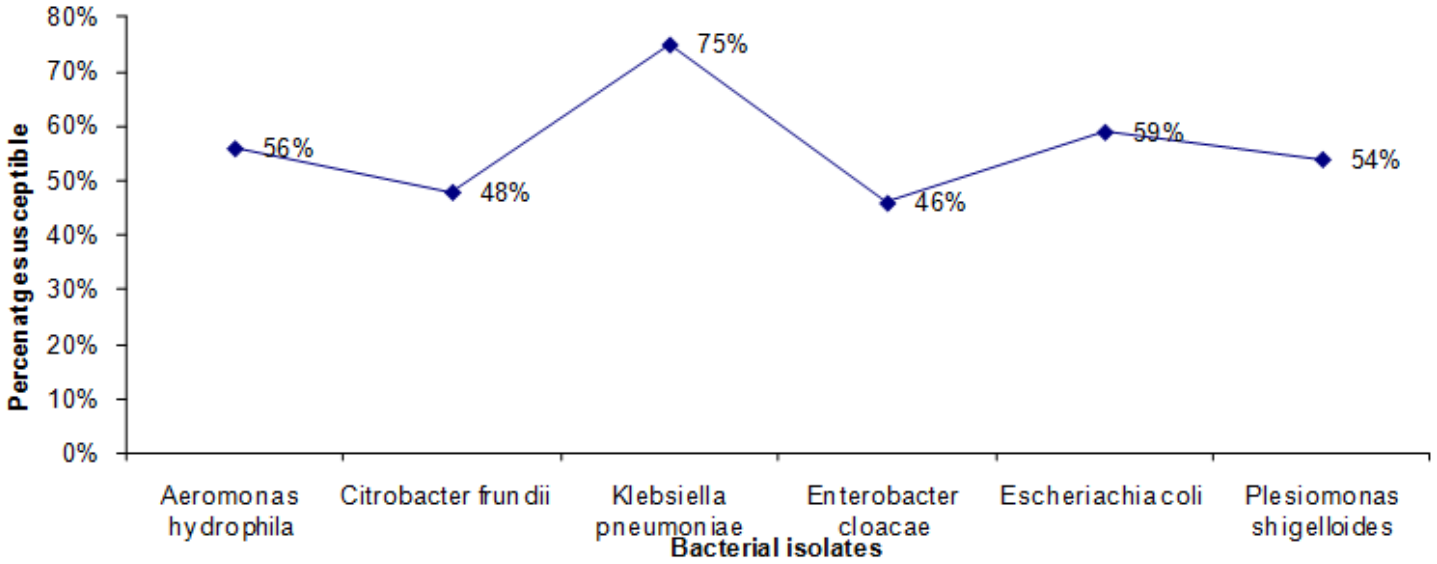

Figure 6. Antibacterial activity of Honey F against Gram negative bacteria. Honey F extract had the highest antibacterial effect on *Klebsiella pneumonia* while against the other test isolates it was moderate.

DISCUSSION

The antibacterial activities of six South African honeys and medicinal plants were investigated on some common entero pathogens isolated from geophagist and water samples. In this study, we found that extracts of different honeys and medicinal plants commonly used in South Africa has variable but broad-spectrum activities against many different species of enteric bacteria (Figure 8). Lusby et al. (2005) reported that honeys other than the commercially available honeys can have

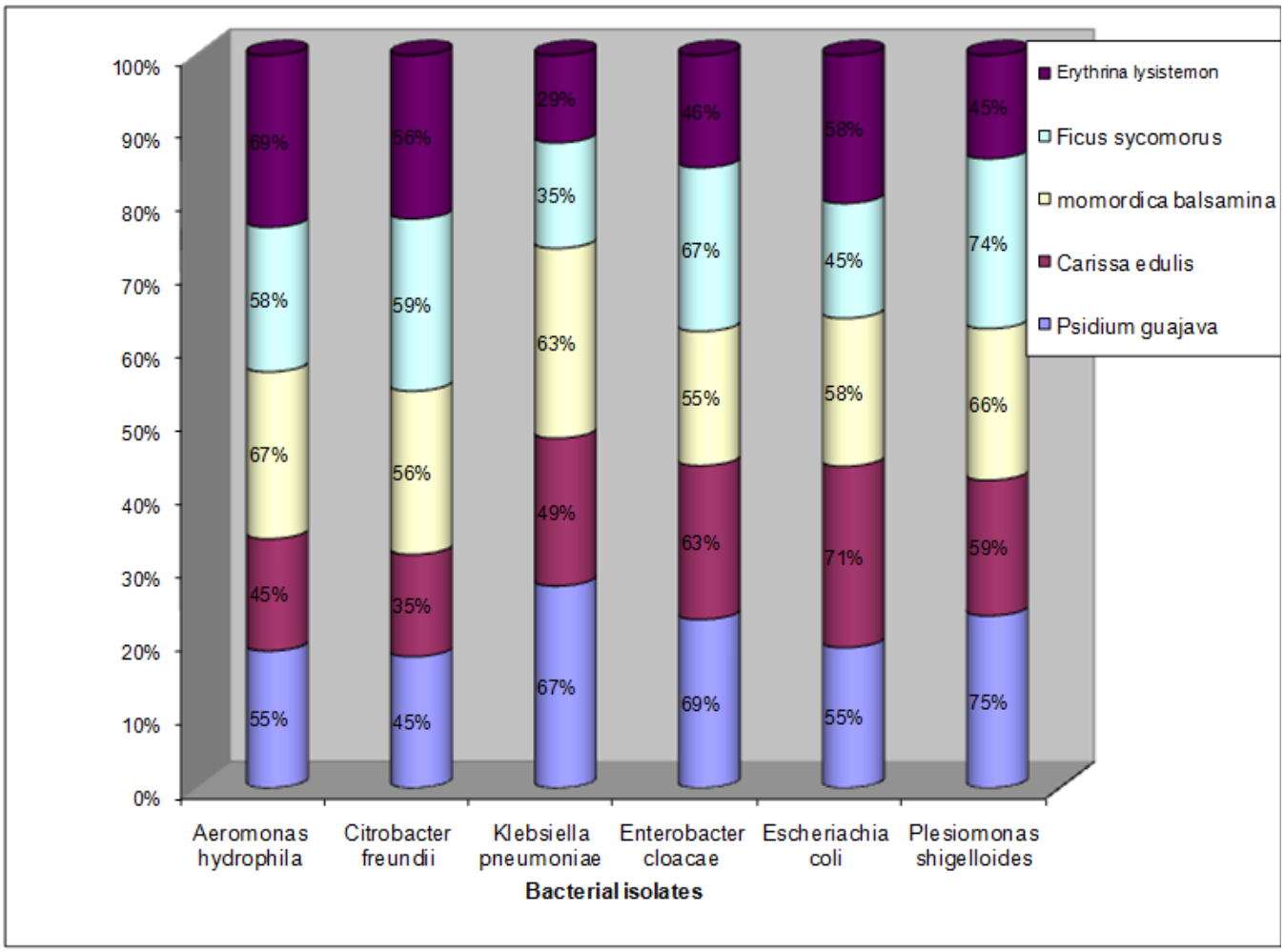

Figure 7. Antimicrobial activity of medicinal plants against Gram negative bacteria. The graph depicts the percentage activities of the different medicinal plants extracts against the test bacterial isolates. The highest antimicrobial activity of 89% was on *A.hydrophila* by the extract of *E. lysistemon* followed by 75% of *P. guajava* extract on *P. shigelloids* and 71% of *C. edulis* on *E. coli*.

equivalent antibacterial activity against some bacteria, whereas Basson and Grobler (2008) found no exceptionally high antimicrobial activity of honeys from indigenous wild flowers from South Africa. Ali et al., (1991), reported the inhibitory effects of natural honey on *Helicobacter pylori*. Analysis of the inhibition of bacterial growth caused by different honey extracts revealed similarities in the pattern of inhibition exhibited by the 6 bacterial isolates tested in this study.

Most bacteria showed similar growth inhibition patterns for all the six honeys tested, but some variations were detected. The observed differences might reflect how each type of bacteria reacts to honey treatment. That honey is effective in treating bacterial gastroenteritis in infants has been reported by Haffejee and Moosa (1985). Honey was reported to be effective when used as a substitute for glucose in oral rehydration and its antibacterial activity shortened the duration of bacterial diarrhoea (Tan et al., 2009). In our study, the growth of bacterial species that cause gastric infections, such as *C. frundii, P. shigelloides* and *E. coli*, were inhibited by honey extracts.

Results obtained reveal the varying levels of the antibacterial activities of honey against bacterial isolates studied. The observations are consistent with the reports of Ibrahim (1985) on the bactericidal activity of aqueous solution of honey on *Salmonella spp.* and *Shigella spp.* and other enteropathogenens such as *E. coli*, *Vibrio cholera,*other Gram-negative and Gram-positive bacteria. Similarly, Allen et al. (2000) reported the antibacterial properties of honey against two laboratory isolates of *P. aeruginosa* and *E. coli.*

Our study is also in agreement with the study done by Samie et al. (2007) who reported on the activities of medicinal plants against 14 Gram negative microogranisms. Similarly, Obi et al. (2003) reported on the inhibitory properties of medicinal plants against a total number of fifty isolates of *E. coli* from various pathologic sources.

Table 3. MBC values of different extracts of honey and medicinal plants.

Isolate	Honey A	Honey B	Honey C	Honey D	Honey E	Honey F	*Psidium guajava*	*Momordica balsamina*	*Dodonea angeostifolia*	*Ficus sycomorus*	*Erythrina lysistemon*
	MBC(mg/ml)										
Aeromonas hydrophila (n= 200)	2.5	1.25	0.625	1.25	0.625	2.5	1.25	1.25	0.625	1.25	0.625
Citrobacter freundi (n=49)	0.625	0.625	0.3125	0.3125	2.5	0.625	0.625	2.5	1.25	1.25	0.625
Klebsiella pneumoniae(n=55)	0.3125	2.5	2.5	5	1.25	0.3125	0.625	0.625	2.5	2.5	1.25
Enterobacter cloacae (n=46)	0.625	0.625	2.5	2.5	1.25	0.625	0.625	0.625	2.5	2.5	1.25
Escheriachia coli (n=88)	0.3125	1.25	1.25	1.25	0.625	0.625	0.3125	0.3125	2.5	1.25	0.3125
Plesiomonas shigelloides (n=89)	2.5	1.25	0.625	1.25	0.625	2.5	1.25	1.25	0.625	1.25	0.625

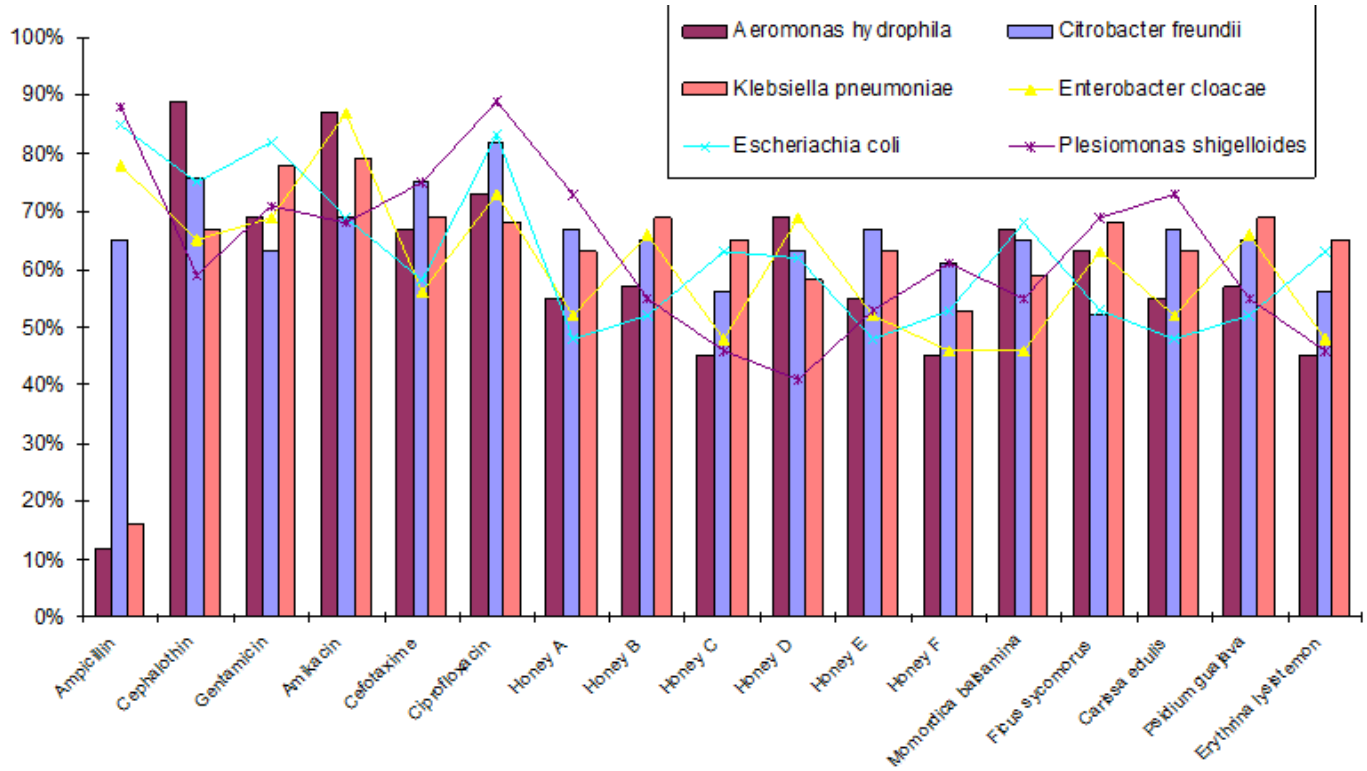

Figure 8. Summary of antimicrobial activities of antibiotics, medicinal plants and honey on bacterial isolates.

Conclusion

The six honeys and plant extracts exhibited variable activities against many different microorganisms and in some cases they showed equivalent or better activities than some antibiotics. The potency of these honeys and plants against certain microorganisms suggests their potential to be used as an alternative therapeutic agent in the face of antibiotic resistance. It will also be of great advantage if they are administered together as they could have synergistic action.

ACKNOWLEDGEMENTS

We thank the National Research Foundation for the financial assistance and traditional Practitioners for their help and willingness to share knowledge on traditional medicine

REFERENCES

Adeleke OE, Olaitan JO, Okepekpe EI (2006). Comparative antibacterial activity of honey and Gentamicin against *Escherichia coli* and *P. aeruginosa*. Ann. Burn, fire disasters 19: 4.

Ali AT, Chowdhury MN, Al-Humayyd MS (1991). Inhibitory effects of natural honey on *Helicobacter pylori.* Trop. Gastroenterol. 12: 139-143.

Allen KI, Radwan S, Reid GM (2000). Antimicrobial activity of honey on some microbial isolates. J. Medic. Pharmaceut.Sci,2:75-79.

Bang LM, Buntting C, Molan PC (2003). The effect of dilution on the rate of hydrogen peroxide production in honey and its implications for wound healing. J. Alter. Compl. Med. 9(2):267-273.

Basson NJ, Grobler SR (2008). Antimicrobial activity of two South African honeys produced from indigenous *Leucospermum cordifolium* and *Erica* species on selected micro-organisms. BMC Compl. Alter. Med. 8:41.

Basualdo C, Sgroy V, Finola MS, Juam M, et al. (2007). Comparison of the antibacterial activity of honey from different provenance against bacteria usually isolated from skin wounds. Vet. Microbiol. 124(3-4): 375-381.

Blair SE, Carter DA (2005). The potential for honey in the management of wounds and infections. J. Austr. Infect. Control 10(1):24-31.

Bogdanov S (1984). Characterization of antibacterial substance in honey. Lebensm Wiss Technol, 17(2): 74-76.

Farouk HF, Kash SA, Khalid M, Mahasin W et al (1988). Studies on sudanese bee honey. Int. J. Crude Drug Res, 26: 161-168.

Haffejee IE, Moosa A (1985). Honey in the treatment of infantile gastroenteritis. Br. Med. J. 290:1866-1867.

Heerng W (1998). Immunochemical screening for antimicrobial drug residue in commercial honey, 123(12): 2759-2762.

Ibrahim AS (1985). Antibacterial action of honey. Bull. Islam Med, 1: 363-365.

Ilechie AA, Kwapong PK, Mate-Kole E, Kyei S, Darko-Takyi C (2012).The efficacy of stingless bee honey for the treatment of bacteria-induced on conjunctivitis in guinea pigs. J. Expt. Pharm. 4: 63-68.

Kaushik P, Goyal P, Chauhan A, Chauhan G (2009). *In vitro* evaluation of antibacterial potential of dry fruit extracts of *Elettaria cardamomum* Maton (Chhoti Elaichi). Iran. J. Pharm. Res. 9(3).

Kingsley A (2001). Supplements. The use of Honey in treatment of infected wounds. Case studies. J. Nurs. 10(22):Sup 13-Sup 20.

Lusby PE, Coombes A, Wilkinson JM (2002) Honey: A potent agent for wound healing? J. Wound Ostomy Continence Nurs. 29:295-300.

Lusby PE, Coombes AL, Wilkinson JM (2005). Bactericidal activity of different honeys against pathogenic bacteria. Arch. Med. Res. 36(5):464-467.

Molan PC (1992). The antibacterial activity of honey. Bee world 73(1):5-28.

Molan PC, Cooper RA (2000). Why honey and sugar as dressing for wound and ulcer. Trop. Doc. 4:249-258.

Namias S (2003) Honey in the management of infections. Surg. Infect (Larchmt), 4:219-226.

NCCLS (1997). National Committee for Clinical Laboratory Standards. Methods for dilution antimicobial susceptibility tests for bacteria that grows aerobically. Approved Standards, M7-A4, Wayne, Pa.

Nzeako BC, Hamdi J (2000). Antimicrobial potential of Honey. Med. Sci. 2:75-79.

Radwan SA, El-Essawy Sarhan MM et al (1984). Experimental evidence for the occurrence in honey of specific substances active against microorganisms. Zentral Mikrobiol, 39:249-255.

Shears P (2000) Antimicrobial resistance in the tropics. *Tropical Doctor*, 30(2):114-116.

Tan HT, Rahman RA, Gan SH, Halim AS, Hassan SA, Sulaiman SA, Kirnpal-Kaur BS (2009). The antibacterial properties of Malaysian tualang honey against wound and enteric microorganisms in comparison to manuka honey.BMC Complementary and Alternative Medicine 9:34 doi:10.1186/1472-6882-9-24.

World Health Organization (1999). Drug information. *WHO, Geneva,* 13(4):230-233.