UJMR, Volume 1 Number 1 December, 2016 *ISSN: 2616 - 0668*

https://doi.org/10.47430/ujmr.1611.020

Received: 7th July, 2016 **Accepted:** 5th Oct., 2016

Evaluation of Synergetic Activity of Honey and Ginger Extracts on *Pseudomonas aeruginosa* and Methicillin-Resistant *Staphylococcus aureus*

*** Idris, U. A., Tijjani, M. B. and Aliyu, M. S.**

Department of Microbiology, Ahmadu Bello University, Zaria.

*Corresponding author: usmanureyn@gmail.com, 08032826354

Abstract

This study was conducted to determine the antimicrobial and synergistic activity of honey and ginger extracts on *Pseudomonas aeruginosa* and methicillin-resistant *Staphylococcus aureus* (MRSA).Agar well diffusion method was used to determine susceptibility of the test organisms using honey and ginger extracts individually, and the Minimum Inhibitory Concentrations of the antimicrobial agents was measured using the tube dilution method. Different proportions of 10% v/v concentrations of the antimicrobials were mixed and their activity against the test organisms determined also using the agar diffusion method. The most active proportion was taken as the proportion with the widest diameter zone of inhibition. The MIC and MBC of the most active proportions on the different test organisms were then determined. Honey-ginger powder extract mixtures produced the highest inhibition compared to the use of honey or ginger extract individually. The zones of inhibitions produced by a mixture of ginger extract and honey each of 10%v/v concentration on both *Pseudomonas aeruginosa* and MRSA was higher than zones of inhibition by honey and ginger extract of the same concentration used individually. Minimum inhibitory concentration (MIC) of the most active proportions of honey-ginger extract mixtures on both *Pseudomonas aeruginosa* and MRSA was 1.25% v/v while minimum bactericidal concentrations (MBCs) of the different most active proportions were 2.5% v/v for both of the test organisms.

Keywords: Synergy, Honey, Ginger, MRSA, *Pseudomonas aeruginosa.*

INTRODUCTION

Antimicrobial agents are substances known to have therapeutic effect on microorganisms either as a control, prevention or cure of diseases with microbial origin. These antimicrobial agents are synthesized chemotherapeutic substances obtained majorly from microorganisms, plants and some animal products (Omoya and Akharaiyi, 2012). Medicinal plants, particularly their active components, have been a dependable source of therapeutics for the treatment of various ailments since time immemorial (Hemaiswarya and Doble, 2009). With the emergence of antibiotic-resistant strains of many microorganisms, the search for antimicrobial agents other than antibiotic is of great concern (Aamer *et al.,* 2015). Therefore, the importance of identifying new effective antimicrobial agents cannot be overemphasized (Ghaleb *et al*., 2009).

The medicinal importance of honey has been documented in the world's oldest medical literatures, and since the ancient times, it has been known to possess antimicrobial property as well as wound-healing activity (Manisha and Shyamapada, 2011). Currently, many researchers have reported the antibacterial activity of honey and found that natural unheated honey has some broad-spectrum antibacterial activity when tested against pathogenic bacteria, oral bacteria as well as food spoilage bacteria (Manisha and Shyamapada, 2011).

Honey possesses therapeutic potential and its antimicrobial activity is widely documented as a large number of in vitro studies confirmed its broad-spectrum antimicrobial properties either in solo (Aamer *et al.,* 2015) or in combination (Yalemwork *et al.,* 2014). No microbial resistance against honey has been observed, making it attractive as a treatment for wound infections (Cooper *et al*., 1999). Several mechanisms have been postulated for honey's antimicrobial activity, including high osmolarity (Mavric *et al.,* 2008), hydrogen peroxide activity (Manisha and Shyamapada, 2011), low pH (Haniyeh *et al.,* 2010) and the presence of flavonoids.

Ginger is the most well-known member of the family Zingibereceae (Omoya and Akharaiyi, 2012). It has long been used as naturopathy due to their potential antimicrobial activity against different microbial pathogens and has been widely used all over the world, since antiquity, for a wide array of unrelated ailments including arthritis, cramps, rheumatism, sprains, sore throats, muscular aches, pains, constipation, vomiting, hypertension, indigestion, dementia, fever and infectious diseases (Islam *et al.,* 2014). It contains the volatile oil gingerol and other pungent principles which not only give ginger its pungent aroma, but are the most medically powerful because they inhibit prostaglandin and leukotriene formation, which are products that influence blood flow and inflammation (Omoya and Akharaiyi, 2012).

Owing to the new attraction to the properties of new antimicrobial products like combating multi-drug resistant bacteria, it is important showcase the potency of local products such as honey and ginger against disease-causing organisms. Therefore, this work is meant to evaluate the activity of honey and ginger against bacteria and to analyse the type of association between the two antimicrobial agents.

MATERIALS AND METHODS

Sample Collection and Identification

Fresh ginger rhizomes were purchased from Samaru market, Sabon Gari Local Government and honey was obtained from the National Research Institute on Chemical Technology, Zaria both in Kaduna State in May, 2015. The samples were identified at the Department of Biological Sciences, Ahmadu Bello University, Zaria, and their antimicrobial activities, including honey-ginger extract mixtures, were analysed in Microbiology laboratory in Ahmadu Bello University from June to August, 2015.

Characterisation of Isolates

After collection of Clinical isolates of *Staphlococcus aureus* suspected to be methicillin-resistant, and *Pseudomonas aeruginosa* from the University Health Services, Ahmadu Bello University Main Campus, Samaru, Zaria, the organisms were both sub cultured on their respective selective media for isolation. The *Staphylococcus aureus* was sub cultured on Mannitol Salt Agar while the *Pseudomonas aeruginosa* was sub cultured on Cetrimide agar. After overnight incubation, the isolates were confirmed using cultural and biochemical characteristics (Coopper *et al.,* 2009).

Screening for Methicillin-Resistant Strains of *S. aureus*

The confirmed isolates of *S. aureus* were used to make a standard inoculum by comparison with McFarland 0.5 turbidity standard and were inoculated onto freshly prepared sterile Mueller Hinton agar plates. Commercially prepared oxacillin antibiotic discs were then aseptically placed and the plates incubated for 18-24 hours. Strains that show resistance according to CLSI standard were picked and stored on Nutrient agar slants which were to be used for the study.

Preparation of Ginger Extracts

Ginger rhizomes were washed with distilled water and sliced into small pieces using sterile knife before air-drying for about 2 weeks in the laboratory where there was sufficient air flow. The dried ginger pieces was grounded using pestle and mortar to obtain ginger powder. Ethanolic ginger extraction was done by adding 100 g of ginger powder into 500 mL ethanol.

The mixture was agitated for three hours and allowed to stand for 24 hours. It was then filtered using a Whatmann filter paper of diameter 125mm and pore size 0.7mm, the residue discarded and the filtrate evaporated to dryness over a water bath. Ginger powder water extract was also obtained using the same procedure but with distilled water in place of the ethanol (Al Tahtawy *et al.,* 2011).

Preparation of 0.5 McFarland Standards and Standardization of Inoculum Concentration

In this study, 0.5 mL of 0.048 M $BaCl_2$ (1.175% w/v $BaCl_2 2H_2O$) was added to 99.5 mL of 0.18 M H_2SO_4 (1% v/v) with constant stirring to make 0.5 McFarland Standards. The standard was distributed into a screw capped test tube for colour comparison of the test inoculums. 18-24 hour old colonies of bacteria were taken from solid media and were added into 5 mL Saline and the concentration was adjusted to $1–2\times10^8$ colony forming units per millilitre (cfu/mL) by comparing with McFarland 0.5 standard in bright light (Yalemwork *et al.,* 2014).

Preparation of Different Concentrations of the Antimicrobials

Stock solutions of the ginger extracts were prepared by aseptically weighing 1g of the extract and dissolving in 5ml of Dimethyl Sulfoxide (DMSO) to make a 20% (200mg/ml) solution. Twenty percent (20%) solution of honey was also prepared by dispersing 1ml of honey in 4ml of distilled water. Double dilutions of the antimicrobials were then carried out to obtain 10%, 5% and 2.5% concentrations of the solutions (CLSI, 2012).

Assay of Antibacterial Activities

The standardised inocula were seeded onto freshly prepared Mueller Hinton Agar plates. Five wells were then punched into the agar using a sterile cork borer (No. 7). The wells were properly labeled as 1, 2, 3, 4 and 5 to indicate the different concentrations of the antimicrobial agents to be applied. The wells were then filled as follows: Hole 1 contains 200mg/ml of ginger extract or 20% v/v concentration of honey, hole 2 contains 100mg/ml or 10% v/v, hole 3 contains 50mg/ml or 5% v/v, hole 4 contains 25mg/ml or 2.5% v/v while hole 5 contains 100µl of distilled water to serve as negative control.

The plates were allowed to stand and diffuse for about 3 hours and incubated aerobically at 37ºC for 18-24 hours. At the end of the incubation period, the culture plates were examined for evidence of bacterial growth inhibition which appeared as clear zones that were completely devoid of bacterial growth around the wells. The diameter of each zone was measured using a transparent ruler calibrated in millimetres (Mounyr *et al.,* 2016).

Determination of Minimum Inhibitory Concentration (MIC)

The MIC of the ginger extract and honey was determined using the tube dilution method. Serial dilutions of the extracts and honey were carried out in well labeled test tubes using Mueller Hinton broth as diluents. The tubes were then inoculated with 0.1ml of standard inocula and incubated for 20-24 hours at 37°C to observe turbidity (growth). The least concentration showing no visible sign of growth which gave no turbidity of the medium was taken as the MIC as compared with controls of (1) the broth with extract only and (2) broth with test organism only (Mounyr *et al.,* 2016).

Determination of Minimum Bactericidal Concentration (MBC)

The results from the MIC tubes were used to determine the MBC. The contents of all of the test tubes were inoculated onto sterile nutrient agar plate using a wire loop and the plates incubated aerobically at 37°C for about 24 hours.

The MBC value was read as the least concentration that totally killed the test organisms, which was indicated by the complete absence of growth (Mounyr *et al.,* 2016).

Demonstration of Synergetic activity

Ten percent (10%) concentrations of honey and ethanolic extract of ginger were prepared by mixing 1ml of honey with 9ml of distilled water and 1mg of the ginger ethanolic extract dispersed in 10ml of DMSO. Different proportions of the concentrations were prepared and labeled as follows:

1. A contains 90% ginger extract and 10% honey, made by mixing 2.7ml of ethanolic ginger extract with 0.3ml of honey.
2. B contains 80% ginger extract and 20% honey, made by mixing 2.4ml of ethanolic ginger extract with 0.6ml of honey.
3. C contains 70% ginger extract and 30% honey, made by mixing 2.1ml of ethanolic ginger extract with 0.9ml of honey.
4. D contains 60% ginger extract and 40% honey, made by mixing 1.8ml of ethanolic ginger extract with 1.2ml of honey.
5. E contains 50% ginger extract and 50% honey, made by mixing 1.5ml of ethanolic ginger extract with 1.5ml of honey.
6. F contains 40% ginger extract and 60% honey, made by mixing 1.2ml of ethanolic ginger extract with 1.8ml of honey.
7. G contains 30% ginger extract and 70% honey, made by mixing 0.9ml of ethanolic ginger extract with 2.1ml of honey.
8. H contains 20% ginger extract and 80% honey, made by mixing 0.6ml of ethanolic ginger extract with 2.4ml of honey.
9. I contains 10% ginger extract and 90% honey, made by mixing 0.3ml of ethanolic ginger extract with 2.7ml of honey.

Ten wells were punched into the agar plate using a sterile cork borer (No. 7). The wells were properly labeled as A, B, C, D, E, F, G, H, I and J to indicate the different proportions of the antimicrobial agents to be applied. The wells were filled accordingly with the right proportions with hole J containing 100µl of distilled water to serve as negative control. The plates were allowed to stand and diffuse for about 3 hours and then incubated aerobically at 37ºC for 18-24 hours. At the end of the incubation period, the culture plates were examined for evidence of inhibition which appeared as clear zones that were completely devoid of bacterial growth around the wells. The diameter of each zone was measured using a transparent ruler calibrated in millimetres. The most active proportion for each organism was taken as the proportion with the widest zone of inhibition.

Minimum Inhibitory and Minimum Bactericidal Concentrations of the Most Active Proportions

The MIC of the most active proportion was determined using the tube dilution method. Serial dilutions of the proportion were carried out in well labeled test tubes using Mueller Hinton broth as diluent. The tubes were then inoculated with 0.1ml of standard inocula and incubated for 20-24 hours at 37°C to observe turbidity (growth). The least concentration of the most active proportion showing no visible sign of growth which gave no turbidity of the medium was taken as the MIC as compared with controls of (1) the broth with antimicrobial mixture only and (2) broth with test organism only.

The contents of all of the test tubes were inoculated onto sterile nutrient agar plate using a wire loop and the plates incubated aerobically at 37°C for about 24 hours. The MBC value was read for the least concentration that totally killed the test organisms, which was indicated by the complete absence of growth (Mounyr *et al.,* 2016).

RESULTS

Antibacterial Activity Profile of the Antimicrobial Agents

The results show that the antimicrobial activity increases with increase in concentration for the honey and ethanolic extract of ginger while the aqueous extract has no activity against the test organisms as shown in table 1.

Table 1: Antibacterial Activity of the Ethanolic Ginger Extract

Organisms	Ethanolic ginger extract concentration (mg/ml)/ Zones of inhibition (mm)				Aqueous ginger extract concentration (mg/ml)/ Zones of inhibition (mm)				Honey concentration(% v/v)/ Zones of inhibition (mm)			
	200	**100**	**50**	**25**	**200**	**100**	**50**	**25**	**20**	**10**	**5**	**2.5**
MRSA	16	13	12	0	0	0	0	0	18	14	14	0
P. aeruginosa	13	12	12	0	0	0	0	0	15	13	14	0

Minimum Inhibitory Concentrations of the honey and Ginger Extracts

The minimum inhibitory concentration results show that a higher concentration (25mg/ml) of ethanolic ginger extract was required to inhibit the growth of MRSA than *Pseudomonas aeruginosa* (12.5mg/ml). The results also show that the same concentration (2.5%v/v) of honey is needed to inhibit both organisms. The MBC of honey was found to be 5% v/v for both organisms while the MBC of ethanolic ginger extract on MRSA and *Pseudomonas aeruginosa* were found to be 50mg/ml and 25mg/ml respectively.

Minimum Bactericidal Concentration of the Honey and Ginger Extract

After sub culturing the contents of all the tubes, the MBC of honey was found to be 5% v/v for both organisms while the MBC of ethanolic ginger extract on MRSA and *Pseudomonas aeruginosa* was found to be 50mg/ml and 25mg/ml respectively.

Antimicrobial Activities of Different Proportions of 10% Concentrations of Honey and Ethanolic Extract of Ginger

The result of the activity of the different proportions on the isolates shows that MRSA is most susceptible to a mixture of 70% ethanolic ginger extract and 30% honey with a zone of inhibition of 21 mm, while a 60% ginger and 40% honey combination is most active against *Pseudomonas aeruginosa* as shown in the figure below:

Figure 1: Antimicrobial Activities of Mixtures of Honey and Ethanolic Ginger Extract on *Pseudomonas aeruginosa* and Methicillin-Resistant *Staphylococcus aureus*

MIC and MBC of the most Active Proportions

Even though different proportions of the mixtures were active on the organisms, the MIC and MBC of the most active proportions were 1.25% v/v and 2.5% v/v respectively for both organisms.

DISCUSSION

The ginger powder water extract did not show bacterial growth inhibition on the test organisms as it has already been reported by Malu *et al* (2009) and Yalemwork *et al* (2014). The highest inhibition (16 mm) produced by the ginger powder extract on methicillin-resistant *S. aureus* was less than 30 mm for *S. aureus* using fresh ginger rhizome ethanol extract in similar study by Sebiomo *et al* (2011). This difference could be explained by the loss of water soluble antioxidant volatile oils from the ginger powder up on dehydration (Al-Tahtawy *et al.,* 2011; Roy *et al.,* 2006). Despite the loss of some antibacterial agents by evaporation during making ginger powder, antibacterial agents extracted by the organic solvents were enough to produce inhibition on both *P. aeruginosa* and methicillin-resistant *S. aureus*.

The zone diameter of inhibition (ZDI) produced by the honey sample in this study ranges from 14-18mm in 5-20% concentration for MRSA and 14-15mm for *Pseudomonas aeruginosa* as has been determined by Badawy *et al* (2004) using same concentration of honeys against *E. coli* O157: H7 (12 mm – 24 mm) and *S. typhimurium* (0 mm – 20 mm). Rajeswari *et al* (2010) also found the ZDIs of Nilgiris honeys to be (20–21) mm, (15-16) mm and (13–14) mm for *S. aureus*, *P. aeruginosa* and *E. coli*, respectively.

Antimicrobial activities (antibacterial, antiviral, antifungal, and ant parasitic) of honeys were reported due to high osmolality, acidity, hydrogen peroxide, and phytochemicals (Taormina *et al.,* 2001; Ndip *et al.,* 2007; Tanih *et al.,* 2009; Manyi-Loh *et al.,* 2010). In vivo use of honey for human as therapeutic agent depends on the evaluation of the non-peroxide phytochemical components of honey as hydrogen peroxide can be destroyed by catalase in the body tissues and serum (Yalemwork *et al.,* 2014).

Similarly, the high osmolality and acidity of honeys are destroyed in the digestion system or blood circulation of human. The non-peroxide phytochemical components of Manuka Apinae honey (after removing hydrogen per oxide by treating with enzyme catalase) from New Zealand have been found to have substantial levels of antibacterial activity (Iurlina and Rosalia, 2005). Such manuka honey was tested against seven species of bacteria and was found to have MIC (Minimum Inhibitory Concentration) that range from 1.8% to 10.8% (v/v) (Willix *et al.,* 1992) which is in consistency with the MIC found for MRSA (2.5%) and *Pseudomonas aeruginosa* (2.5%) in this study.

The highest (21mm) and the mean (17.06mm) of inhibitions produced by honey-ginger extract mixtures on MRSA was greater than the inhibitions produced by same concentration of ginger (13mm) and honey (14mm) used individually. Likewise the highest (23mm) and the mean zone of inhibition (18.44 mm) produced by the mixtures on *Pseudomonas aeruginosa* was greater than the zones of inhibition cleared by the same concentration of ginger extract (12mm) and honey (13mm). The inhibitions of ginger powder ethanol extract was enhanced by mixing with honey due to the synergetic antibacterial effects of honey-ginger extract mixtures as already reported by Omoya and Akhairaiyi (2011) and Yalemwork *et al.* (2014). The ginger powder ethanol extracts were positive for known antimicrobial agents such as saponin, alkaloids, phlobatannin, flavonoids, and cardiac glycosides according to Omoya and Akharaiyi (2011).

The increased activity of the antimicrobial agents when used in combination was accounted for by the synergetic activity between the antimicrobial agents as reported by earlier researchers.

CONCLUSION

The result of this study showed that honey-ginger extract mixtures have the potential to serve as cheap source of antibacterial agents especially for drug resistant bacteria strains.

REFERENCES

Aamer, A. A., Abdul-Hafeez, M. M. & Sayed, S. M. (2015): Minimum Inhibitory and Bactericidal Concentrations (MIC & MBC) of Honey and Bee Propolis against Multidrug Resistant (MDR) *Staphylococcus* Sp. Isolated from Bovine Clinical Mastitis: *Global Journal of Science Frontier Research: D Agriculture and Veterinary*: **15**(2): 21-28

Al-Tahtawy, R.., El-Bastawesy, Monem, A., Zekry, M., Z., Al- Mehdar, H. and El-Merzabani, M., (2011): Antioxidant activity of the volatile oils of *Zingiber officinale* (ginger): *Spatula DD:* **1**(1): 1–8.

Badawy, O. F. H.; Shafii, S. S. A.; Tharwat, E. E., Kamal, A. M.: (2004): Antibacterial activity of bee honey and its therapeutic usefulness against *Escherichia coli* O157:H7 and *Salmonella typhimurium* infection: *Rev Science and Technology International*: 23: 1011–1122.

CLSI, Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically, approved standard, 9th edition, CLSI document M07-A9. Clinical and laboratory standards institute, 950 West Valley Road, Suite 2500, Wayne, Pennsylvania 19087, USA.

Cooper, R. A., Molan, P. C., and Harding, K. G. (1999): Antibacterial activity of honey against strains of *Staphylococcus aureus* from infected wounds: *Journal of the royal society of medicine:* **92**(6): 283-285

Ghaleb, M. A., Bassam, A. A. and Kamel M. A. (2009): *In vitro* activity of certain drugs in combination with plant extracts against *Staphylococcus aureus* infections: *African Journal of Biotechnology*: **8**(17): 4239-4241

Haniyeh K, Seyyed MS, Hussein M. Preliminary study on the antibacterial activity of some medicinal plants of Khuzestan (Iran): *Asian Pac J Trop Med*. 2010: **3**(3):180–184.

Hemaiswarya, S. and Doble, M. (2009): Synergistic interaction of eugenol with antibiotics against Gram negative bacteria: *Phytomedicine*: **16**(2009) 997–1005

Islam, K., Asma, A. R., Md. Murad K. and Md. Shahidul K. (2014): Antimicrobial activity of ginger (*Zingiber Officinale*) extracts against food-borne pathogenic bacteria: *International Journal of Science, Environment and Technology*: **3**(3): 867 – 871

Iurlina M. & Rosalia F., (2005): Characterization of microorganisms in Argentinean honeys from different sources: *International Journal of Food Microbiology*: **105**(3): 297–304.

Malu, S. P., Obochi, G. O., Tawo, E. N and. Nyong, B. E (2009): Antibacterial activity and medicinal properties of Ginger (*Zingiber officinale*): *Global Journal of Pure and Applied Sciences*: **15**(3): 365–368

Manisha Deb Mandal and Shyamapada Mandal (2011): Honey: its medicinal property and antibacterial activity: *Asian Pac J Trop Biomed*: **1**(2): 154-160

Manyi-Loh C. E., Clarke A. M., and Ndip R. N., (2011): An overview of honey: therapeutic properties and contribution in nutrition and human health: *African Journal of Microbiology Research*: **5**(8): 844–852.

Mavric E, Wittmann S, Barth G, and Henle T. (2008) Identification and quantification of methylglyoxal as the dominant antibacterial constituent of manuka (*Leptospermum scoparium*) honeys from New Zealand. *Mol Nutr Food Res*.; **52**(4):483–489

Mounyr B, Moulay S. And Saad K. I. (2016): Methods for *in vitro* evaluating antimicrobial activity: a review. *Journal of Pharmaceutical Activity* **6**(2): 71-79.

Ndip R. N., Malange Takang A. E., Echakachi C. M. et al., (2007): In-vitro antimicrobial activity of selected honeys on clinical isolates of *Helicobacter pylori*: *African Health Sciences*: **7**(4): 228–232.

Omoya, F. and Akharaiyi F.: (2011): Mixture of honey and ginger extract for antibacterial assessment on some clinical isolates: *International Journal of Pharmaceutical and Biomedical Research* **2**(1): 39–47.

Omoya, F. O. and F.C. Akharaiyi, F. C. (2012): Mixture of Honey and Ginger Extract for Antibacterial Assessment on Some Clinical Isolates: *Int. Res. J. of Pharmaceuticals*: **02**(05): 127-132

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

Roy, J., Shakleya, D. M., Callery P. S. and Thomas J. G., (2006): Chemical constituents and antimicrobial activity of a traditional herbal medicine containing garlic and black cumin: *African Journal of Traditional, Complementary and Alternative Medicines* **3**(2): 1–7.

Sebiomo, A., Awofodu, A. D., Awosanya, A. O., Awotona, F. E. and Ajayi A. J., (2011): Comparative studies of antibacterial effect of some antibiotics and ginger (Zingiber officinale) on two pathogenic bacteria: *Journal of Microbiology and antimicrobials*: **3**: 18–22.

Tanih, N. F., Dube C., E., Green (2009): An African perspective on *Helicobacter pylori:* relevance of human infection, drug resistance, and alternative approaches to treatment: *Annals of Tropical Medicine and Parasitology*: **103**(3): 189–204.

Taormina, P. J., Niemira, B. A., and Beuchat, L. R.: (2001): Inhibitory activity of honey against food borne pathogens as influenced by the presence of hydrogen peroxide and level of antioxidant power: *International Journal of Food Microbiology***: 69**(3): 217–225.

Willix D. J., Molan P. C., and Harfoot C. G.: (1992): A comparison of the sensitivity of wound-infecting species of bacteria to the antibacterial activity of manuka honey and other honey: *Journal of Applied Bacteriology* **73**(5): 388– 394.

Yalemwork, E., Wossenseged,L. and Nega, B., (2014): Synergetic Antimicrobial Effects of Mixtures of Ethiopian Honeys and Ginger Powder Extracts on Standard and Resistant Clinical Bacteria Isolates: *Evidence-Based Complementary and Alternative Medicine*: vol. 2014, Article ID 562804, 8 pages.