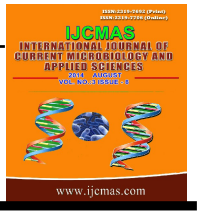
International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 3 Number 8 (2014) pp. 312-325**
http://www.ijcmas.com



Original Research Article

Synergistic Antibacterial Activity of Egyptian Honey and Common Antibiotics against Clostridium Reference Strains

Ahmed Hegazi[1]*, Sherein I. Abd El-Moez[2], Amr M. Abdou[2] and Fyrouz Abd Allah[3]

[1]Department of Zoonotic Diseases, National Research Center, Dokki, Giza, Egypt
[2]Department of Microbiology and Immunology, National Research Center, Dokki, Giza, Egypt
[3]Department of Parasitology and animal diseases, National Research Center, Dokki, Giza, Egypt
**Corresponding author*

Keywords

Antimicrobial activity, Synergistic antibacterial effect, *Clostridium acetobutylicum*, *Clostridium perfringens*, Well diffusion assay, Disk diffusion method.

A B S T R A C T

The aim of the present study was to evaluate the synergistic antibacterial effect between the two Egyptian honey brands, sesame and eucalyptus, and common antibiotics against clostridium reference strains including *Clostridium acetobutylicum* DSM1731 and *Clostridium perfringens* (KF383123). Sesame and eucalyptus honey exhibited a potent antibacterial activity against DSM1731 and KF383123 when used alone. The combined effect of honey and antibiotics against clostridial strains was found to be in general advantageous when compared to the antibiotic alone. Sesame honey and Cefotaxime (30 µg/disc) CTX showed a great synergistic effect with an increase in zone of inhibition (ZI) of 40.67±0.67 mm against *C. perfringens* when compared with 29.16±0.60 mm and 8.67±0.33 mm for honey alone and antibiotic alone respectively. Sesame honey also exhibited a clear synergistic effect against *C. acetobutylicum* when combined with CTX, Ciprofloxacin (5µg/disc) CIP and Tobramycin (30 µg/disc) TOB. Eucalyptus honey also showed a clear synergistic effect when combined with CTX, CIP, Cephalexin (Cephem/Cephalosporin I) (10 µg/disc) CN, TOB and Sulphamethoxazole (100 µg/disc) RL against *C. perfringens*. Meanwhile, eucalyptus honey did not show any synergistic effect when combined with antibiotics against *C. acetobutylicum.* Interestingly, the antibacterial effect of honey alone showed to be more effective in some cases than both the antibiotic alone and the combination of honey and antibiotic. The total flavonoid content in sesame honey was 3.16 mg/100g while it was 7.23 mg/100g for eucalyptus honey. The results revealed that sesame and eucalyptus honey can be used for the development of potent and novel antibacterial agents that can be used either separately or in synergistic combination with commonly used antibiotics to safely enhance their antibacterial activity as well as to overcome the growing problem of antibiotic resistance.

Introduction

Clostridium is the second largest bacterial genera next to *Streptomyces* and it is classified as Gram-positive endospore-forming obligate anaerobes (Andreesen et al., 1989, Rehner and Samuels 1994; Garrity 2005). Many species of *Clostridium* are known to cause a broad spectrum of human and animal disease (Miyakawa et al., 2007; ESR, 2010). On the other hand, some species are of biotechnological importance

including *C. acetobutylicum* which is used for solvent production (Jones and Woods, 1986). There are five types of *C. perfringens* based on toxin type including A, B, C, D, and E. Most *C. perfringens* food poisoning cases reported in developed countries are caused by *Clostridium perfringens* type A strains (Bates and Bodnaruk, 2003). Antibiotic resistance of *C. perfringens* strains to antibiotics are becoming a major health concern. The intestine is an environment that favors *C. perfringens* to multiply and sporulation. *Clostridium perfringens* enterotoxin (CPE) is expressed during the sporulation of cells in the small intestines, after CPE binds to intestinal epithelial and cause damage to intestinal cells, which is clinically manifest as diarrhea (Veshnyakova *et al.,* 2010).

Honey has been used in medical practice since ancient times (Hegazi, 1998, Ayaad et al., 2009 and Richard, 2009). The therapeutic use of honey has been rediscovered by medical provincial as it inhibits both Gram-positive and Gram-negative bacteria (Hegazi, 2011; Hegazi and Abd Allah, 2012; Khalil et al., 2001)**.** Honey exhibits several biological activities (Molan, 2002) including antioxidant (Hegazi and Abd El-Hady, 2009). It was also used for the treatment of burns and wounds (Snow and Manley-Harris, 2004; Brudzynski, 2006; Mullai and Menon, 2007), post-surgical wound infection (Namias, 2003), ulcers and bed sore (Brudzynski, 2006 and Tousson et al., 1997), bacterial gastroenteritis in infants (Haffeejee and Moosa 1985) and liver diseases (Yoirish, 1977). The antibacterial activity of different honey was of specific interest for many authors (Kwakman et al., 2010; Halawani and Shohayeb, 2011; Hegazi, 2011; Abd El-Moez et al., 2013). The aim of the present investigation was to evaluate the synergistic antibacterial effect of sesame and eucalyptus honey brands and seven reference antibiotics against *Clostridium acetobutylicum* DSM1731 and *Clostridium perfringens* KF383123 reference strains using agar well diffusion assay.

Materials and Methods

Honey samples

Two monofloral honey brands including sesame and eucalyptus were obtained from apiary farm in Egypt. Honey samples were stored at 5 ^{o}C in dark glass container to prevent photo degradation until being used (Pimentel et al., 2004).

Preparation of microbial suspensions

A total of two clostridium reference strains were used in this study including *Clostridium acetobutylicum* DSM1731 and *Clostridium perfringens* KF383123. A suspension of each bacterial strain was freshly prepared by inoculating fresh stock culture from the tested reference strain into broth tube containing 7 ml of Muller Hinton Broth. The inoculated tubes were incubated anaerobically at 37°C for 24 h. Serial dilutions were carried out for each strain and dilution matching with 0.5 Mc-Farland scale standard was selected for the screening of antimicrobial activities.

Antibiotic sensitivity testing (AST)

Seven common antibiotics were used as reference in this study including Cefotaxime (30 µg/disc) CTX, Ciprofloxacin (5µg/disc) CIP, Erythromycin (15 µg/disc) E, Oxytetracycline (30 µg/disc) OT, Cephalexin (Cephem/Cephalosporin I) (10 µg/disc) CN, Tobramycin (30 µg/disc) TOB and Sulphamethoxazole (100 µg/disc) RL. Antibiotic susceptibility was determined using disc diffusion method according to the

guidelines published by the British Society for Antimicrobial Chemotherapy (BSAC) standardized disc susceptibility testing method (Andrews, 2007), except that Mueller-Hinton agar (MHA; Oxoid, Cambridge, UK) was used in place of iso sensitest agar (Poilane et al., 2007).

A volume of 100 µl of cell culture suspension matching with 0.5 Mc-Farland of reference strains; *Clostridium acetobutylicum* DSM1731 and *Clostridium perfringens* KF383123 were spread onto Muller Hinton agar plates. Antibiotic discs were firmly applied to the surface of agar with a maximum of 6 discs for a 90 mm plate. To investigate the antibiotic activity against tested reference strains, plates were left for 1 h at 25 °C to allow a period of pre-incubation diffusion in order to minimize the effects of variation in time between the applications of different discs. The plates were re-incubated anaerobically at 37 °C for 24 h to allow bacterial growth. After incubation, plates were observed and the zones of inhibition were measured to evaluate the antimicrobial activity for each of the tested antibiotics. The experiment was carried out in triplicates for statistical relevance and the Mean±SE of the results was calculated.

Antimicrobial activity of pure monofloral honey brands using agar-well diffusion method

The antimicrobial activity of honey against bacterial strain was evaluated by using agar-well diffusion method (Katirciolu and Mercan, 2006)**.** A volume of 100 µl of cell culture suspension matching with 0.5 Mc-Farland of target isolate was spread onto the plates. To investigate the antibacterial activity, 50 µl of different honey samples were added in individual wells. Plates were left for 1 h at 25 °C to allow a period of pre-incubation diffusion in order to minimize the effect of variation in time between the applications of different solutions. The plates were re-incubated anaerobically at 37 °C for 24 h to allow bacterial growth. After incubation, plates were observed and the zones of inhibition were measured to evaluate the antimicrobial activity for each of the tested honey samples. The experiment was carried out in triplicates for statistical relevance and the Mean± SE of results was calculated.

Testing for synergistic antibiotic and honey combinations by AST

To evaluate the combined antimicrobial activity of antibiotics and honey to check if there is any synergistic activity, disc diffusion tests were repeated on MHA. Antibiotic disks used for sensitivity test were saturated with 50µl of either sesame or eucalyptus honey. The same procedure as in AST was applied. The experiment was carried out in triplicates for statistical relevance and the Mean± SE of results was calculated. The resulted means were compared with both the means obtained when both types of honey were used alone as well as the means obtained from antibiotic discs alone to check the presence of synergism.

Measurement of Total Flavonoid Content Using Folin-Ciocalteu Assay

Total phenolic contents of the two honey brands were determined spectrophotometrically according to the Folin-Ciocalteu colorimetric method (Singleton et al., 1999)**.** Total flavonoid content was determined using the method of Meda et al. (2005) with minor modifications. In brief, 0.25 mL of sample (0.1 mg/mL) was added to a tube containing 1 mL of double-distilled water followed by

0.075 mL of 5% $NaNO_2$, 0.075 mL of 10% $AlCl_3$ and 0.5 mL of 1 M NaOH at 0, 5 and 6 min, sequentially. Finally, the volume of the reaction solution was adjusted to 2.5 mL with double-distilled water. The absorbance of the solution was measured at 410 nm wave length using a spectrophotometer. Caffeic acid, a ubiquitous flavonoid was used as a standard to quantify the total flavonoid content of honey and the results were expressed in microgram Catechin Equivalents (CE) mg/100g honey.

Statistical analysis

The *in vitro* antibacterial activity was conducted in triplicates. The data were then subjected to SPSS Ver. 21(IBM, New York, US) software for statistical analysis. Duncan Test of Post Hoc Multiple Comparisons in one way ANOVA was applied for comparison between and within the groups. All the data were given in mean± standard error (SE). A probability value $P<0.05$ was taken as significant (Steel and Torrie, 1980).

Results and Discussion

The antibacterial activity of the two brands of Egyptian honey, sesame and eucalyptus were evaluated according to the following criteria: zone of inhibition range >18 showed significant activity, 16-18 good activity, 13-15 low activity, 9-12 non-significant activity, and <8 no activity. Sesame and eucalyptus branded honey exhibited significant activities against *C. acetobutylicum* DSM1731 with zones of inhibition 18.33±0.88 mm and 25.00±0.58 mm, respectively. While only sesame honey was significantly effective against *C. perfringens* KF383123 strain with zone of inhibition reaching 29.16±0.60 mm. On the contrary, eucalyptus honey showed non-significant activity against *C. perfringens* KF383123 with zone of inhibition of 9.00±0.58 mm as shown in (Table 1 and Chart 2).

Antibiotic sensitivity test was carried out to detect the antimicrobial activities of seven reference antibiotics; Cefotaxime, Ciprofloxacin, Erythromycin, Oxytetracycline, Cephalexin, Tobramycin and Sulphamethoxazole against tested reference strains; *C. acetobutylicum* DSM1731 and *C. perfringens* KF383123 as well as the synergistic effect of the sesame honey and eucalyptus honey when added individually to the reference antibiotics.

The results revealed that CIP showed the best antimicrobial activities with zone of inhibition of 18.00±0.58 mm against both tested reference strains (Table 1, Chart 1 and Chart 2). The activity of CIP was followed by CN with inhibition zone of 16.00±0.58 and 17.00±0.58 against *C. acetobutylicum* DSM1731 and *C. perfringens* KF383123, respectively. Then TOB exhibited lower activities with ZI of 11.33±0.88 mm and 10.00±0.58 mm respectively. This was followed by RL and CTX with ZI of 8.00±0.58 mm and 10.33±0.88 mm for RL against DSM1731 and KF383123 respectively and 8.33±0.33 mm and 8.67±0.88 for CTX against DSM1731 and KF383123 respectively. Meanwhile, antibiotics E and OT showed no hindrance activities against tested strains.

The results of synergistic activity of sesame and eucalyptus honey were presented in Table (1) as well as Chart (1) and Chart (2). A combination of honey and antibiotic was considered synergistic when the scored ZI for the combination is bigger than ZI of honey and antibiotic separately. The results revealed that the addition of sesame honey showed great synergistic effect with CTX with an increase of inhibition zone against *C. perfringens* KF383123, from 29.16±0.60

mm for sesame honey alone and 8.67±0.33 mm for CTX alone to 40.67±0.67 mm for the combination. Although the combination of sesame honey and CN caused an increase in the zone of inhibition from 17.00±0.58 mm for CN alone to 26.00±0.58 mm for the combination against *C. perfringens* KF383123, this combination is not considered synergistic. as the antimicrobial activity of sesame honey alone scored ZI of 29.16±0.60 mm which is bigger than that of the combination.

Similar results were recorded for the combination of sesame honey with CIP and TOB, with ZI of 24.00±0.58 mm for both combinations when compared with CIP and TOB alone, with ZI of 18.00±0.58 mm and 10.00±0.58 mm, respectively against *C. perfringens* KF383123. Both OT and E alone were not effective against tested strains but when combined with sesame honey, an increase they scored ZI of 29.33±1.85 mm and 23.00±0.58 mm respectively against *C. acetobutylicum* DSM1731 and 17.00±0.58 mm and 15.00±0.00 mm, respectively against *C. perfringens* KF383123. This effect could be totally due to sesame honey alone.

The results revealed the presence of synergistic effect between eucalyptus honey and CTX, CN, TOB and RL against *C. perfringens* KF383123 with an increased ZI of 10.00±0.58 mm, 20.33±0.88 mm, 12.00±1.15 mm and 24.00±1.15 mm respectively compared with ZI of 8.67±0.33 mm, 17.00±0.58 mm, 10.00±0.58 mm and 10.33±0.88 mm respectively for antibiotics alone and 9.00±0.58 mm for eucalyptus honey alone.

Although, eucalyptus honey did not show any synergistic effect with antibiotics against *C. acetobutylicum* DSM1731, eucalyptus honey alone scored higher ZI than those scored by individual antibiotics. On the other hand sesame honey exhibited a synergistic effect against *C. acetobutylicum* when combined with the antibiotics CTX, CIP and TOB with ZI of 21.33±0.88 mm, 23.00±0.58 mm and 22.00±0.58 mm respectively as compared with 8.33±0.33 mm, 18.00±0.58 mm and 11.33±0.88 mm for antibiotics alone respectively and ZI of 18.33±0.88 mm for sesame honey alone.

Quantitative determination of the total flavonoid content was done photo metrically using Caffeic acid as a standard. The total flavonoid content in sesame honey was 3.16 mg/100g and the total flavonoid content in eucalyptus honey was 7.23 mg/100g.

Honey has been principally used for its antibacterial effects since ancient times (Zumla and Lulat, 1989; Hegazi, 1998). It was believed that honey could be used in the topical treatment of wounds and burns due to its antibacterial and wound healing promotion activity (Khan et al.,, 2007; Wijesinghe et al., 2009). In the present study, the antibacterial activities of sesame and eucalyptus honey obtained from apiary farm in Egypt were estimated against *C. acetobutylicum* DSM1731 and *C. perfringens* KF383123 strains. The results revealed that sesame and eucalyptus branded honey exhibited significant activities against *C. acetobutylicum* DSM1731. Only sesame honey was significantly effective while eucalyptus honey showed non-significant activity against *C. perfringens* KF383123. Researchers have failed to point out the active ingredient responsible for the antibacterial activities of honey. Over 100 substances were found to be candidates for such antibacterial activity (Simon et al., 2009). While antibiotics destroy bacteria by attacking the cell wall honey works in a different way. Honey is hygroscopic, meaning that it draws moisture out of the

environment and dehydrates and prevents the growth of bacteria with the aid of its hyperosmolar properties (Khan et al., 2007; Simon et al., 2009; Molan, 2006). Furthermore, honey has a mean pH of 4.4, so the acidification of honey can reduce bacterial colonization (Molan, 1992; Rushton, 2007; Schneider et al., 2007). Beside the low pH, other factors that contribute to antimicrobial activities of honey include the high sugar concentration, hydrogen peroxide, methylglyoxal and the antimicrobial peptide bee defensin-1 (Kwakman and Zaat., 2012). It was found that both hydrogen peroxide and the non-peroxide components contribute to the bacteriostatic and bactericidal activity of honey. Also, H_2O_2 in honey was involved in oxidative damage causing bacterial growth inhibition and DNA degradation, but these effects were modulated by other honey components (Brudzynski et al., 2011).

Antibiotic sensitivity test was carried out to detect the antimicrobial activities of seven reference antibiotics; Cefotaxime, Ciprofloxacin, Erythromycin, Oxytetracycline, Cephalexin, Tobramycin and Sulphamethoxazole against tested reference strains; *C. acetobutylicum* DSM1731 and *C. perfringens* KF383123 as well as the synergistic effect of sesame honey and eucalyptus honey when combined individually with each of the reference antibiotics. The results revealed that CIP showed the best antimicrobial activities against both tested reference strains followed by CN, TOB, RL and CTX. While both E and OT showed no hindrance activities against tested strains. Though there is hardly any data on antibacterial effect of sesame and eucalyptus honey against *C. acetobutylicum* and *C. perfringens*, several studies investigated the antibacterial activity of several honey brands against different bacterial strains, which can be used for comparison with our data. The MIC and MBC of Manuka honey for three *C. difficile* strains were investigated by Hammond and Donkor, 2013. The MIC values of the three *C. difficile* strains were the same (6.25% v/v). Similarly, MBC values of the three *C. difficile* strains were the same (6.25% v/v). Cooper et al., 1999 reported the antibacterial activity of Manuka honey against 58 isolates of *S.aureus*. In another report, Cooper and Mulan., 1999 determined the MIC of Manuka honey for 20 strains of *P. aeruginosa*. Furthermore, Cooper et al., 2002 proved medium level of activity of Manuka honey against 17 strains of *P. aeruginosa.* Wilkinson and Cavanagh, 2005 reported that Manuka honey was effective against many organisms including *S. aureus, E. coli, S. typhimurium* and *P. mirabilis*. In general, earlier studies indicated that antimicrobial activities of honey are among several health beneficial effects of honey (Tan et al., 2009; Hegazi, 2011; Hegazi and Abd Allah, 2012; Koc et al., 2011). The antimicrobial activities of sesame and eucalyptus honey may be due to the presence of sugars such as monosaccharides, disaccharides, oligosaccharides and polysaccharides (Bogdanov et al., 2008; Erejuwa et al., 2012a; Erejuwa, et al., 2012b). Earlier studies also indicated that honey contains enzymes such as glucose oxidase, diastase, invertase, catalase and peroxidase (Bogdanov et al., 2008) and these enzymes may play an important role in the antimicrobial activity of honey. Also, honey contains other bioactive constituents such as organic acids, ascorbic acid, trace elements, vitamins, amino acids, proteins and Maillard reaction products (Bogdanov et al., 2008).

These results indicated that the total flavonoid contents varied considerably between the two types of honey. The total flavonoid content in sesame honey was 3.16

mg/100g and the total flavonoid content in eucalyptus honey was 7.23 mg/100g. Honey was found to contain a great variety of minor components, including phenolic acids and flavonoids, the enzymes glucose oxidase and catalase, ascorbic acid, carotenoids, organic acids, amino acids, proteins, and α-tocopherol (Ferreres et al., 1993). Although the actual composition of honey varies, depending on many factors such as the pollen source, climate and environmental conditions (Gheldof et al., 2002; Azeredo et al., 2003), the phenolic compounds in their many forms are the main components responsible for the functional properties, such as antioxidant capacity (Kerem et al., 2006; Almaraz-Abarca et al., 2007; Hegazi and Abd el Hady, 2009), antibacterial capacity (Huang et al., 2006; Theodori et al., 2006; Hegazi and Abd Allah 2012), and antiviral capacity (Evers et al., 2005; Ozcelik et al., 2006).

Synergistic effect of honey brands with tested antibiotics against *C. acetobutylicum* and *C. perfringens* were evaluated. The results revealed that the combination of sesame with CTX showed great synergistic antibacterial effect against *C.perfringens* KF383123, The combination of sesame honey with CTX, CIP, and TOB was also synergistic against *C. acetobutylicum*. On the other hand combinations of eucalyptus honey with CTX, CIP, CN, TOB and RL was synergistic against *C.perfringens* KF383123. Meanwhile, there was no synergistic antibacterial effect for the combination of eucalyptus honey with any of the antibiotics against *C. acetobutylicum.*

Recently synergistic action between piperacillin and methylglyoxal (an antibacterial component characteristically found in manuka honey) was demonstrated by disc diffusion method and checkerboard procedure against multi-drug resistant (MDR) clinical isolates of *P. aeruginosa* (Mukherjee et al., 2011). Synergistic combinations between methylglyoxal and carbenicillin as well as amikacin were also noted against *P. aeruginosa*. Furthermore, synergy between oxacillin and manuka honey in the inhibition of methicillin-resistant *Staphylococcus aureus* (MRSA) has been reported (Jenkins and Cooper, 2012). Manuka honey, therefore, seems to offer real potential in providing novel synergistic combinations with antibiotics for treating wound infections of MDR bacteria. In this study a selection of antibiotics which affect a wide variety of cellular target sites was tested for synergistic activity with medical grade Manuka honey in order to identify novel therapies and five combinations were identified.

It has been also shown that combinations of antibiotics with non-antibiotic substances can enhance the efficacy of a number of currently used antibiotics by forming synergetic combinations (Ejim et al., 2011; Jayaraman et al., 2010). Many natural compounds have previously been shown to have potential to inhibit antibiotic resistance in bacteria (Gibbons, 2008).

Three antibiotics, from an initial selection of fifteen antibiotics proved to be synergistic in combination with Manuka honey against MRSA and three were additive against *P. aeruginosa*. One combination (Manuka honey and tetracycline) exhibited enhanced activity against the tested bacteria.

Another two research groups have reported synergy between gentamicin and honey (Karayil et al., 1998; Al-Jabri et al., 2005). It is likely that the botanical origin of honey influences its biological activity because different antibacterial components have been identified in different honey sample (Kwakman *et al.,* 2011). This fact confirms the importance of selecting an appropriate honey for specific antibacterial use.

The use of antibiotics exerts selection pressure that favours the emergence of mutants with antibiotic resistance determinants. While training experiments with honey indicated that bacteria failed to manifest resistance to honey in the laboratory (Blair et al., 2009; Cooper et al., 2010). It can be postulated that combinations of antibiotic and honey would be less likely to encourage the emergence of MRD bacteria than antibiotics alone. These findings also highlight the great importance and usability of synergistic activities of sesame and eucalyptus honey when combined with antibiotics against clostridial strains. Further studies that involve the evaluation of the antibacterial activity of honey *in vivo* are of great importance to underline the effect of honey on the host immune system during infection.

Table.1 Antibacterial activity of sesame and eucalyptus honey separate and combined against *Cl. acetobutylicum* (DSM1731) and *Cl. perfringens* (KF383123)

Antibacterial agent	*Cl. acetobutylicum* (DSM1731)	*Synergistic*	*Cl. perfringens* (KF383123)	*Synergistic*
Sesame	18.33±0.88	-	29.16±0.60	-
Eucalyptus	25.00±0.58	-	9.00±0.58	-
CTX+ Sesame	21.33±0.88	Yes	40.67±0.67	Yes
CIP+ Sesame	23.00±0.58	Yes	24.00±0.58	No
E+ Sesame	23.00±0.58	-	15.00±0.00	-
OT+ Sesame	29.33±1.85	-	17.00±0.58	-
CN+ Sesame	16.00±0.58	No	26.00±0.58	No
TOB+ Sesame	22.00±0.58	Yes	24.00±0.58	No
RL + Sesame	16.00±1.15	No	20.33±1.45	No
CTX+ Eucalyptus	10.33±0.88	No	10.00±0.58	Yes
CIP+ Eucalyptus	16.33±0.88	No	15.33±0.33	Yes
E+ Eucalyptus	9.67±0.33	No	8.33±0.33	No
OT+ Eucalyptus	0.00±0.00	No	0.00±0.00	No
CN+ Eucalyptus	19.00±0.58	No	20.33±0.88	Yes
TOB+ Eucalyptus	11.00±0.58	No	12.00±1.15	Yes
RL+ Eucalyptus	11.00±0.58	No	24.00±1.15	Yes
CTX	8.33±0.33	-	8.67±0.33	-
CIP	18.00±0.58	-	18.00±0.58	-
E	0.00±0.00	-	0.00±0.00	-
OT	0.00±0.00	-	0.00±0.00	-
CN	16.00±0.58	-	17.00±0.58	-
TOB	11.33±0.88	-	10.00±0.58	-
RL	8.00±0.58	-	10.33±0.88	-

Chart.1 Antibacterial activity of sesame honey alone and in combination with antibiotics against *Cl. acetobutylicum* (DSM1731) and *Cl. perfringens* (KF383123)

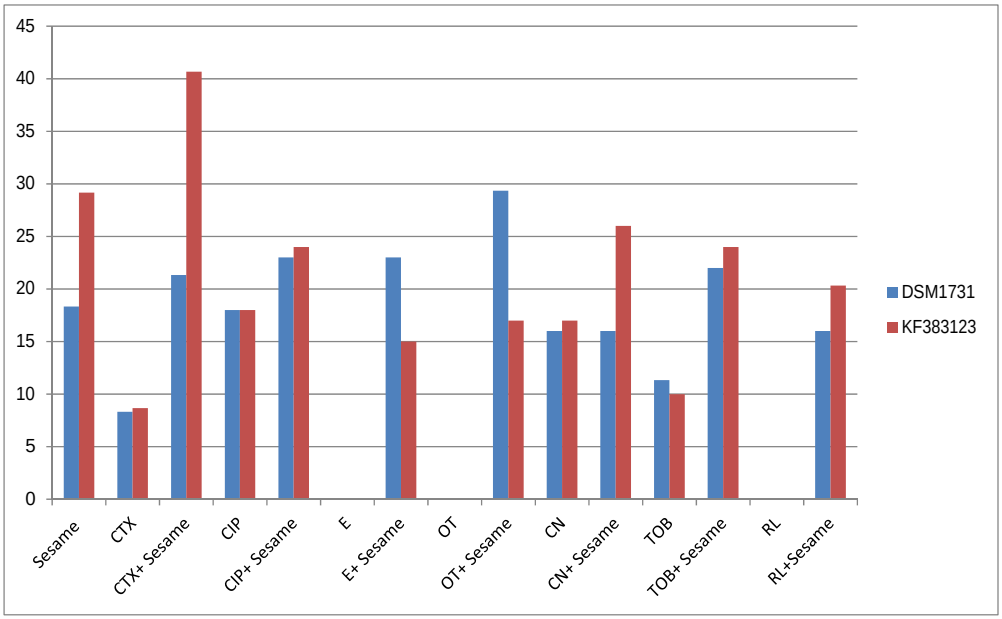
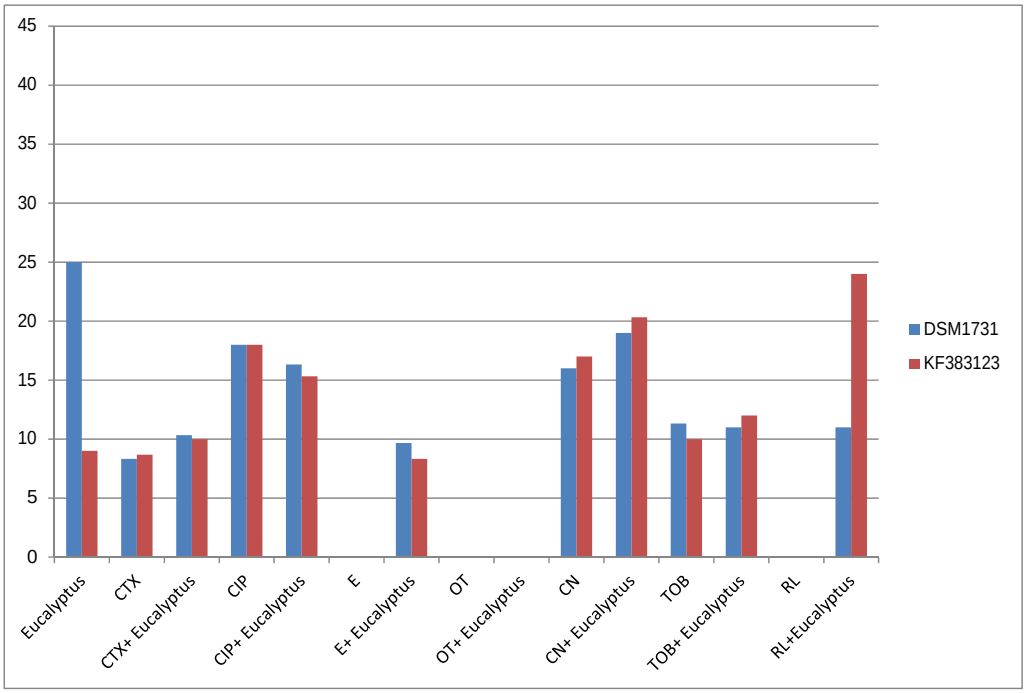


Chart.2 Antibacterial activity of eucalyptus honey alone and in combination with antibiotics against *Cl. acetobutylicum* (DSM1731) and *Cl. perfringens* (KF383123)



References

Abd El-Moez, S.I., A.S.S. Abdoon, H.A. Sabra, O.M. Kandil and H.A. Sabra, 2013. Antimicrobial Activity of Egyptian Cotton Flower Honey. Global Veterinaria, 11(5): 568-574.

Al-Jabri, A.A, S.A. Al-Hosni and B.C., Nzeako, 2005. Antibacterial activity of Omani honey alone and in combination with gentamicin. Saudi Med. J., 26: 767–771

Almaraz-Abarca, N., M.G. Campos, J.A. Avila-Reyes, N. Naranio-Jimenez, J. Herrera-Corral, L.S. Gonzalez-Valdez, 2007. Antioxidant activity of polyphenolic extract of monofloral honeybee-collected pollen from mesquite (Prosopis juliflora, Leguminosae). Journal of Food Composition and Analysis, 20: 119-124.

Andreesen, J.R, H. Bahl and G. Gottschalk, 1989. Introduction to the physiology and biochemistry of the genus Clostridium. Clostridia: 27-62.

Andrews, J.M., 2007. BSAC standardized disc susceptibility testing method (version 6). Journal of Antimicrobial Chemotherapy, 60: 20–41.

Ayaad, T.H., M.A. Dorrah, A.A. Mohamed, and T.T.M. Bassal, 2009. Specificity and developmental changes of hemagglutination activity of serum of the desert locust Schistocerca gregaria (Orthoptera: Acridadae). Journal of Orthoptera Research, 18: 51-56.

Azeredo, L. da C., M.A.A. Azeredo, S.R. de Souza and V.M.L. Dutra, 2003. Protein contents and physicochemical properties in honey samples of Apis mellifera of different floral origins. Food Chem., 80: 249–254.

Bates, J.R. and P.W. Bodnaruk, 2003. Clostridium perfringens. In: Foodborne Microorganisms of Public Health Significance. 6th edition. Ed: A.D. Hocking. Australian Institute of Food Science and Technology Inc., Food Microbiology Group, Waterloo NSW, 505-542.

Blair, S.E., N.N. Cokcetin, E.J. Harry and D.A. Carter, 2009. The unusual antibacterial activity of medical-grade Leptospermum honey: antibacterial spectrum, resistance and transcriptome analysis. Eur. J. Clin. Microbiol. Infect. Dis., 28: 1199–1208.

Bogdanov, S., T. Jurendic, R. Sieber and P. Gallmann, 2008. Honey for nutrition and health. A review. J. Am. Coll. Nutr., 27: 677–689.

Brudzynski, K., 2006. Effect of hydrogen peroxide on antibacterial activities of Canadian honeys. Can. J. Microbiol., 52: 1228-1237.

Brudzynski, K., K. Abubaker, L. St-Martin, and A. Castle, 2011. Re-examining the role of hydrogen peroxide in bacteriostatic and bactericidal activities of honey. Front Microbiol., 2: 213.

Cooper, R.A. and P.C. Molan, 1999. The use of honey as an antiseptic in managing Pseudomonas in fection. J. Wound Care, 8(4): 161–164.

Cooper, R.A., E. Halas and P.C. Molan, 2002. The efficacy of honey in inhibiting strains of Pseudomonas aeruginosa from infected burns. J. Burn Care Rehabil., 23(6): 366–370.

Cooper, R.A., L. Jenkins, A.F.M. Henriques, R.S. Duggan and N.F. Burton, 2010. Absence of bacterial resistance to medical-grade manuka honey. Eur. J. Clin. Microbiol. Infect. Dis. 29: 1237–1241.

Cooper, R.A., P.C. Molan and K.G.

Harding, 1999. Antibacterial activity of honey against strains of Staphylococcus aureus from infected wounds. J. R. Soc. Med., 92: 283–285.

Ejim, L., M.A. Farha, S.B. Falconer, J. Wildenhain, B.K. Coombes, M. Tyers, E.D. Brown, G.D. Wright, 2011. Combinations of antibiotics and non antibiotic drugs enhance antimicrobial efficacy. Nat. Chem. Biol., 7: 348–350.

Erejuwa, O.O., S.A. Sulaiman and M.S. Wahab, 2012b. Oligosaccharides might contribute to the antidiabetic effect of honey: A review of the literature. Molecules, 2012b, 17, 248–266.

Erejuwa, O.O., S.A. Sulaiman, and M.S. Wahab, 2012a Fructose might contribute to the hypoglycemic effect of honey. Molecules, 17: 1900–1915.

ESR, 2010. Annual summary of outbreaks in New Zealand 2009. Client report FW10048 for the Ministry of Health. ESR: Kenepuru Science Centre.

Evers, D.L., C.F. Chao, X. Wang, Z.G. Zhang, S.M. Huong and E.S. Huang, 2005. Human cytomegalovirus-inhibitory flavonoids: studies on antiviral activity and mechanism of action. Antiviral Res., 68(3): 124–134.

Ferreres, F., C. Garciaviguera, F. Tomaslorente and F.A Tomasbarberan, 1993. Hesperetin C a marker of the floral origin of citrus honey. J. Sci. Food Agric., 61: 121–123.

Garrity, G., 2005. Bergey's manual of systematic bacteriology. New York: Springer. P. 2816

Gheldof, N., X.H. Wang and N.J. Engeseth, 2002. Identification and quantification of antioxidant components of honeys from various floral sources. J. Agric. Food Chem., 50:.5870–5877.

Gibbons, S., 2008. Phytochemicals for Bacterial Resistance-Strengths, Weaknesses and Opportunities. Planta Med., 74: 594–602.

Haffejee, I.E. and A. Moosa, 1985. Honey in the treatment of infantile gastroenteritis. Br. Med. J. (Clin Res Ed), 290(6485): 1866-1867.

Halawani, E. and M. Shohayeb, 2011. Survey of the antibacterial activity of Saudi and some international honeys. Journal of Microbiology and Antimicrobials, 3(4), 94-101.

Hammond, E.N. and E.S. Donkor, 2013. Antibacterial effect of Manuka honey on Clostridium difficile. BMC Research Notes, 6:188

Hegazi, A.G. and F.M. Abd Allah, 2012. Antimicrobial Activity of Different Saudi Arabia Honeys. Global Veterinaria, 9 (1): 53-59.

Hegazi, A.G., 1998. Propolis an over view. J. Bee Informed, 5: 22–23, 6: 723-728.

Hegazi, A.G., 2011. Antimicrobial Activity of Different Egyptian Honeys as Comparison of Saudi Arabia Honey. Research Journal of Microbiology, 6 (5): 488-495

Hegazi, A.G., and F.K. Abd El Hady, 2009. Influence of Honey on the Suppression of Human Low Density Lipoprotein (LDL) Peroxidation (In–vitro). Journal of Evidence Based Complementary and Alternative Medicine, 6(1): 113-121.

Huang, W.Z., X.J. Dai, Y.Q. Liu, C.F. Zhang, M. Zhang and Z.T. Wang, 2006. Studies on antibacterial activity of flavonoids and diarylheptanoids from Alpinia katsumadai. Journal of Plant Resources and Environment15(1): 37-40.

Jayaraman, P., M.K. Sakharkar, C.S. Lim, T.H. Tang and K.R. Sakharkar, 2010. Activity and interactions of antibiotic and phytochemical combinations against Pseudomonas aeruginosa in vitro. Int. J. Biol. Sci., 6: 556–568.

Jenkins, R.E. and R.A. Cooper, 2012. Synergy between oxacillin and manuka honey sensitizes methicillin-resistant Staphylococcus aureus to oxacillin. J. Antimicrob. Chemother., 67: 1405–1407.

Jones, D. and D. Woods, 1986. Acetone-butanol fermentation revisited. Microbiol. Mol. Biol .Rev. 50: 484–524.

Karayil, S.1., S.D. Deshpande and G.V. Koppikar, 1998. Effect of honey on multidrug resistant organisms and its synergistic action with three common antibiotics. J. Postgrad. Med., 44(4): 93-96.

Katirciolu, H. and N. Mercan, 2006. Antimicrobial activity and chemical compositions of Turkish propolis from different regions. African Journal of Biotechnology, 5 (11): 1151-1153.

Kerem, Z., D. Chetrit, O. Shoseyov and G. Regev-Shoshani, 2006. Protection of lipids from oxidation by epicatechin, trans-resveratrol, and gallic and caffeic acids in intestinal, J. Agric. Food Chem., 54(26):10288-10293.

Khalil, M.I., M. Abdul-Motallib, A.S.M. Anisuzzaman, Z.S. Sathi, M.A. Hye and M. Shahjahan, 2001. Antibacterial activities of different brands of unifloral honey available at the Northern region of Bangladesh. J. Med. Sci., 1: 389-392.

Khan, F.R, Z. Ul-Abadin, N. Rauf, 2007. Honey: nutritional and medicinal value. Int. J. Clin. Pract., 61(10): 1705-1707.

Koc, A.N., S. Silici, F. Kasap, H.T. Hormet-Oz, H. Mavus-Buldu and B.D. Ercal, 2011. Antifungal activity of the honeybee products against Candida spp. and Trichosporon spp. J. Med. Food, 14: 128–134

Kwakman, P.H.S. and S.A.J. Zaat, 2012. Antibacterial Components of Honey: Critical Review. IUBMB Life, 64(1): 48–55, January 2012. Resour. Environ. 15(1): 37–40.

Kwakman, P.H.S., A.A. te Velde, L. Boer, C.M.J.E. Vandenbroucke-Graulsn and S.A.J. Zaat, 2011. Two major medicinal honeys have different mechanisms of bactericidal activity. PLoS One, 6(3): e17790.

Kwakman, P.H.S., A.A. te Velde, L. de Boer, D. Speijer, C.M.J.E. Vandenbroucke-Graulsn and S.A.J. Zaat, 2010. How honey kills bacteria. FASEB J., 24: 2576–2582.

Meda, A., C.E. Lamien, M. Romito, J. Millogo, and O.G. Nacoulma, 2005. Determination of the total phenolic, flavonoid and proline contents in Burkina Fasan honey, as well as their radical scavenging activity. Food Chemistry, 91: 571–577.

Miyakawa, M.E.1., J. Saputo, J.S. Leger, B. Puschner, D.J. Fisher, B.A. McClane and F.A. Uzal, 2007. Necrotizing enterocolitis and death in a goat kid associated with enterotoxin (CPE)-producing Clostridium perfringens type A. Can. Vet. J., 48(12): 1266

Molan, P.C., 1992. The antibacterial activity of honey 1. The nature of the antibacterial activity. Bee World, 73: 5-28.

Molan, P.C., 2002. Re-introducing honey in the management of wounds and ulcers-theory and practice. Ostomy Wound Manage, 48 (11): 28-40.

Molan, P.C., 2006. The evidence supporting the use of honey as a wound dressing. Int. J. Low Extreme Wounds, 5 (1):40-54.

Mukherjee, S., S. Chaki, S. Das, S. Sen, S.K. Dutta, S.G. Dastidar, 2011. Distinct synergistic action of piperacillin and methylglyoxal against Pseudomonas aeruginosa. Indian J. Exp. Biol., 49: 547–551.

Mullai, V. and T. Menon, 2007. Bactericidal activity of different types of honey against clinical and environmental isolates of *Pseudomonas aeruginosa*. J. Altern. Complementary Med., 13: 439-442.

Namias, N., 2003. Honey in the management of infections. Surg. Infect. (Larchmt). Summer, 4(2): 219-226.

Ozcelik, B., I. Orhan and G. Toker, 2006. Antiviral and antimicrobial assessment of some selected flavonoids. Z. Naturforsch C., Biosci. 61(9): 632–638.

Pimentel, R.B.dQ., C.A. da Costa, P.M. Albuquerque and S. Duvoisin Junior, 2013. Antimicrobial activity and rutin identification of honey produced by the stingless bee Melipona compressipes manaosensis and commercial honey. BMC Complementary and Alternative Medicine, 13: 151.

Poilane, I., F. Bert , P. Cruaud , M.H. Nicolas-Chanoine and A. Collignon, 2007. Interest of the disk diffusion method for screening Clostridium difficile isolates with decreased susceptibility to antibiotics. Pathol. Biol. (Paris), 55(8-9): 429-33.

Rehner, S. and G. Samuels, 1994. Taxonomy and phylogeny of Gliocladium analysed from nuclear large subunit ribosomal DNA sequences. Mycol. Res. (United Kingdom) 98: 625–634.

Richard J., 2009. Honey and healing through the ages. Journal of ApiProduct and ApiMedical Science 1(1): 2-5

Rushton, I., 2007. Understanding the role of proteases and pH in wound healing. Nurs Stand., 21(32): 68-70.

Schneider, L.A., A. Korber, S. Grabbe and J. Dissemond, 2007. Influence of pH on wound-healing: a new perspective for wound-therapy. Arch. Dermatol. Res., 298(9): 413-420.

Simon, A., K. Traynor, K. Santos, G. Blaser, U. Bode and P. Molan, 2009. Medical honey for wound care-still the 'latest resort'?. Evid. Based Complement Alternat. Med., 6(2):165-173.

Singleton, V.L., R. Orthofer and R.M. Lamuela-Raventos, 1999. Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. Oxidants and antioxidants. Methods in Enzymology. U.S.A.: Academic Press. 299: 152-178

Snow M.J. and Manley-Harris, 2004. On the nature of non peroxide antibacterial activity in New Zealand Manuka honey. Food Chem., 84(1): 145-147.

Steel, R.G.D. and J.H. Torrie, 1980. Principles and Procedures of Statistics. 2nd ed. McGraw Hill Book Company, New York.

Tan, H.T.; Rahman, R.A.; Gan, S.H.; Halim, A.S.; Hassan, S.A.; Sulaiman, S.A.; Kirnpal-Kaur, B. The antibacterial properties of Malaysian tualang honey against wound and enteric microorganisms in comparison to manuka honey. BMC Complement. Altern. Med. 2009, 9, 34.

Theodori, R., A. Karioti, A. Racnic and H. Skaltsa, 2006. Linear sesquiterpene lactones from Anthemis auriculata and their antibacterial activity. J. Nat. Prod., 69(4): 662–664.

Toussoun, Z., A. Rashed and A.G. Hegazi, 1997. Honey and propolis as management of some chronic skin ulcers. Proceeding International Symposium On Apitherapy, Cairo 8-9th, March, 1997 p 71.

Veshnyakova, A., J. Protze, J. Rossa, I.E. Blasig, G. Krause and J. Piontek, 2010. On the Interaction of Clostridium perfringens Enterotoxin with Claudins. Toxins, 2: 1336-1356

Wijesinghe, M., M. Weatherall, K. Perrin and R. Beasley, 2009. Honey in the treatment of burns: a systematic review and meta-analysis of its efficacy. N. Z. Med. J, 122(1295): 47-60.

Wilkinson, J.M. and M.A. Cavanagh, 2005 Antibacterial activity of 13 honeys against Escherichia coli and Pseudomonas aeruginosa. J. Med. Food, 8(1): 100–103.

Yoirish, N., 1977. Curative Properties of Honey and Bee Venom. New Glide Publication, San Francisco, USA., Pages: 198.

Zumla, A.and A. Lulat 1989. Honey--a remedy rediscovered. J. R. Soc. Med., 82 (7): 384-385.