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Efficacy of some Natural and Trade Saudi Honeys as an Antibacterial agent against Multidrug Resistant (MDR) Human Bacterial Pathogens

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ABSTRACT

For centuries, honey had a valued place in traditional medicine especially in remedy of microbial infections. In Saudi Arabia, the consumption of honey is increasing daily and there are many types of Saudi consumed honey (local and exotic), so comparing these honeys with their biological activities is greatly required. The main objective of the study was to evaluate the antibacterial potency of three natural mountain honeys (Al-Sail Al-Kabir, Wadi Al-Dibaa &Wadi Sahl) and three trade honeys (Al-Shifa, Haley & Polyflora) randomly selected from different regions of Saudi Arabia country and evaluated for antibacterial activity against multidrug resistant (MDR) bacteria by agar diffusion methods. Generally, all of the tested natural and trade honey types have inhibitory effect on growth of all tested bacterial strains, recording higher activity for natural than trade types. Among the tested honeys, Al-Sail Al-Kabir and Haley were the most potent ones with inhibition zone diameter (IZD) 14.5–16.5and 12.0 - 15.0 mm respectively. Also it was found that, Al-Sail Al-Kabir retains with its activity up to 25%, while Haley was found to have weak activity when diluted to 75%. The minimum inhibition concentrations (MICs) and minimum bactericidal concentrations (MBCs) of the two honeys showed that, Al-Sail Al-Kabir honey possessed lowest MIC values against *E. faecium* & *P. aeruginosa* with 5 mg/ml MIC and 10 mg/ml for MBC. While the highest MICs were recorded for Haley honey against *S. aureus* (VRSA) &*A. baumanii* with 20 & 40 mg/ml MIC and 40 &>40 mg/ml MBC values. From the obtained values it was observed statistically significant difference($P \leq 0.05$) between the tested organisms and the concentrations of the honey. It could be concluded that, Saudi Arabia honeys, namely Al-Sail Al-Kabir and Haley have high antibacterial potency and can be used as alternative strategy for treatment of multidrug resistant bacteria.
KEY WORDS: Antibacterial activity, Multidrug-resistant bacteria, Natural and trade Saudi honey, MIC, MBC.

INTRODUCTION

Using of antimicrobial agents in reducing the global burden of the infectious microbial diseases is essentially important. The developing and spreading of the resistant microbial pathogens led to diminishing the effectiveness of the known antibiotics. Antibiotic bacterial resistance is a serious problem to the public health and frequencies of this resistance are increased worldwide [1-2]. The availability of antibiotics on shelf for the public in Saudi Arabia contributes to the spread of multidrug[3].

MDR bacteria present an increasing problem in medical care [4]. Cases reported with MDR bacteria increased in number and failure of routine antimicrobial therapy has extended to cover more patients even those who are not seriously ill or complicated [5].In Saudi Arabia MDR bacteria represents a major problem causes length of hospitalization, increases in morbidity and mortality, limited therapeutic options as well as high cost of health care. MDR bacteria are retained to several types of Gram-positive and Gram-negative bacterial pathogens [6-9].

Currently, as a result of arising of those MDR organisms options for treatment with antimicrobial agents are limited, and newly introduced drugs placed on the market are decreasing, and because it is quite important problem, many efforts are directed to limit those bacteria from spreading[10]. Infection control represents the first steps towards limiting those resistant bacterial types, secondly comes introduction of new effective antibiotics in the market. In the wake of increasing interest in complementary and alternative medicine, herbal extracts are attracted recently in many countries [11-13].

The Arab region is rich in natural resources, including plants and herbs. However, most of these remain unexplored for their biological activities including those used in traditional medicine. With the growing problem of

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bacterial resistance to major classes of antibiotics, which is associated with many of the healthy and economic problems, the search for alternative treatments from natural resources becomes a pressing issue[14]. According to estimation of the World Health Organization, 80% of developing countries peoples rely on the harvested wild plants for their primary health care [15]. In Arab countries honey has the first rank in folk medicine. In Saudi Arabia, the consumption of honey is increasing, since it is one of the principle ingredients in foods, as remedy and in natural mixtures [16].

Honey is a unique food product having several of bioactive compounds which derived from bees and plants [17]. The average content of honey was recorded as, monosaccharides (75% fructose and glucose), disaccharides (3–10% sucrose), complex sugars and other materials as well as water (20%) [18-19], also it contains antioxidants [20-22]. Some reports mentioned that honey contains more than 200 components [23].

From ancient times, the honey was used in traditional medicine for remedy of the microbial infections. The killing effect of honey to the microorganisms has been attributed to several factors such as its phytochemical nature, its high concentration of hydrogen peroxide, high acidic nature as well as its high osmotic effect [24]. The antimicrobial potency of honey is also dependent on several causes such as flower from which it is derived, geographical location and the type of the used honey [25].

Recently, honey has been reported to have an antibacterial activity against about 60 species of bacteria including Gram positives, and Gram negatives, aerobic and anaerobic, hence it was known that honey, had antibacterial of broad spectrum activity [26].

Since honey types differ from one country to another and in different regions in the same country due to floral origin, soil composition and other factors consequently, quality criteria differ from one honey type to another. So these criteria vary according to these factors and need to be periodically revised with updating methodologies [27]. There are many types of honey (local and exotic) commonly consumed in Saudi Arabia. Most of these honeys are traded without quality sign or reference to their origins and this may lead to honey adulteration and/or marketing non-standard honeys. So, comparing these honeys with its biological activities is greatly required [28]. The present study was conduct in order to evaluate some of the natural and trade Saudi honeys as antibacterial agents against the MDR human bacterial pathogens.

MATERIALS AND METHODS

Collection of honeys

Three types of natural mountain honeys (Al-Sail Al-Kabir, Wadi Al-Dibaa and Wadi Sahl) originating from three different localities in Saudi Arabia are kindly provided by honey– beekeepers in addition to three trade honeys (Al-Shifa, Haley and Polyflora) purchased from three Saudi local markets were randomly selected from different locations of Saudi Arabia country (Table 1).

Table 1: Types and regional data of the tested honey samples

No.	Type	Name of Honey (Scientific name)	Location
1	Natural Honey	Al-Sail Al Kabir (*Acacia* spp)	Al-Taif, Southwestern KSA (Stored honey)
2	Natural Honey	Wadi Al-Dibaa (*Acacia* spp)	Arar, South KSA (Stored honey)
3	Natural Honey	Wadi Sahl (*Acacia* spp)	Tarif, South KSA (Stored honey)
4	Trade Honey	Al-Shifa	Rafha, Othaim Markets
5	Trade Honey	Haley	Dammam, Carrefour Market
6	Trade Honey	Polyflora	Hafar Al-Batin, Hyper Panda

Preparation of honeys concentrations

Different concentrations 1, 2.5, 5.0 and 7.5% (v/v) of each honey type were prepared using sterile distilled water. To obtain theses concentrations, the respective honey volumes: 9.0, 7.5, 5.0, 2.5 mL were dissolved in sterile distilled water of a corresponding volume of to give 10 mL final volume. 6 mm filter paper discs were prepared [29], and impregnated individually with the prepared honey concentrations.

Bacterial test organisms

The MDR bacterial test organisms used in this study were Gram-positive strains; *Enterococcus faecium* (strain resistant to 15 different antibiotics) [30]and vancomycin-resistant *Staphylococcus aureus* (resistant to 13 different antibiotics) [31] and Gram-negative strains; *Pseudomonas aeruginosa* (resistant to 15 different antibiotics) and

Acinetobacter baumanii (strain resistant to 16 different antibiotics) [30]. In addition to two standard strains; *Staphylococcus aureus* ATCC 29213 as a Gram-positive and *Escherichia coli* ATCC 25922 as a Gram-negative were used for comparison of inhibition zones in the primary antibacterial assay. The previously mentioned strains were obtained from Bacteriology Lab at Department of Botany and Microbiology, Faculty of Science (Boys), Al-Azhar University, Cairo, Egypt.

Culture preparation

The six bacterial strains used in this study were kept on nutrient agar slants at 4°C until use. For purpose of the culture preparation, twenty-five ml of nutrient broth medium were inoculated into six conical flasks of 50 ml volume; each flask was inoculated separately with the six bacterial strains under study then incubated for 24 h at 37 °C.

Testing antibacterial susceptibility

Preparation of the bacterial inoculums

The bacterial slants were incubated at 37ºC for overnight. The bacterial suspension (0.5 McFarland) was prepared according to the method of Koneman et al. [32]. Briefly, three to five colonies were dispersed in sterile normal saline then turbidity of the test tube was adjusted to 1.5×10^8 CFU/mL that corresponding to 0.5 McFarland standard. Mueller Hinton agar plates were swabbed from the prepared standardized bacterial suspension, then the plates were dried for 3 to 5 min.

Paper disc assay

The method of paper disc assay was performed according to Bauer et al. [33]. In this experiment the honey samples were impregnated on paper discs, then, all discs were loaded on the surface of the agar and pressed to be contacted with agar. To prevent overlapping of the inhibition zones, the discs were placed at a distance of 15 mm from the edges of the plates. After incubation at 37°Cfor 24 h, the plates were examined and the inhibition zone diameters (mm) were recorded. Triplicate plates were prepared and measured for each test organism.

Agar well Assay

The honey types gave highest antibacterial activity were selected and different concentrations were performed from each type to detect the highest concentration gives highest antibacterial activity. Plates of Mueller Hinton agar were prepared according to the instructions of the manufacturing company. Wells with 6 mm diameter were cut in the agar using sterile cork borer. Swabs from bacterial suspension (1.5×10^8 CFU/mL) were streaked on the surface of the agar plates. Different concentrations of the honey; 10, 25, 50 & 75% were added to the wells individually. The inoculated plates were incubated for 24 h at 37°C [34].

Determination of MIC and MBC

The highest active natural and trade honey types (types gave highest diameter of inhibition zones) were selected for determination of their MIC and MBC. For determination of MIC, different concentrations 80, 40, 20, 10, 5, 2.5, 1.25 and 0.62 mg/mL were prepared from each honey in tested tubes by serial dilutions using 1 mL of Mueller Hinton broth medium. Inoculum of 20 µl (1.5×10^8 CFU/mL) of the test organisms was dispensed into the tubes. The tube of positive control was contained only each of the organisms in 1 mL broth medium and but not honey, in contrast the negative control tube was contained 1 mL of honey. After incubation at 37°C for 24 h, the turbidity of each tube was visually investigated [35]. For MBC determination, the tubes of MIC that showed visible growth or turbidity, each bacterial test organism was streaked on plates of nutrient agar and incubated for 24 h at 37°C. MBC was recorded as the lowest concentration without any growth of the test organisms.

Statistical analysis

The results of inhibition zone diameters were recorded and expressed as average ± standard deviation then analyzed using ANOVA (analysis of variance), if $P\leq0.05$ the differences were considered significant.

RESULTS AND DISCUSSION

The presence and spreading of multidrug resistant microbes led to re-evaluation of ancient remedies such as honey [36-38]. Honey is highly valued by users for therapeutic purposes as an alternative medicine [39]. Using of honey as a therapeutic is due to ancient times because of its varied therapeutic and nutritional values [40]. Honey is commonly used as an anti-inflammatory, anti-oxidant and anti-bacterial agent [41]. Recent advances in research, literature highlighted that honey has potential biological activities with promising health promoting properties [42].

The selected honeys and bacterial strains

In Saudi Arabia, there are many types of honey, some are locally produced and some are imported from outside the country. Evaluation the antibacterial activity of the most common types of Saudi honeys not studied sufficiently [43]. Most of the available Saudi honeys are traded without quality sign or reference to their origins and this may lead to honey adulteration and/or marketing non-standard honeys. So, comparing these honeys with quality standards such as antibacterial activity especially against MDR bacteria is greatly required. Although, previous studies which were conducted on Saudi honeys focused on antimicrobial activity as a general [44-49], but literatures about evaluating the activity of both natural and trade Saudi honeys as antibacterial agent against multidrug resistant (MDR) human bacterial pathogens are few. In this study, three natural mountain honeys (Al-Sail Al-Kabir, Wadi Al-Dibaa and Wadi Sahl) originating from three different localities in Saudi Arabia were evaluated for antibacterial activity in comparing with three trade honeys (Al-Shifa, Polyflora and Haley) purchased from three Saudi local markets.

Antibacterial activity of the collected honeys against MDR bacteria

The collected six Saudi honeys were tested against four MDR bacterial strains *S. aureus* (VRSA), *E. faecium*, *P. aeruginosa* and *A. baumanii* in addition to two standard strains *S. aureus* ATCC 29213 and *E. coli* ATCC 25922 using paper disc diffusion method. Important MDR bacteria include methicillin-resistant *Staphylococcus aureus* (MRSA), vancomycin-resistant *S. aureus* (VRSA), vancomycin-resistant *Enterococci* (VRE), *Acinetobacter sp.* and *Pseudomonas aeruginosa* cause infections are more difficult and expensive to treat, and result in significant mortality, imposing high economic burden on health care systems [5].

The results of antibacterial assay (Figure 1) showed that the highest antibacterial activity was recorded for natural honeys where the inhibition zone diameter (IZD) was ranged from 12.0±0.54 to 19.5±0.10 mm in comparison with trade honeys which showed lower activity of IZD ranged from 9.5±0.52 to 16.0±0.27 mm against all tested bacterial strains. Generally, among the tested natural honey types, Al-Sail Al-Kabir honey obtained from Al-Taif, Southwestern KSA was the most potent type with IZD 14.5±0.35–19.5±0.10 mm followed by Wadi Al-Dibaa and Wadi Sahl with IZD 12.5±0.44 - 17.0±0.33 and 12.0±0.54 - 15.5±0.10 mm.

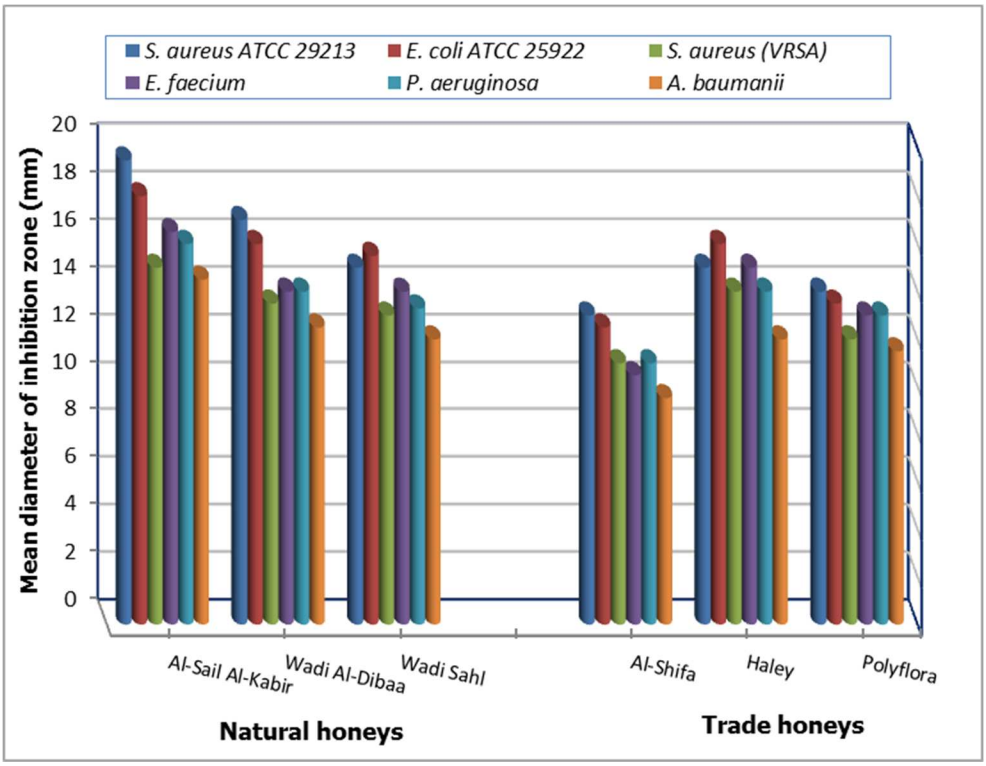

Figure 1: Inhibitory growth activity of natural and trade honeys against MDR bacterial strain.

In case of trade honeys among the tested three honeys, Haley type that obtained from Carrefour Market;

Dammam governorate was the most potent one with IZD ranged from 12.0±0.45 to 16.0±0.27 mm followed by Polyfera honey of IZD ranged from 11.5±0.30 to 14.0±0.35 mm against the tested strains. While Al-Shifa honey was the weakest activity at all of IZD9.5 - 13.0±0.32 mm.

Our finding indicated that, susceptibility of the tested MDR strains to the different honey types was varied where the highest inhibitory effect was recorded in case of *E. faecium* (10.5 – 16.5 mm) followed by *P. aeruginosa* (11.0 – 16.0 mm) and *S. aureus* VRSA (11.0 – 15.0 mm), while the less susceptible strain was *A. baumanii* where IZD was ranged from 9.5 to 14.5mm. Honey has been used for its healing, nutritional and therapeutic properties since ancient times. Its antibacterial potentials even against multi-drug resistant bacteria, such as *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Acinetobacterbaumanni* have been proved[50].

In general, the obtained results showed that, all tested natural and trade honey types showed inhibitory effect on growth of all tested bacterial strains with varying degrees. The inhibition of bacteria growth is depending on the origin of honey for the natural types and manufacturing company for the trade types. Molan [51] reported that, the antibacterial activity of the honey may due to the floral source of honey which acts on its biological properties. Also, many authors established that, the antimicrobial activity of honey is return to many factors [52-53] such as its osmotic effect [24,54-55], its acidity or activity of glucose oxidase [56], its content of hydrogen peroxide [57-60], presence of inhibin [61] that consider as antibacterial agent [62], H_2O_2[63] and non-peroxide contents [64-65], as well as the factors of phytochemicals [66-67] and phytochemical substances [52,62,68-69].

Evaluation of antibacterial activity for the different honey dilutions

Based on the results of antibacterial assay it was found that, both Al-Sail Al-Kabir and Haley were the most potent ones, therefore, it were selected and different concentrations 10, 25, 50 & 75% were performed and assayed against the four test multidrug resistant bacteria by agar well diffusion method to detect the lowest active dilution can be used as a consumable diet.

The results of antibacterial assays for the different dilutions (Figure 2) showed that, Al-Sail Al-Kabir was active up to the concentrations 25% with IZD9.0 – 10.5 mm, while Haley honey was found to have weak activity at the concentration 75% with IZD 8.5 – 11.0 mm. From these results we could concluded that the natural honey can be used if though it diluted until to 25% while the trade honey losses it activity if diluted to 75%. The results of the recorded inhibition zone diameters showed statistical significant difference in the values between the honey concentrations and the microorganisms (P≤0.05). Similar findings were reported by Alqurashi et al. [70].

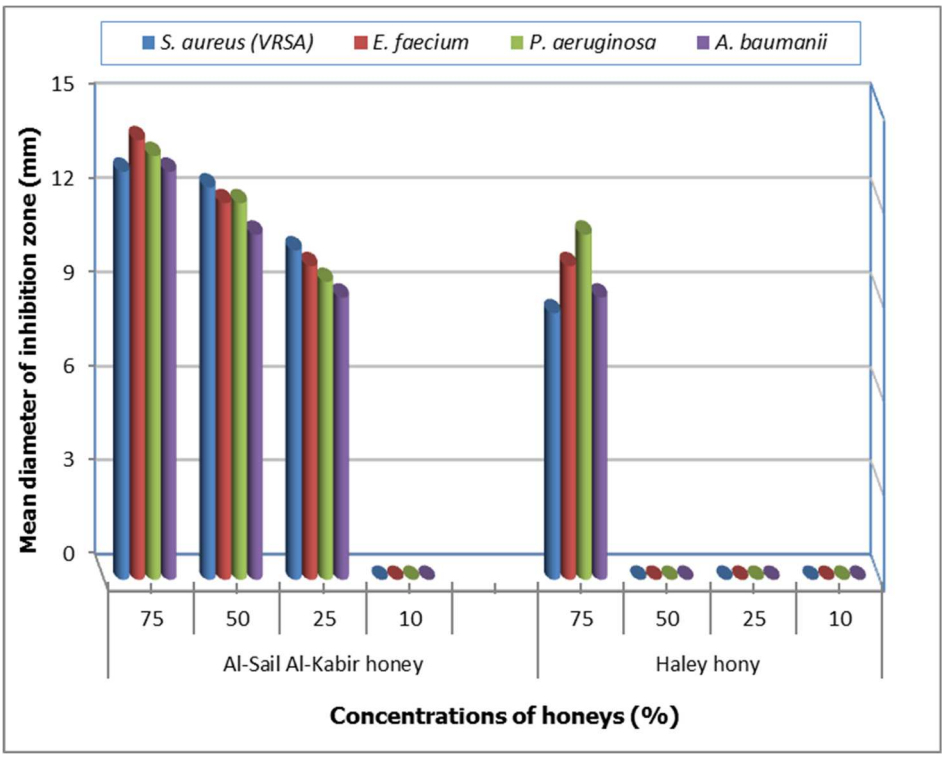

Figure 2: Antibacterial activity of different concentrations of the most potent honeys.

Determination of MIC and MBC of the most potent honeys

From results of antibacterial activity it was clear that Al-Sail Al-Kabir and Haley honeys were the most potent among the tested honey types thus it were selected for determination MIC and MBC against four tested MDR bacterial strains. The MIC and MBC values of the most potent Al-Sail Al-Kabir and Haley honeys against the four tested MDR bacteria are recorded in Table 2. The lowest MIC values were recorded for Al-Sail Al-Kabir against *E. faecium* & *P. aeruginosa* with MIC 5 mg/ml and 10 mg/ml MBC, relative increase in both MIC values 10 & 20 mg/ml and MBC 20 & 40 mg/ml for both *S. aureus* (VRSA) and *A. baumanii* respectively. The Highest MIC values were shown by Haley honey against *S. aureus* (VRSA) &*A. baumanii* with 20 & 40 mg/ml MIC and 40 &>40 mg/ml MBC values. Relative decreasing in MIC values 10 mg/ml and MBC 20 mg/ml for both *E. faecium* & *P. aeruginosa* respectively. The obtained MIC and MBC values are similar to those obtained by many authors discussed antibacterial potency of different Saudi honeys against non-multidrug-resistant bacteria [70, 71-73] and the results reported by Hern et al. [74]. To the best of our knowledge there is no study about evaluation of antibacterial activity of Saudi honeys against MDR bacteria. So, from the results obtained for MIC and MBC of Al-Sail Al-Kabir and Haley honeys we can indicate efficiency of these types as alternative treatment for MDR bacteria at low concentrations.

Table 2: MICs and MBCs of the most potent Saudi honeys

Bacterial strains	MIC/MBC (mg/ml)			
	Al-Sail Al-Kabir honey		Haley honey	
	MIC	MBC	MIC	MBC
***S. aureus* (VRSA)**	10	20	20	40
E. faecium	5	10	10	20
P. aeruginosa	5	10	10	20
A. baumanii	20	40	40	>40[a]

*MIC, Minimum inhibitory concentration; MBC, Minimum bactericidal concentration; [a.] The highest concentration tested.

CONCLUSION

The results obtained in this study established that, not all the common Saudi honeys have the same biological and medical properties, among them Al-Sail Al-Kabir and Haley Saudi honeys are valuable types where they have a promising antibacterial activity against multidrug resistant bacteria (MDRB). Thus they can be used as an alternative and efficient remedy for reducing the infections caused by such bacteria.

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REFERENCES

1. Levy, S.B. and B. Marshall, 2004. Antibacterial Resistance Worldwide: Causes, Challenges and Responses. Nat. Med., 10 (12): 122-129.
2. Mandal, S., N.K. Pal, I.H. Chowdhury and M. Deb Mandal, 2009. Antibacterial Activity of Ciprofloxacin and Trimethoprim, Alone and in Combination, against *Vibrio cholerae* O1 Biotype El-Tor Serotype Ogawa Isolates. Pol. J. Microbiol., 58 (1): 57-60.
3. Carlet, J. and D. Pittet, 2013. Access to Antibiotics: A Safety and Equity Challenge for the Next Decade. Antimicrob. Resist. Infect. Control, 2(1): 1.
4. Jean, S.S. and P.R. Hsueh, 2011. Current Review of Antimicrobial Treatment of Nosocomial Pneumonia Caused by Multidrug Resistant Pathogens. Expert. Opin. Pharmacother., 12 (14): 2145-2148.
5. Savard, P. and T.M. Perl, 2012. A Call for Action: Managing the Emergence of Multidrug-Resistant Enterobacteriaceae in the Acute Care Settings. Curr. Opin. Infect. Dis. 25 (4): 371-377.
6. Saeed, N.K., A.M. Kambal and N.A. El-Khizzi, 2010. Antimicrobial Resistant Bacteria in a General Intensive Care Unit in Saudi Arabia. Saudi Med. J., 31 (12): 1341-1349.

7. Salem-Bekhit, M.M., I.M. Moussa, M.M. Muharram, F.K. Alanazy and H.M. Hefni, 2012. Prevalence and Antimicrobial Resistance Pattern of Multidrug-Resistant Enterococci Isolated from Clinical Specimens. Indian J. Med. Microbiol., 30 (1): 44-51.
8. Somily, A.M., M.M. Absar, M.Z. Arshad, A.I. Al Aska, Z.A. Shakoor, A.J. Fatani, et al., 2012. Antimicrobial Susceptibility Patterns of Multidrug-Resistant *Pseudomonas aeruginosa* and *Acinetobacter baumannii* against Carbapenems, Colistin, and Tigecycline. Saudi Med. J., 33 (7): 750-755.
9. Yezli, S., A.M. Shibl, D.M. Livermore and Z.A. Memish, 2012. Antimicrobial Resistance among Gram-Positive Pathogens in Saudi Arabia. J. Chemother., 24 (3): 125-136.
10. Fishman, N. 2006. Antimicrobial Stewardship. Am. J. Infect. Control, 34 (5 Suppl 1): S55-63; discussion S64-73.
11. Abd Ali, M.A., S.M. Kidem, A.A. Fadhil and N.H. Saddam, 2016. In Vitro Antibacterial Activity of Some Natural and Trade Iraqi Honey against MRSA *Staphylococcus Heamolyticus* Isolated from Some Burned Patients in Misan City.Am. J. Microbiol. Res., 4 (5): 159-163.
12. Dash, N., D. Panigrahi and M. Al-Zarouni, 2016. Antimicrobial Effect of Honey from the Arabian Gulf Region against Bacterial Isolates from Pus and Wound Swabs. Adv. Microbiol., 6 (10): 745-752.
13. Rezvani, M.B., M. Niakan, M. Kamalinejad, F.S. Ahmadi and F. Hamze, 2017. The Synergistic Effect of Honey and Cinnamon against *Streptococcus mutans* Bacteria, Asian Pac. J. Trop. Biomed., 7 (4): 314-320.
14. Abdulhaq, A. 2017. Antibacterial Activity of Extracts from Selected Arabian Plants against Major Human Pathogens Including Multidrug Resistant Strains. J. Med. Plants St., 5 (1): 280-283.
15. World Health Organization, 1999. Drug Information. WHO, Geneva, 13 (4): 230-233.
16. Alqarni, A.S. 2011. Beekeeping in Saudi Arabia: Current and Future (in Arabic), 1st ed., Saudi Society for Agricultural Sciences, King Saud Univ., Riyadh, 40, No. 21.
17. Nzeako, B. and J. Hamdi, 2000. Antibacterial Potential of Honey on Some Microbial Isolates. Med. Sci. 2 (1): 75-79.
18. Celechovska, L.O. 2002. Activity of Enzymes and Trace Element Content in Bee Honey. Acta Vet. Brno., 71: 375–378.
19. Serrano, S., R. Espejo, M. Villarejo and M.L. Jodral, 2007. Diastase and Invertase Activities in Andalusian Honeys. Int. J. Food Sci. Technol., 42 : 76–79.
20. Berettaa, G., P. Granataa and M. Ferrerob, 2005. Standardization of Antioxidant Properties of Honey by a Combination of Spectrophotometric/Fluorimetric Assays and Chemometrics. Anal. Chim. Acta, 533 : 185-191.
21. Baltac, C., S. Candan, B. Manuela, M. Candiracci, A. Accorsi, M.B. Piacentini, M.C. Albertini and E. Piatti, 2006. Raw *Millefiori* Honey is Packed Full of Antioxidants. Food Chem. 97 (2): 217–222.
22. Bertoncelj, J., U. DoberSˇ ek, M. Jamnik and T. Golob, 2007. Evaluation of the Phenolic Content, Antioxidant Activity and Colour of Slovenian Honey. Food Chem., 105 (2): 822–828.
23. Kucuk, M.K., S. Kolayl, S. Karaoglu, E. Ulusoy, C. Baltac and F. Candan, 2007. Biological Activities and Chemical Composition of Three Honeys of Different Types from Anatolia. Food Chem., 100 (2): 526–534.
24. Molan, P.C. 1992. The Antibacterial Activity of Honey. Bee World 73 (1): 5-28.
25. Molan, P.C. and R.A. Cooper, 2000. Honey and Sugar as a Dressing for Wounds and Ulcers. Trop. Doct., 30 (4): 249-250.
26. Hannan, A., M. Barkaat, S. Saleem, M. Usman and W.A. Gilani, 2004. Manuka Honey and Its Antimicrobial Potential against Multi Drug Resistant Strains of *Typhoidal Salmonellae*, Ph.D. Thesis, Department of Microbiology, University of Health Science, Lahore, Pakistan.
27. Codex Alimentarious Commission (CAC), 1998. Codex Alimentarious Draft Revised for Honey CAD CX P 5/102, CI, 1998/12-S 1998, FAO, Rome, Italy.

28. Alqarni, A.S., A.A. Owayss and A.A. Mahmoud, 2016. Physicochemical Characteristics, Total Phenols and Pigments of National and International Honeys in Saudi Arabia. Arab. J. Chem., 9 (1): 114–120.
29. Cheesbrough, M. 2000. District Laboratory Practice in Tropical Countries. PART II. Low Price Edition. The Edinburgh Building Cambridge: Cambridge University Press; Biochemical Test to Identify Bacteria; Antimicrobial Susceptibility Testing. pp: 1933-1934. 63-70. 132-143.
30. El-Sayed, M.H., B.M. Refaat and M.H. Sharaf, 2014. Microbiological Evaluation of Antibacterial Potentiality of some Edible Plant Extracts against Multidrug Resistant (MDR) Human Pathogens.Int. Curr. Pharm. J., 4 (1): 336-339.
31. Moghannem, S.A., M.H. Elsayed, B.M. Refaat, G.M. Elsherbiny, H.A. Abd El-Fadeel and E.A. Mahna 2015. Isolation and Identification of *Staphylococcus* spp. Isolated from Oral Specimens in Egyptian Patient. Egypt. J. Biotechnol., Vol. 48, February, 1/48.
32. Koneman, W.E., D.S. Allen, M.W. Janda, C.P. Scherchenberger and W.C. Winn, 1992. Color Atlas and Text Book of Diagnostic Microbiology. 4th edition. JB Lippincott Company; Antimicrobial Susceptibility Testing; pp. 624, 629, 637.
33. Bauer, A.W., W.M. Kirby, J.C. Sherirs and M. Turck, 1966. Antibiotic Susceptibility Testing by Standard Single Disk Method. Am. J. Clin. Pathol., 45 (4): 433-496.
34. Barry, A.L. and C. Thornsberry, 1985. Susceptibility Test: Diffusion Test Procedures. In: Lennette EA, Balows A, Hausller WJ, Shadomy HJ (eds). Manual of Clinical Microbiology, Washington, DC pp. 978-987.
35. Mackie, McCartney, 1996. Practical Medical Microbiology. International Student 14th Edition New York. Church Livingston.
36. Basualdo, C., V. Sgroy, M.S. Finola and J.M. Marioli, 2007. Comparison of the Antibacterial Activity of Honey from Different Provenance against Bacteria Usually Isolated From Skin Wounds. Vet. Microbiol., 124 (4) : 375-381.
37. Mandal, S., M. Deb Mandal and N.K. Pal, 2010. Synergistic Anti-*Staphylococcusaureus* Activity of Amoxicillin in Combination with *Emblica officinalis* and *Nymphae odorata* Extracts. Asian Pac. J. Trop. Med., 3 (9): 711-714.
38. Mandal, S., M. Deb Mandal, N.K. Pal and K. Saha, 2010. Antibacterial Activity of Honey against Clinical Isolates of *Escherichia coli*, *Pseudomonas aeruginosa* and *Salmonella enterica* serovar Typhi. Asian Pac. J. Trop. Med., 3 (12): 961-964.
39. Denisow, B. and M. Denisow-Pietrzyk, 2016. Biological and Therapeutic Properties of Bee Pollen: A Review. J. Sci. Food Agric.,96 (13): 4303–4309
40. Meo, S.A., S.A. Al-Asiri, A.L. Mahesar and M.J. Ansari, 2017. Role of Honey in Modern Medicine: A Review. Saudi J. Biol. Sci. 24 (5): 975–978.
41. Al-Waili, N.S., F.S. Al-Waili, M. Akmal, A. Ali, K.Y. Salom, A.A. Al Ghamdi, 2014. Effects of Natural Honey on Polymicrobial Culture of Various Human Pathogens. Arch. Med. Sci., 10 (2): 246–250.
42. Muhammad, A., O.A. Odunola, M.A. Ibrahim, A.B. Sallau, O.L. Erukainure, I.A. Aimola and I. Malami, 2016. Potential Biological Activity of Acacia Honey. Front. Biosci. (Elite Ed) 8 (1): 351–357.
43. Eman, H. and S. Mohamed, 2011. Survey of the Antibacterial Activity of Saudi and some International Honeys. J. Microbiol. Antimicrob., 3 (4): 94-101.
44. Doghairi, M.A., S. Al-Rehiayani, G.H. Ibrahim and K.A. Osman, 2007. Physicochemical and Antimicrobial Properties of Natural Honeys Produced in Al-Qassim Region, Saudi Arabia. Met. Environ. Arid Land Agric. Sci., 18 (2): 3–18.
45. Hegazi, A.G. 2011. Antimicrobial Activity of Different Egyptian Honeys as Comparison of Saudi Arabia Honeys. Res. J. Microbiol., 6 (5): 488-495.
46. Hegazi, A.G. and F.M. Abd Allah, 2012. Antimicrobial Activity of Different Saudi Arabia Honeys. Glob. Vet., 9 (1): 53-59.

47. Hegazi, A.G., F.M. Al Guthami, A.M. Al Gethami, F.A. Allah, A.A. Saleh and E.A. Fouad, 2017. Potential Antibacterial Activity of Some Saudi Arabia Honey, Vet. W., 10 (2): 233-237.

48. Halawani, E.M. and M.M. Shohayeb, 2011a. Shaoka and Sidr Honeys Surpass in Their Antibacterial Activity Local and Imported Honeys Available in Saudi Markets against Pathogenic and Food Spoilage Bacteria. Aust. J. Basic Appl. Sci., 5 (4): 187-191.

49. Halawani, E.M. and M.M. Shohayeb, 2011b. Survey of the Antibacterial Activity of Saudi and some International Honeys. Jpn. Meteorol. Agency, 3 (4): 94-101.

50. Vallianou, N.G., P. Gounari, A. Skourtis, J. Panagos and C. Kazazis, 2014. Honey and its Anti-Inflammatory, Anti-Bacterial and Anti-Oxidant Properties. Gen. Med. (Los Angel) 2 (2): 132.

51. Molan, P. 2002. Not All Honeys are the same for Wound Healing. Bull. Eur. Tissue Rep. Soc., 9: 5-6.

52. Halawani, E.M. 2006. A Study on *Salmonella typhimurium* Causing Food Poisoning in Al-Taif City and Antibacterial Effect of *Nigella sativa* Honey and Camels Urine. Ph.D. Thesis. Taif University, Saudi Arabia.

53. Kwakman, P.H., A.A. Te Velde, L. De Boer, D.V. Speijer, C.M. Enbroucke-Grauls and S.A. Zaat, 2010. How Honey Kills Bacteria. FASEB J., 24 (7): 2576-2582.

54. Cooper, R.A., P.C. Molan and K.G. Harding, 2002. The Sensitivity to Honey of Gram-Positive Cocci of Clinical Significance Isolated from Wounds. J. Appl. Microbiol., 93 (5): 857-863.

55. Feás, X., A. Iglesias, S. Rodrigues and L.M. Estevinho, 2013. Effect of *Erica sp.* Honey against Microorganisms of Clinical Importance: Study of the Factors Underlying this Biological Activity. Molecules, 18 (4): 4233-4246.

56. Sherlock, O., A. Dolan, R. Athman, A. Power, G. Gethin, S. Cowman and H. Humphreys, 2010. Comparison of the Antimicrobial Activity of Ulmo Honey from Chile and Manuka Honey against Methicillin-Resistant *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa*. BMC Complement. Altern. Med., 10 (2): 47.

57. Roth, L.A., S. Kwan and P. Sporns, 1986. Use of a Disc Assay System to Detect Oxytetracycline Residues in Honey. J. Food Prot., 49 (6): 436-441.

58. Nassar, H.M., M. Li and R.L. Gregory, 2012. Effect of Honey on *Streptococcus mutans* Growth and Biofilm Formation. Appl. Environ. Microbiol., 78 (2): 536-540.

59. Brudzynski, K. 2006. Effect of Hydrogen Peroxide on Antibacterial Activities of Canadian Honeys. Can. J. Microbiol., 52 (12): 1228-1237.

60. Mercan, N., A. Guvensen, A. Celik and H. Katircioglu, 2007. Antimicrobial Activity and Pollen Composition of Honey Samples Collected from Different Provinces in Turkey. Nat. Prod. Res., 21 (3): 187-195.

61. Nour, M.E. 1988. Some Factors Affecting Quality of Egyptian Honeys. Ph.D. Thesis. Faculty of Agriculture, Cairo University.

62. Irish, J., S. Blair and D.A. Carter, 2011. The Antibacterial Activity of Honey Derived from Australian Flora. Otto M, ed. PLoS One, 6 (3): e18229.

63. Watt, B.E., A.T. Proudfoot and J.A. Vale, 2004. Hydrogen Peroxide Poisoning. Toxicol. Rev., 23 (1): 51-57.

64. Sufya, N., N. Matar, R. Kaddura and A. Zorgani, 2014. Evaluation of Bactericidal Activity of Hannon Honey on Slowly Growing Bacteria in the Chemostat. Drug Healthc. Patient Saf., 6 : 139-144.

65. Zainol, M.I., K. Mohd Yusoff and M.Y. Mohd Yusof, 2013. Antibacterial Activity of Selected Malaysian Honey. BMC Complement. Altern. Med., 13 (10): 129.

66. Mandal, M.D. and S. Mandal, 2011. Honey: Its Medicinal Property and Antibacterial Activity. Asian Pac. J. Trop. Biomed., 1 (2): 154-160.

67. Moniruzzaman, M., S.A. Sulaiman, M.I. Khalil and S.H. Gan, 2013. Evaluation of Physicochemical and Antioxidant Properties of Sourwood and other Malaysian Honeys: A Comparison with Manuka Honey. Chem. Cent. J., 7 (12): 138.

68. Yao, L., Y. Jiang, R. Singanusong, N. Datta, and K. Raymont, 2004. Phenolic Acids and Abscisic Acid in Australian Eucalyptus Honeys and Their Potential for Floral Authentication. Food Chem., 86 : 169-177.

69. Mavric, E., S. Wittmann, G. Barth and T. Henle, 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.

70. Alqurashi A.M., E.A. Masoud and M.A. Alamin, 2013. Antibacterial Activity of Saudi Honey against Gram Negative Bacteria. J. Microbiol Antimicrob. 5 (1): 1-5.

71. Chauhan, A., V. Pandey, K.M. Chacko and R.K. Khandal, 2010. Antibacterial Activity of Raw and Processed Honey. Elect. J. Biol., 5 (3): 58-66.

72. Ghanem, N.B. 2011. The Antimicrobial Activity of Some Honey Bee Products and some Saudi Folkloric Plant Extracts. JKAU: Sci., 23 (2): 47-62.

73. Zeedan, G.S., S.A. Alharbi, A.M. Abdalhamed and E.S. Khatar 2016. Antimicrobial Effect of Honey and some Herbal Plant Extracts against Multidrug Resistance Bacteria Isolated from Patient in Local Riyadh Hospital. Int. J. Adv. Res., 4 (4): 283-291.

74. Hern, T.T., A.R. Rosliza, H.G. Siew, S.H. Ahmad, A.H. Siti, A.S. Siti and B.S. Kirnpal-Kaur, 2009. The Antibacterial Properties of Malaysian Tualang Honey against Wound and Enteric Microorganisms in Comparison to Manuka Honey. BMC Complementary Altern. Med., 9 : 34.