Trop J Nat Prod Res, March 2021; 5(3):470-477 **ISSN 2616-0684 (Print)**
ISSN 2616-0692 (Electronic)

Tropical Journal of Natural Product Research

Available online at https://www.tjnpr.org

Original Research Article

Antibacterial and Antioxidant Activities of Local Honey from Jordan

Khaled M. Khleifat[1], Haitham Qaralleh[2]*, Muhamad O. Al-limoun[1], Enas M. Al-khlifeh[3], Sundus A. Aladaileh[1], Nafi Tawarah[2], Ibrahim S. Almajali[2]

[1]*Biology Department, Mutah University, Mutah, Karak, 61710, Jordan*
[2]*Department of Medical Laboratory Sciences, Mutah University, Mutah, Karak, 61710, Jordan*
[3]*Department of Medical Laboratory Science, Faculty of Science, Al-Balqa Applied University, Al-Salt, 19117, Jordan*

ARTICLE INFO

Article history:
Received 21 November 2020
Revised 30 January 2021
Accepted 05 March 2021
Published online 01 April 2021

ABSTRACT

The medical usage of honey has become popular as an alternative and complementary treatment in recent time. This study was aimed at determining the inhibitory effect of honey and propolis against various species of Gram-positive and Gram-negative bacteria. The antibacterial activity was assessed using the disc diffusion and macro-dilution methods. The antioxidant activity was measured using the 2,2-diphenyl-1-picryl hydrazyl (DPPH) radical scavenging method. The tested honey showed antibacterial activity only against *Escherichia coli* and *Staphylococcus saprophyticus*. All bacteria tested showed less degree of susceptibility to propolis treatment; producing less than 15 mm zone of inhibition. The propolis exhibited different antibacterial activity compared to the honey, the propolis inhibited the growth of gram-positive bacteria (*S. aureus* and *B. cereus*) while the honey inhibited *S. saprophyticus* and *E. coli*. In the DPPH scavenging assay, the scavenging activities of local honey and propolis ranged from 9.81 to 90.70% and from 25.33 to 73.47%, respectively, with IC_{50} values of 4.5 mg/mL and 7.5 µg/mL, respectively. Therefore, the locally available natural honey may be used as a rich source of antioxidant and as an effective antibacterial agent that may be used to combat bacterial infections due to susceptible organisms.

Keywords: Honey, Propolis, Antibacterial, Antioxidant.

Introduction

In recent times, an obvious rise in antibiotic resistance by pathogenic bacteria has been noticed. This rise is due to the overuse and misuse of antibiotics and has resulted in many highly resistant bacterial strains such as Methicillin-resistant *Staphylococcus aureus* (MRSA. For this reason, better alternative agents apart from the traditional antibiotics is being explored. Traditionally, bee products including honey, propolis and the royal jelly have been applied to heal wounds, treat gastrointestinal tract, respiratory tract and eyes infections. Besides, honey is used as a nutrient for health maintenance and to enhance vital body functions.[1,2]

Currently, honey and other bee products receive great attention in medicinal and pharmaceuticals research.[3–7] In addition to their broad biological activities, honey consist of a variety of bioactive compounds. Honey and propolis possess antimicrobial, antioxidant, anti-leishmanicidal,[8] anticancer,[9] hepatoprotective,[10] and cardioprotective[11] activities. Honey is rich with several bioactive compounds such as α-tocopherol, phenolic acid, flavonoid, ascorbic acid, carotenoids and proteins.[1,2,12] In addition to vitamins and minerals, propolis appears to be rich in phenolic compounds, flavonoids and terpenes.[13,14] The antibacterial activity of honey is due to their osmolarity, acidity or constituents from flora sources.[15] Studies have demonstrated the antimicrobial activity of honey and evaluated the effects of the high osmolarity, the acidity, sugar content on the antimicrobial activity its high sugar content. It was concluded that the honey has antimicrobial substances.[2,5,15-17]

Due to the variation of botanical origin, honey differs in their appearance and this may reflect in their chemicals in term of composition and concentration. Therefore, the study of the bioactivity of honey on the basis of the differences in the environmental conditions and plant variation is highly recommended. This study was aimed at evaluating the antibacterial and antioxidant activities of honey and propolis samples collected from Jordan.

Materials and Methods

Sample collection

Honey samples were collected from the local bee farmers in northern Ghore, Karak, Jordan. The first harvesting took place in March 2017 (springtime) while the second harvesting time was in June, 2017. Three samples were collected on each harvesting time. Propolis samples were also provided by the same farmers in March and June 2017. The samples were collected in sterile glass screwed bottles.

Extract of propolis

The propolis was extracted using 70% ethanol. Thirty grams (30 g) of raw propolis were soaked in 100 mL of 70% ethanol for 7 days at room temperature. Then the solvent was filtered and clarified using Millipore filter syringe. The dry weight was estimated after evaporation of the solvent at 50°C.[18]

Bacterial strains

Four Gram-positive bacterial species including *Staphylococcus aureus* (25923), *Staphylococcus saprophyticus*, *Bacillus cereus* (ATCC 11778) and *Enterococcus faecalis* and six Gram-negative bacterial species including *Shigella flexneri* (ATCC 12022), *Salmonella* Typhimurium (ATCC 14028), *Klebsiella oxytoca* (ATCC 700324), *Pseudomonas aeruginosa* (ATCC 10145), *Escherichia coli* (ATCC 25922) and *Enterobacter aerogenes* (ATCC 13048) were used in this study. The species were provided by the medical laboratory of the Al-

*Corresponding author. E mail: haitham@mutah.edu.jo
Tel: 00962-797489248

Citation: Khleifat KM, Qaralleh H, Al-limoun MO, Al-khlifeh EM, Aladaileh SA, Tawarah N, Almajali IS. Antibacterial and Antioxidant Activities of Local Honey from Jordan. Trop J Nat Prod Res. 2021; 5(3):470-477. doi.org/10.26538/tjnpr/v5i3.10

Official Journal of Natural Product Research Group, Faculty of Pharmacy, University of Benin, Benin City, Nigeria.

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

Karak Governorate hospital, Karak, Jordan.[12,19–21]. Overnight bacterial suspension adjusted to 0.5 McFarland's standard ($1.5x10^8$ CFU/mL) was used in all the antibacterial tests.

Disc diffusion method

Disc diffusion method was performed using Mueller-Hinton agar. Hundred microliter of each bacterial species ($1.5x10^8$ CFU/mL) was streaked on the surface of the agar plate. Then, sterile blank disc impregnated in 100 µL of each sample was transferred to the surface of the inoculated plate at equal distance with the control. Standard antibiotics including cefoxitin (10 µg), cloxacillin (10 µg), lincomycin (10 µg) and gentamicin (10 µg) were used as positive controls. After incubation for 24 h at 37°C, the inhibition zone diameter was measured in mm.[22]

MIC determination

The minimum inhibitory concentration was determined using macrodilution method according to Kacaniova *et al*.[16]. Briefly, eight sterile test tubes containing 1 mL nutrient broth were prepared and labelled (1 to 8). Honey control tube (HC) and growth control tube (GC) was used as quality control. Then, 1 mL of honey sample (undiluted) was added to tube no. 1 and tube HC. Then serial dilution using the same broth media was performed to give 1:1; 1:2; 1:4; 1:8; 1:16 and 1:32, fractions respectively. The GC tube received no honey and served as a the growth control while the HC tube received no bacterial inoculums and served as a the honey control. Then, 1 mL of the cultured bacteria was transferred to all tubes except HC tube. After 24 h incubation at 37°C, the concentration that showed no visible growth using spectrophotometer (600 nm) was assigned as MIC. Using the same method, the MIC values of propolis and sugar solution were determined.[15]

To determine the MBC, the incubated tubes having no sign of visible growth (growth/turbidity) in MIC, were sub-cultured onto sterile nutrient agar plates by streak plate method with 24 hours of aerobic incubation at 37°C. The lowest concentration of honey that did not show growth of tested organisms was considered as the MBC.[16]

Inhibition effect of different concentrations of honey and sugar solution on the tested strains

Decreasing concentrations of honey and sugar solution were each prepared in two-fold serial dilutions using nutrient broth. The sugar solution was prepared to contain the following: 46.5% fructose, 34% glucose, 1.5% sucrose and 18% water. Bacteria suspension adjusted to $1.5x10^8$ CFU/mL was added to an equal volume (5 mL) of each concentration. Growth control tube was prepared without honey or the sugar solution. An uninoculated tube of nutrient broth was incubated to serve as the negative growth control. After overnight incubation at 37°C, the tubes were examined for turbidity indicating growth of the microorganism at 600 nm.

2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity

The antioxidant potential of honey and propolis samples was measured using DPPH· as already described by Tarawneh *et al*.[23] with some modifications. Briefly, various volumes of the tested samples solutions (2.0 mg/mL) were mixed with 2.0 mL of DPPH methanol solution (0.1192 mmol/L). The mixtures were incubated in the dark for 30 min at room temperature and the absorbance was measured at 517 nm. Methanol and Trolox were used as negative control and positive control, respectively.

The antioxidant activity of the tested samples was measured by calculating the scavenging capacity of the DPPH radical according to the following formula:

% of Inhibition DPPH = ((Abs DPPH –Abs sample) / Abs DPPH)*100

The results were expressed as IC_{50} value (µg/mL).[23,24]

Statistical analysis

All experiments were performed in triplicate. Data were expressed as mean ± standard deviation (SD). Statistical analysis was performed using SPSS. Data are considered different if it had significance value of $P < 0.05$.

Results and Discussion

Antibacterial activity of honey

Disc diffusion method

Preliminary screening using disc diffusion assay showed that the honey sample tested had no antimicrobial activity against most of the bacterial isolates except *E. coli* and *S. saprophyticus* (Table 1). Comparing the inhibition diameters between the control antibiotics and the honey at different concentrations, no significant differences in the values between these antibiotics were observed. This may suggest that the antibacterial activities of honey at these concentrations are comparable to that of the tested antibiotics. Antibiotic evaluation tests were made, by using commercial antibiotic permeated discs; cefoxitin, cloxacillin, lincomycin and gentamicin. Of the antibiotics tested, cefoxitin and gentamicin were the most effective against all ten bacteria, except for *S. aureus* and *S. saprophyticus*, which were most susceptible to cefoxitin, cloxacillin and lincomycin (Table 1). Of the four antibiotics, lincomycin displayed less inhibitory effects with the zone of inhibition produced being 16 mm against *S. saprophyticus*. The honey was in certain instances, comparable to cefoxitin and gentamicin, which were the most effective treatments overall. On the other hand, honey was the most effective product against *E. coli* and *S. saprophyticus*.

Using honey as antibacterial agent was generally attained by applying high concentration ranging from undiluted to 1:4 ratio (for example, against *S. flexneri*). The antibacterial activity of honey was achieved mainly by the high concentrations. This suggests that, honey is commonly beneficial in the treatment of bacterial infections as it can be directly applied to the bacteria infected body surfaces such as skin diseases, septic wounds and eye infections.[15] This was also in parallel with the results of clinical study where honey was used in the treatment of septic wound and burns. For example, it was shown that honey reduced superficial septic wounds better than using Savlon antiseptic.[25,26] Research showed that cutaneous infected wounds in buffalo calves which were experimentally induced healed significantly faster with natural honey than ampicillin ointment and honey-ampicillin mixture.[27] There are great antimicrobial activity dissimilarities shown by some natural honeys, because of the spatial and temporal variation in sources of nectar. Other reports have confirmed that variation in antibacterial activity of honey can be traced to the available nectars and pollens.[17,28] It can be observed that both Gram-positive (*S. saprophyticus*) and Gram-negative (*E. coli*) bacteria were inhibited by the honey collected during the springtime. Many studies have demonstrated the antibacterial activity of honey against Gram-positive and Gram-negative bacteria. *S. aureus* and *E. coli* are among the most studied microorganisms.[29]

Bacteriostatic and bactericidal activity of honey

The results of antibacterial activity achieved from the macro-dilution assays for honey samples are presented in tables 2 and 3. The inoculated plates were scored as bactericidal if no growth; bacteriostatic if there is minor to moderate growth and no antibacterial activity if there is substantial growth according the record of Payveld (1986). Table 2 shows the results of antibacterial activity attained by honey and the sugar solution against 10 species of bacteria. It is clear that the growth of *S. typhimuruim, K. oxytoca, E. coli* and *E. faecalis* were mostly inhibited by the highest ratio of honey:distilled water mixtures used as compared with the rest of tested bacterial genera used (Table 2). The minimal bactericidal concentrations (MBCs) for *K. oxytoca, S. aureus, B. cereus* and *E. aerogenes* were undiluted, *S. saprophyticus* and *E. faecalis* was 1:1(50%), *S. typhimurium* and *E. coli* were 1:2 (33%) and finally *S. flexneri* was 1:4 (25%).

The honey is known to have low water activity as a result of high sugar concentration or super-saturated solution of sugars. In this study, high concentration of sugar solution was made in a manner similar to the property of high sugar concentration in honey, which leads to the high osmolarity that could produce antimicrobial activity (Table 2). Because the sugar solution was similar to honey in its high sugar composition, the bacteriostatic and bactericidal activity achieved by pure honey (undiluted). Tables 3 and 4 showed the significance of the high sugar concentration in the antibacterial activity of honey. As

recorded in Table 3, the clear differences between the bacteriostatic activity of the diluted forms (1/2, 1/4, 1/8) of honey and the sugar solution and between the bactericidal activity of the undiluted as well as the diluted forms (1/2, 1/4, 1/8). In this study, the bacterial broth macrodilution method presents higher sensitivity compared to the disc diffusion assay, probably due to a greater availability of the active molecules in the liquid broth than in the agar. This behaviour can be explained by the possibility of honey being composed of substances that do not diffuse properly within the solid media, which could happen because of variances linked to the polarity of both molecule and the agar medium. In the liquid medium, however, this diffusion rate obstacle is naturally reduced. Previous studies had predicted that the diffusion of extracts of natural products have more hydrophobic characteristics that lead to the hindering of their spread in agar. In other words, inhibiting the diffusion of natural products is related to their water solubility and molecular weight. As recorded in Table 2 the clear differences between the bacteriostatic activity of the diluted forms (1/2, 1/4, 1/8) of honey and the sugar solution and between the bactericidal activity of the undiluted as well as the diluted forms (1/2, 1/4, 1/8) suggest the presence of other factors responsible for the antibacterial activity of the honey.[15] The honey samples showed bactericidal activity against the tested organisms up to the dilutions of 25%. This result is similar to those reported previously by Nzeako and Hamdi[30] but is at variance with the results of Willix *et al*.[31] Since honey's antimicrobial property is dependent on its water activity (the free water molecules in honey which is usually between 15 and 21%) in part, this will directly affect its osmotic effect. It is then rational to assume that a more diluted honey may have lost its antibacterial ability. The range of total content of reducing sugars in honey is generally between 69.6 - 80% depending on which seasons the honey is harvested or if it wild honey.[32,33] Fructose concentration in honey may be between 36.9 and 47.3%.[33] Glucose concentration may be between 27 and 34.9%.[32,33] Sucrose concentration may be between 0.2 and 2.7%. Maltose concentration may be between 0.7 and 11%. Melezitose concentration may be 0.6%.[15,34]. The water content of honey may comprise 12.4-20.3%;[34] 19.9-20.3%;[32] or 16.3-18.5%.[35]

Inhibitory effect of different concentrations of honey and sugar solution on the tested strains

The inhibitory effect of different concentrations of honey and sugar solution on the tested strains was evaluated using spectrophotometer at 600 nm. As shown in figures 1a-d and 2a-f, honey exhibited stronger inhibitory effects than the sugar solution against all strains tested. These results are consistent with the honey's antibacterial activity tests in tables 1-4. In addition, the IC_{50} values of the honey and sugar solution against the Gram-positive bacteria were lower than the IC_{50} against the gram-negative bacteria. In particular, the IC_{50} of honey against *S. saprophyticus*, *S. aureus*, *B. cereus* and *E. faecalis* were 10, 10, 20 and 10%, respectively. The IC_{50} of sugar solution were 30, 30, 40 and 60%, respectively. Regarding Gram-negative bacteria, the IC_{50} of honey against *E. coli*, *P. aeruginosa*, *K. oxytoca*, *S. Flexneri*, *E. aerogenes* and *S. typhimurium* were 10, 30, 10, 10, 20 and 10%, respectively, whereas the IC_{50} of sugar solution were 30, 60, 30, 30, 30 and 10, respectively.

Antibacterial activity of propolis

Disc diffusion assays

The bacteria tested showed the least amount of susceptibility to propolis with each producing a zone of inhibition less than 15 mm (Table 5). *S. aureus* showed 13 mm zone of inhibition while *B. cereus* showed 14 mm. The ethanol and blank disc controls run for each bacterium produced no zone of inhibition, indicating that the ethanol solvent used has no effect on the antibacterial activity of the propolis. The action of propolis was different from that of honey, the propolis inhibited the growth of *S. aureus* and *B. cereus* (Table 5) whereas its honey harvested from the same hives inhibited the growth of *S. saprophyticus* and *E. coli*. Thus, it is favorable to use it as a mixture of both. It was reported that the use of propolis at concentrations below the MIC could increase the growth of the microorganisms, whereas at higher levels they may inhibit or even kill them. This event is known as "hormesis".[36]

Bacterial broth macrodilution assays

It is clear that local propolis when using macrodilution technique affected bacterial growth in the same manner as in the disc diffusion method (Table 4). *S. typhimurium*, *S. flexneri* and *S. saprophyticus* showed no susceptibility effect toward local propolis. At the same time a 1:1 (50%) of local propolis inhibited the growth of *S. aureus* and *B. cereus*. In contrast, Brazilian propolis as control showed better activity mainly with undiluted and 1:1(50%). The growth of all ten bacteria used were inhibited by 100% Brazilian propolis and five of the ten tested bacterial genera had susceptibility effect. These are *S. typhimurium*, *E. coil*, *B. cereus*, *E. aeruginosa* and *E. faecalis*. The bacteria chosen in this study are common infectious bacterial organisms. For example, *S. aureus* is a common agent in skin infections, food poisonings, and toxic shock syndrome. *E. coli*, while part of the body's normal intestinal flora, can be infectious and toxins producing, like *S. aureus* that resulted in food and water borne poisonings. *B. cereus* also, is a food borne pathogen that is prevalent in cream sauces, soups and rice. To treat such infections, honey and propolis have the potential to be useful as either prophylaxis for pre-infection control of contaminated foods and surfaces or as a post-infection treatment.[37] The observed differences in the activity of the two propolis samples with different concentrations, may also suggest that there could be regional differences along with the nature of honey and propolis production influencing the inhibitory activity as previously suggested.[7] In addition, the variations could be attributed to the occurrence of other components resulting from the nature of honey production, in that bees are capable of taking nectar from any kind of source that is available to them at the time.

The antioxidant activity of honey and Propolis

In the DPPH assay, the scavenging activity of local honey (Figure 3) and propolis (Figure 4) ranged from 9.81 – 90.7% and 25.33 – 73.47%, respectively, with the IC_{50} values of 4.5 mg/mL and 7.5 µg/mL, respectively. Therefore, the lowest inhibition caused by local honey and propolis samples were 9.81% and 25.33%, respectively, at concentrations of 5 mg/mL and 0.6 µg/mL, respectively. The highest concentrations of the honey and propolis samples used were 20 mg/mL and 20 µg/mL, respectively at which the scavenging activity were decreased to 90.7% for local honey and decreased to 73.47% for propolis. The locally available natural honey may be used as a rich source of antioxidant. The locally available natural as well as commercially available processed honeys (around the world) are reported to contain antioxidant components (such as phenolics and flavonoids), and to exert antioxidative activity with wide-ranging capacities.[38,39] The honey samples obtained from several countries had higher antioxidative ability for the sample with higher quantities of phenolic compounds. From Croatia, the IC_{50} values of chestnut honey were reported as 14.24-24.56 mg/mL, acacia honey as 52.06-176.57 mg/mL.[40] The excellent antioxidative activity with IC_{50} values of 3.1-5.05% was shown for Romanian honeydew honey and 2.39 - 5.11% for Polish honeydew honey.[40,41] The DPPH scavenging activity was estimated for Chinese honey samples from various floral sources. The IC_{50} values of the raw and processed honeys ranged from 126 - 625.79 µg/mL, with highest activity being noted in Trigona honey from Trivandrum, Kerala, India, having 97.21% inhibition at 500 µg/mL. The multifloral honey had DPPH free radical scavenging activity of 19.04-71.92%.[6,42] As shown earlier, the commercial honeys had antioxidant activity in the DPPH assay, with IC_{50} values between 66.73 and 132.24 mg/mL, which were higher than the IC_{50} values of tested honey and propolis samples used in the current study (5.5 mg/mL and 7.5 µg/mL, respectively) indicating that the locally produced honeys are more effective as antioxidant than the imported ones. The locally available natural honey may be used as rich source of antioxidant and can be used as effective antibacterial agent in order to combat the bacterial infection to humans.

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

Table 1: The inhibition Zone (mm) of the honey sample against the bacteria isolates

Bacterial species	Inhibition Zone (mm)								
	Honey					Antibiotics			
	0 .4 g/mL	0 .7 g/mL	1 g/mL	2 g/mL	2.5 g/mL	Cefoxitin	Cloxacillin	Lincomycin	Gentamicin
S. typhimurium	-	-	-	-	-	-	10	-	-
S. flexneri	-	-	-	-	-	20	-	-	-
K. oxytoca	-	-	-	-	-	19	-	-	14
E. coil	21.0 ± 0.0	20.0 ± 0.0	19.0 ± 0.5	15.0 ± 0.5	-	26	-	-	14
S. aureus	-	-	-	-	-	25	26	15	18
S. saprophyticus	30.0 ± 0.5	20.0 ± 0.0	19.0 ± 0.0	16.0 ± 0.0	-	35	23	16	-
P. aeruginosa	-	-	-	-	-	-	-		26
B. cereus	-	-	-	-	-	-	-	11	15
E. aerogenes	-	-	-	-	-	-	-	-	23
E. faecalis	-	-	-	-	-	8	15	-	-

-: no activity

Table 2: Inhibitory effect of different concentrations of honey and sugar solution on the bacterial isolates

Bacterial species	Honey							Sugar Solution		
	Undiluted	1/1	1/2	1/4	1/8	1/16	1/32	Undiluted	1/1	1/2
S. typhimurium	0	0	0MBC	T	T	T	T	0MBC	52000000	T
S. flexneri	0	0	0	0 mbc	100	T	T	0 mbc	16000000	T
K. oxytoca	0mbc	10000	15000000	T	T	T	T	620000	T	T
E. coil	0	0	0mbc	2000	6000000	T	T	12000000	T	T
S. aureus	0mbc	800000	1000000	4000000	T	T	T	T	T	T
S. saprophyticus	0	0mbc	20000	5000000	6000000	T	T	28000000	T	T
P. aeruginosa	0	0	T	T	T	T	T	0 mbc	3000000	T
B. cereus	0	10000	40000	T	T	T	T	0mbc	500000	T
E. aerogenes	0mbc	5000	10000	4000000	T	T	T	10000000	T	T
E. faecalis	0	0	5000	400000	500000	T	T	0	3200000	T

T = Turbid tubes (not counted); (MBC) = minimum bactericidal concentration: The minimum inhibitory concentration (MIC) is the concentration before the turbid tubes (T). All concentrations greater than MIC are inhibitory concentrations and all concentrations greater than MBC are bactericidal concentrations.

Table 3: The Bacteriostatic activity and Bactericidal activity of honey

Bactericidal activity				Bacteriostatic activity				Concentrations
Sugar solution		Honey		Sugar solution		Honey		
%	No.(T=10)	%	No.(T=10)	%	No.(T=10)	%	No.(T=10)	
50%	5	100%	10	100%	10	100%	10	Undiluted
0%	0	50%	5	50%	5	100%	10	1l1
0%	0	33%	3	0%	0	66%	6	1l2
0%	0	10%	1	0%	0	20%	2	1l4
0%	0	0%	0	0%	0	10%	1	1l8
0%	0	0%	0	0%	0	0%	0	1l16
0%	0	0%	0	0%	0	0%	0	1l32

No. = number of bacterial species; T = total number of bacterial species;. Honey had bactericidal concentration with ten species of bacteria (100%), whereas the sugar solution had bactericidal concentration with five species (50%) .and 34.9%.[32,33] Sucrose concentration may be between 0.2 and 2.7%. Maltose concentration may be between 0.7 and 11%. Melezitose concentration may be 0.6%.[15,34]. The water content of honey may comprise 12.4-20.3%;[34] 19.9-20.3%;[32] or 16.3-18.5%.[35]

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

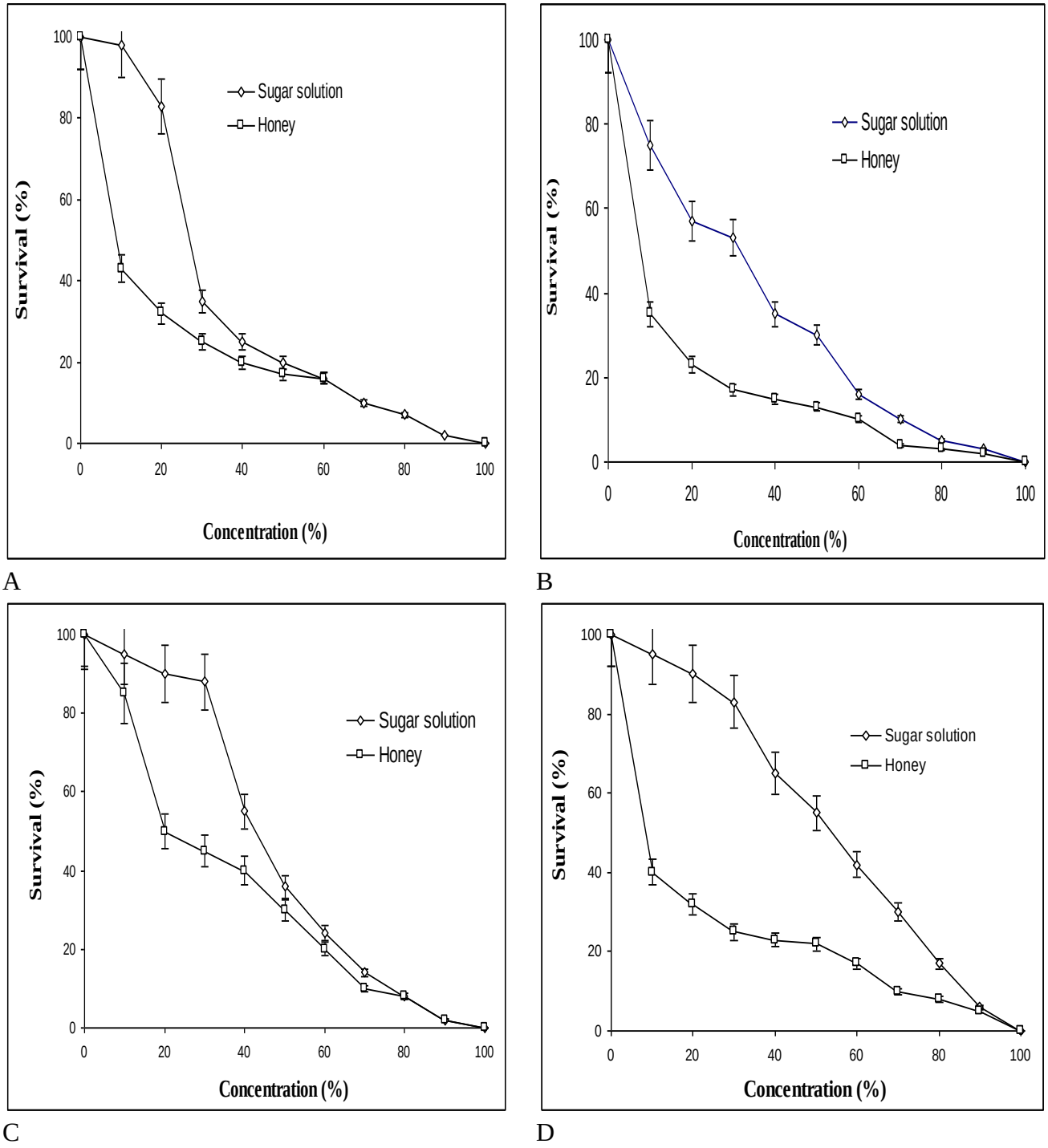

Figure 1: Effect of different concentrations of honey and sugars (v/v) on the growth of gram-positive bacteria (a) *Staphylococcus saprophyticus*, (b) *Staphylococcus aureus*, (c) *Bacillus cereus* and (d) *Enterococcus faecalis.* Growth was measured at 600 nm

Table 4: The inhibition zone (mm) of the propolis sample against *B. cereus* and *S. aureus*

Bacterial species	Inhibition zone (mm)						
	10 mg/mL	30 mg/mL	50 mg/mL	70 mg/mL	100 mg/mL	200 mg/mL	Honey + propolis
S. aureus	-	-	9.0 ± 0.5	11 ± 0.0	11 ± 0.0	11 ± 0.0	13 ± 0.0
B. cereus	-	12.0 ± 0.0	14.0 ± 0.0	14 ± 0.0	14 ± 0.0	14 ± 0.5	16 ± 0.0

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

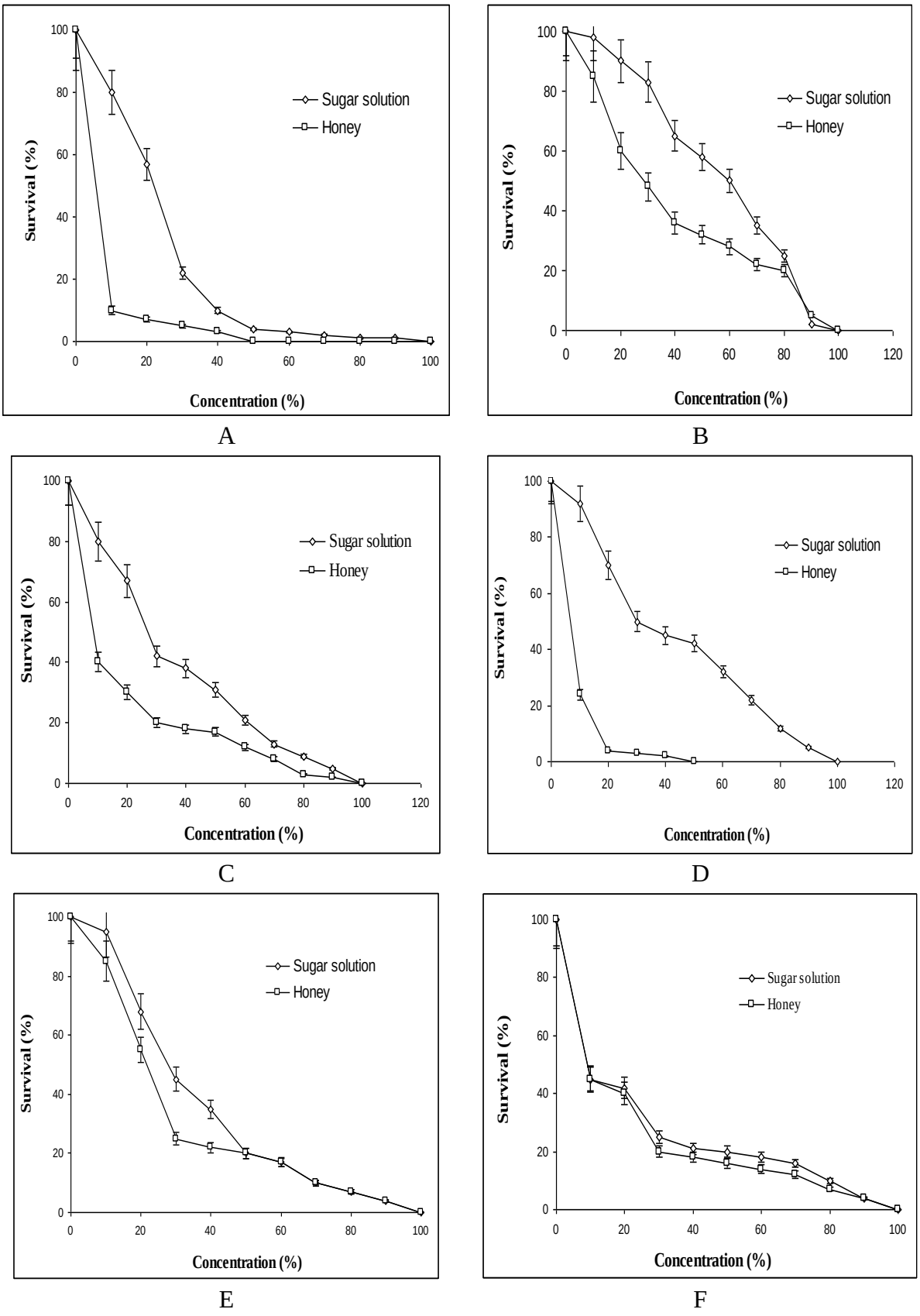

Figure 2: Effect of different concentrations of honey and sugars (v/v) on the growth of gram-negative bacteria; (a) *Escherichia coli*, (b) *P. aeruginosa*, (c) *Klebsiella oxytoca* and (d) *Shigella flexneri* (e) *Enterobacter aerogenes* and (f) *Salmonella typhimurium*. Growth was measured at 600 nm

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

Table 5: Inhibition effect of different concentrations of local propolis and Brazilian propolis on the bacterial isolates

	local propolis					Brazilian propolis				
	Undiluted	1/1	1/2	1/4	1/8	Undiluted	1/1	1/2	1/4	1/8
S. typhimurium	0	0 mbc	80000000	T	T	32000000	T	T	T	T
S. flexneri	0MBC	20000000	40000000	T	T	2000000	44000000	T	T	T
K. oxytoca	0MBC	10000000	16000000	T	T	0	1000000	T	T	T
E. coil	0	0MBC	8000000	T	T	0	2000000	40000000	T	T
S. aureus	0MBC	16000000	28000000	T	T	0	0	8000000	T	T
S. saprophyticus	0MBC	2000000	T	T	T	4000000	6000000	T	T	T
P. aeruginosa	0MBC	2800000	T	T	T	T	T	T	T	T
B. cereus	0	0MBC	20000000	16000000	T	0	0	4000000	T	T
E. aerogenes	0	0MBC	T	T	T	T	T	T	T	T
E. faecalis	0	0MBC	T	T	T	T	T	T	T	T

T = Turbid tubes (not counted); (MBC) = minimum bactericidal concentration: The minimum inhibitory concentration (MIC) is the concentration before the turbid tubes (T). All concentrations greater than MIC are inhibitory concentrations and all concentrations greater than MBC are bactericidal concentrations.

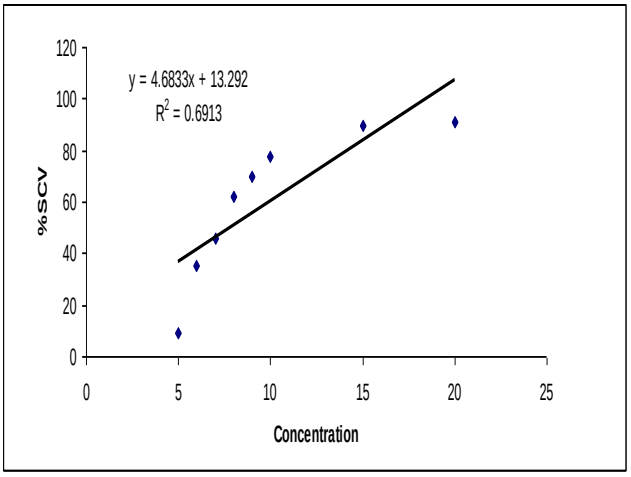

Figure 3: The antioxidant of honey (mg/mL) by using DPPH

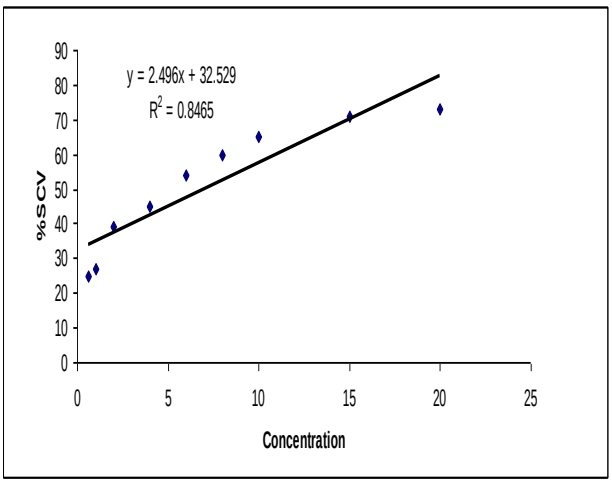

Figure 4: The antioxidant of *Propolis* (mg/mL) by using DPPH

Conclusion

The locally available natural honey showed antibacterial activity at lower concentrations against both gram-positive and gram-negative bacteria. At higher concentration, propolis was also active against gram-positive bacteria. Honey and its propolis as a mixture can be recommended for preventative and therapeutic uses against infections caused by the tested bacterial isolates.

Conflict of interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

References

1. Ferreres F, García-Viguera C, Tomás-Lorente F, Tomás-Barberán FA. Hesperetin: A marker of the floral origin of citrus honey. J Sci Food Agric. 1993; 61(1):121-123.
2. Moniruzzaman M, Khalil MI, Sulaiman SA, Gan SH. Advances in the analytical methods for determining the antioxidant properties of honey: a review. Afr J Trad Compl Altern Med. 2012; 9(1):36-42.
3. Shapla UM, Solayman M, Alam N, Khalil MI, Gan SH. 5-Hydroxymethylfurfural (HMF) levels in honey and other food products: effects on bees and human health. Chem Cent J. 2018; 12(1):1-18.
4. Kurek-Górecka A, Górecki M, Rzepecka-Stojko A, Balwierz R, Stojko J. Bee products in dermatology and skin care. Molecules. 2020; 25(3):556.
5. Maroof K and Gan SH. A Review on Chemical Compositions, Biological Activity and Formulation Techniques of Malaysian Honey Bee and Meliponine Propolis. J Biol Act Prod Nat. 2020; 10(6):507-523.

6. Temizer İK, Güder A, Çelemli ÖG. Botanic origin, various physicochemical and antioxidant properties of honey samples from Giresun, Turkey. J Biol Chem. 2016; 44:209-215.
7. Osato MS, Reddy SG, Graham DY. Osmotic effect of honey on growth and viability of Helicobacterpylori. Dig Dis Sci. 1999; 44(3):462-464.
8. Devequi-Nunes D, Machado BAS, Barreto G de A, Rebouças Silva J, da Silva DF, da Rocha JLC, Brandão HN, Borges VM, Umsza-Guez MA. Chemical characterization and biological activity of six different extracts of propolis through conventional methods and supercritical extraction. PLoS One. 2018; 13(12):e0207676.
9. Elnakady YA, Rushdi AI, Franke R, Abutaha N, Ebaid H, Baabbad M, Omar MOM, Al Ghamdi AA. Characteristics, chemical compositions and biological activities of propolis from Al-Bahah, Saudi Arabia. Sci Rep. 2017; 7(1):1-13.
10. Wang Y, Li D, Cheng N, Gao H, Xue X, Cao W, Sun L. Antioxidant and hepatoprotective activity of Vitex honey against paracetamol induced liver damage in mice. Food Func. 2015; 6(7):2339-2349.
11. Afroz R, Tanvir EM, Karim N, Hossain M, Alam N, Gan SH, Khalil M. Sundarban honey confers protection against isoproterenol-induced myocardial infarction in Wistar rats. Biomed Res Int. 2016; 2016:6437641.
12. Abboud MM, Aljundi IH, Khleifat KM, Dmour S. Biodegradation kinetics and modeling of whey lactose by bacterial hemoglobin VHb-expressing Escherichia coli strain. Biochem Eng J. 2010; 48(2):166-172.
13. Lotfy M. Biological activity of bee propolis in health and disease. Asian Pac J Cancer Prev. 2006; 7(1):22-31.
14. Huang S, Zhang C-P, Wang K, Li GQ, Hu F-L. Recent advances in the chemical composition of propolis. Molecules. 2014; 19(12):19610-19632.
15. Wahdan. Causes of the Antimicrobial Activity of Honey Infection. Food Microbiology Laboratory, Central Health Laboratories, Ministry of Health, 19 El Sheik Rihan St El Falaky, Cairo Egypt. 1998; 26(1):26-31p.
16. Kačániová M, Vuković N, Bobková A, Fikselová M, Rovná K, Haščík P, Čuboň J, Hleba L, Bobko M. Antimicrobial and antiradical activity of Slovakian honeydew honey samples. J Microbiol Biotechnol Food Sci. 2020; 9(6):354-368.
17. Allen KL, Molan PC, Reid GM. The variability of the antibacterial activity of honey. Apiacta. 1991; 26(4):114-121.
18. Fernandes JA, Balestrin EC, Betoni JEC, Orsi R de O, Cunha M de LR de S da, Montelli AC. Propolis: anti-Staphylococcus aureus activity and synergism with antimicrobial drugs. Mem Inst Oswaldo Cruz. 2005; 100(5):563-566.
19. Khleifat KM, Abboud MM, Al-Mustafa AH, Al-Sharafa KY. Effects of carbon source and Vitreoscilla hemoglobin (VHb) on the production of β-galactosidase in Enterobacter aerogenes. Curr Microbiol. 2006; 53(4):277-281.
20. Tarawneh KA, Al-Tawarah N, Abdel-Ghani AH, Al-Majali AM, Khleifat KM. Characterization of verotoxigenic Escherichia coli (VTEC) isolates from faeces of small ruminants and environmental samples in Southern Jordan. J Basic Microbiol. 2009; 49(3):310-317.
21. Qaralleh H, Al-Limoun M, Khlaifat A, Khleifat K, Al-Tawarah N, Hashem A-H, Alsharafa K. Antibacterial and Antibiofilm Activities of a Traditional Herbal Formula against Respiratory Infection Causing Bacteria. Trop J Nat Prod Res. 2020; 4(9):527-534.
22. Al-Asoufi A, Khlaifat A, Tarawneh AA, Alsharafa K, Al-Limoun M, Khleifat K. Bacterial Quality of Urinary Tract Infections in Diabetic and Non Diabetics of the Population of Ma'an Province, Jordan. Pakistan journal of biological sciences: PJBS. 2017 ;20(4):179-88.
23. Tarawneh KA, Irshaid F, Jaran AS, Ezealarab M, Khleifat KM. Evaluation of antibacterial and antioxidant activities of methanolic extracts of some medicinal plants in northern part of Jordan. J Biol Sci. 2010; 10(4):325-332.
24. Jaafreh M, Khleifat KM, Qaralleh H, Al-limoun MO. Antibacterial and Antioxidant Activities of Centeurea damascena Methanolic Extract. J Basic Appl Res Biomed. 2019; 5(1):55-63.
25. Hamdy MH, El-Banby MA, Khakifa KI, Gad EM, Hassanein EM. antimicrobial effect of honey in the management of septic wounds. In: Proceedings of the Fourth International Conference on Apiculture in Tropical Climates, Cairo, Egypt, 6-10 November 1988/Hosted by the Government of Egypt; Convened by the International Bee Research Association. London: Int Bee Res Assoc. 1989; 1989.
26. Althunibat OY, Qaralleh Q, Al-Dalin SYA, Abboud M, Khleifat K, Majali IS, Aldal'in HKH, Rayyan WA, Jaafraa A. Effect of Thymol and Carvacrol, the Major Components of Thymus capitatus on the Growth of Pseudomonas aeruginosa. J Pure Appl Microbiol. 2016; 10(1):367-374.
27. Qaralleh H, Khleifat KM, Al-Limoun MO, Alzedaneen FY, Al-Tawarah N. Antibacterial and synergistic effect of biosynthesized silver nanoparticles using the fungi Tritirachium oryzae W5H with essential oil of Centaurea damascena to enhance conventional antibiotics activity. Adv Nat Sci Nanosci Nanotechnol. 2019; 10(2):25016.
28. Allen KL, Molan PC, Reid GM. A survey of the antibacterial activity of some New Zealand honeys. J Pharm Pharmacol. 1991; 43(12):817-822.
29. Boukraâ L, Niar A, Benbarek H, Benhanifia M. Additive action of royal jelly and honey against Staphylococcus aureus. J Med Food. 2008; 11(1):190-192.
30. Nzeako BC and Hamdi J. Antimicrobial potential of honey on some microbial isolates. Sultan Qaboos Univ Med J [SQUMJ]. 2000; 2(2):75-79.
31. Willix DJ, Molan PC, Harfoot CG. A comparison of the sensitivity of wound-infecting species of bacteria to the antibacterial activity of manuka honey and other honey. J Appl Bacteriol. 1992; 73(5):388-394.
32. Kim JG, Kim MK, Lee SH. Study on the content of Oligosaccharides in honey by honey plants. Korea J Apic (Korea Republic). 1994; 8(2):165-196.
33. Chou T-C, Wu A-B, Chen CY. Sugar content of honey from wild and kept honeybees. J Food Drug-Analysis. 1994; 2(2):97-102.
34. Huidohro JF and Simal J. Estimation of sugars in honey. Ann Bromatol. 1985; 36(2):247-264.
35. Harman AW. Hive products for therapeutic use. Am Bee J. Published online. 1983; 39-44.
36. Calabrese EJ and Baldwin LA. Hormesis as a biological hypothesis. Environ Health Perspect. 1998; 106(suppl 1):357-362.
37. Viuda-Martos M, Ruiz-Navajas Y, Fernández-López J, Pérez-Álvarez JA. Functional properties of honey, propolis, and royal jelly. J Food Sci. 2008; 73(9):R117-R124.
38. Moussa A, Saad A, Noureddine D, Alvarez-Suarez JM, Tulipani S, Romandini S, Bertoli E, B. How Honey Acts as an Antioxidant?. Mediter J Nutr Metab. 2012; 3:15-23.
39. Chua LS, Rahaman NLA, Adnan NA, Eddie Tan TT. Antioxidant activity of three honey samples in relation with their biochemical components. J Anal Methods Chem. 2013; 2013.
40. Roy S, Mandal M, Pal NK, Das MK, Halder D, Sircar B, Mandal S. Exploration of antibacterial and antioxidative property of two natural honey samples from Malda district, India. Transl Med. 2016; 6(4):1000187.
41. da Silva Silveira LM, Olea RSG, Mesquita JS, da Cruz A de LN, Mendes JC. Metodologias de atividade antimicrobiana aplicadas a extratos de plantas: comparação entre duas técnicas de ágar difusão. Rev Bras Farm. 2009; 90:2.
42. Ukkuru VKPM. Phytochemical screening and antioxidant activity of diff erent bee honeys. J Med Herbs Ethnomed. 2015; 1(1):38-44.