Pharmacogn J. 2021; 13(1): 217-225
A Multifaceted Journal in the field of Natural Products and Pharmacognosy
www.phcogj.com

Research Article

Antimicrobial Effect of Different Types of Honey on Selected ATCC Bacterial Strains

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ABSTRACT

Honey is a complex sweet highly viscous liquid and is composed of various ingredients such as sugar, proteins, minerals, and polyphenolic compounds. Honey is used in traditional medicine systems for centuries because of its ability to inhibit the pathogenesis of various diseases through modulating various biological activities. In this study, antimicrobial potential of different types of honey was tested against selected pathogenic bacterial strains through agar well diffusion method. Four types of honey were used in the present investigation, and the potential antimicrobial activities of these varieties were further compared with that of antibiotics commonly used against targeted microbial strains. Among all of the four tested honey, three types were classified as blossom honey, being Talha *(Acacia sp)* honey (TH), Zahoor (mixed flower) honey (ZH), and Manuka (*Leptospermum* based honey) honey (MH). Both gram positive and gram negative bacterial species were used for this investigation. The pathogenic gram-positive bacterial strains included *Staphylococcus aureus* ATCC 29213, *Enterococcus faecalis* ATCC 29212 and methicillin-resistant Staphylococcus (MRSA) ATCC 43300. Three gram negative bacteria like *Escherichia coli* ATCC 25922, *Klebsiella pneumonia* ATCC 700603, and *Pseudomonas aeruginosa* ATCC 27853 were also used to confirm the antimicrobial activities. As reflected from our study, we found that three varieties of honey including TH, ZH, and MH (with different UMF +20, +16, +5) displayed a broad spectrum antibacterial activity against all tested microbial strains. However, all strains showed a high frequency of resistance to BF honey. Gram-positive (G+) bacteria were found to be more sensitive to all tested honey types except (BF) as indicated by significantly higher zone of inhibition (ZOI) values than those of gram-negative (G-) bacteria. As a conclusion, this study suggests that antimicrobial potential of honey types might be helpful in order to treat the pathogenic microorganisms threatening the public health and changing antibiotics into last-resort drugs.

Key Words: Talha honey, Zahoor honey and Manuka honey, *Staphylococcus aureus*, *Enterococcus faecalis*.

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History

- Submission Date: 05-11-2020;
- Review completed: 28-11-2020;
- Accepted Date: 08-12-2020.

DOI : 10.5530/pj.2021.13.31

Article Available online

http://www.phcogj.com/v13/i1

INTRODUCTION

Honey is a complex sweet liquid with greater viscosity made by bees. It is graded by color, with clear, golden amber and darker honey. The flavor of each type of honey varies, depending on the types of flowers.[1] Honey is a complex food substance, comprised of approximately 200 deferent substances, including sugar, water, proteins, vitamins, minerals, polyphenolic compounds and plant derivatives.[2,3] Honey can be classified as honeydew and blossom. Honeydew honey is produced by collection of living plant, aphid and insect secretions whereas blossom honey is produced by collection of flower nectar and characterized by pollen content. Blossom honey can be subdivided into uni-floral, which is from one flower species, or multiflora where multiple sources of flower species. The composition of active compounds present within plant nectar can vary, depending on geographical location and climate conditions.[4,5] Honey has antimicrobial and antioxidant properties in which the first observations of the antimicrobial activity of honey described in 1892. The honey has then been observed to have a broad spectrum of activity. It can also inhibit both Gram positive and Gram negative organisms including *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneuomniae*, *Staphylococcus aureus*, *Bacillus subtilis* and *Listeria monocytogens* and their multidrug-resistant counterparts.[6,7] Observing the broad spectrum of activity showed by honey, mostly against MDR and many types of antibiotics have led to investigate honey–antibiotic synergy with some promising effects.[8] Honey contain different types of substances contributing to eradicate and kill microbial pathogen and these substances are classified into two categories, being direct inhibitory factors affecting the cellular mechanism such as polyphonic component, hydrogen peroxide, methylglyoxal (MGO) and bee-defensin1, and indirect inhibitory factors which have a wider ranging effect on the bacterial cell such as high osmatic pressure, high sugar content and low pH.[9] A recent study showed that Manuka +20, Manuka +16 and Manuka +10 have strong antibacterial activities against both sensitive and resistant *P. aeruginosa* strains in addition to *P. aeruginosa* ATCC 27853 which served as control. A previous study showed that Manuka honey of lower UMF grade demonstrated equal to significantly increased antimicrobial activity compared to higher

Cite this article: Aljaghwani A, Allemailem KS, Aljaghwani LF, Alrumaihi F, Joseph RJ, Khan AA, *et al.* Antimicrobial Effect of Different Types of Honey on Selected ATCC Bacterial Strains. Pharmacog J. 2021;13(1): 217-25

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UMF grade honey for all tested organisms. The study included 128 bacterial organisms that were selected for antimicrobial susceptibility testing, being coagulase-positive and coagulase-negative *Staphylococci*, enteric gram-negative bacilli and *P. aeruginosa.* MRSA and some MDR bacteria were included.[10,11]

A very recent study demonstrated that Talha honey had higher antimicrobial activity than Sidr honey which showed strong antibacterial activities against pathogenic gram-positive bacteria including *Bacillus cereus* and *S. aureus,* and gram-negative bacteria including *E coli* and *Salmonella enteritidis*.[12] A previous study showed that black forest honey had highest antibacterial activity against *P. aeruginosa* ATCC 27853 and least with *E. coli* ATCC 25922.[13]

This study aimed to evaluate the antimicrobial potential of different types of honey including Talha (Acacia sp.) honey, Zahoor honey (mixed flower) and Manuka honey (Leptospermum based honey) with different UMF (+20, +16, +5) against selected pathogenic bacterial strains. Their potential antimicrobial activities were also compared with that of antibiotics commonly used against targeted microbial strains.

MATERIALS AND METHODS

Honey samples

Four types of honey were used. Three types were classified as blossom honey, being Talha *(Acacia sp*) honey, Zahoor honey (mixed flower) and Manuka honey (*Leptospermum based honey*) with different UMF (+20, +16, +5). These honeys are usually made from the nectar of the flowers. One type is classified as honeydew, being Black Forest honey which is produced from trees. It can also be produced from larger species of grasses and plants (Table 1).

All types of honey were collected from apiaries of selected regions among countries, such as Saudi Arabia, Germany and New Zealand for *in vitro* analysis purposes in order to determine their antimicrobial activities against selected pathogenic bacterial strains.

Microbial strains

The pathogenic bacterial strains included three gram-positive bacteria, being *Staphylococcus aureus* ATCC 29213, *Enterococcus faecalis* ATCC 29212 and methicillin-resistant Staphylococcus (MRSA) ATCC 43300. Three gram-negative bacteria, being *Escherichia coli* ATCC 25922, *Klebsiella pneumonia* ATCC 700603 and *Pseudomonas aeruginosa* ATCC 27853 were also included. All strains were obtained from the bacterial cultures stocks and supplied by the Department of microbiology, College of Applied medical sciences, Qassim University.

Assessment of antibacterial activity

Agar well diffusion method was used to determine the antimicrobial potential of different types of honey. Muller-Hinton agar and Muller-Hinton agar with 5% sheep blood were used. Bacterial suspension from pure isolates of each microbe were prepared in Muller-Hinton broth under aseptic condition. The density of each suspension was adjusted with 0.5% McFarland standard. The tested organisms were then inoculated into MHA using lawn culture technique.

After inoculation; 3 wells were made in agar plate using a sterile cork borer (10 mm diameter). The lower portion of the wells was sealed with melted MHA agar. The wells were completely filled with 330 µl of the honey. The agar plates were then incubated at 37 ºC for overnight. The zone of inhibition was checked and the diameter of zone of inhibition was measured using measuring ruler. Vancomycin 30, imipenem 10, amikacin 30 were used as positive controls. All experiments were performed as triplicate.

Table 1: Honey samples collected from different regions.

Honey type	Botanical origin	Honey source
Talh honey (TH)	*Acacia gerrardii* Benth	Saudi Arabia
Zahoor honey (ZH)	(mixed flower)	Saudi Arabia
Black forest honey (B.F)	Honeydew honey is made from the aphids secretion on the leaves of trees	Germany
Manuka +20	(*Leptospermum based honey*)	New Zealand
Manuka +16	(*Leptospermum based honey*)	New Zealand
Manuka +5	(*Leptospermum based honey*)	New Zealand

Statistical analysis

The mean antimicrobial activity of all tested honey types against each tested bacterial strain was measured. The data were analyzed using analysis of Microsoft Excel Software and analyzed using IPE INFO 7. Statistical means were compared for significant differences at $p \leq 0.05$.

RESULTS

Different types of honey including Talha (TH), Zahoor (ZH), and Manuka (MH) with different UMF +20, +16, +5 and Black Forest (BF) were tested for in vitro antimicrobial activities and evaluated against selected pathogenic bacterial strains. Three Gram-positive bacteria, being *S. aureus* ATCC 29213, *E. faecalis* ATCC 29212 and MRSA ATCC 43300 and three Gram-negative bacteria including *E.coli* ATCC 25922, *K. pneumonia* ATCC 700603 and *P. aeruginosa* ATCC 27853 were used. Natural forms of honey were used for testing their potential antimicrobial activity. The data revealed that TH, (ZH), MH with different UMF +20, +16, +5 honey types have significant antimicrobial potentialities against microbial strains. BF honey has no significant antimicrobial potentialities as microbial strains were found to be resistant to this type of honey. The microbial strains were significantly inhibited as measured in terms of their zone of inhibition (ZOI), and a large ZOI reflected a high sensitivity of tested microbial strains. Gram-positive (G+) bacteria were found to be more sensitive to all tested honey types except (BF) with significantly higher ZOI values than those of Gram-positive (G-) bacteria as stated in Table 2.

S. aureus showed the greatest inhibition zone with MA 20+ (24.6 mm), followed by MRSA showing 23 mm ZOI. The least inhibition zone was recorded for MRSA, *E. faecalis* and *P. aeruginosa* with BF showing only 10 mm ZOI (Table 2).

Manuka honey

MA 20+ was found to be the most effective type of MH probably due to the concentration of MGO. It was effective against the most tested strain such as MRSA, *S. aureus* and *E. faecalis* showing 25 mm ZOI, whereas the least ZOI was *P. aeruginosa* showing 14 mm ZOI (Figure 1). On the other hand, MA +16 showed slightly smaller ZOI, being MRSA as 21 mm, *S. aureus* showing 21.6 mm and *E. faecalis* showed 19.3 mm ZOI. *P. aeruginosa* had 12 mm ZOI (Figure 2).

MA +5 was found to be the least effective Manuka honey type. It was very effective against MRSA, being 22 mm followed by *S. aureus* with 20.6 mm ZOI. ZOI of *P. aeruginosa* was the least showing only 13.6 mm (Figure 3).

The inhibition zones due to TH were found to be quite similar to the MH results compared to others honey types. MRSA was the most effected bacteria showing 20.6 mm ZOI followed by *S. aureus* showing 20.3 mm ZOI. TH was also found to be the most effective honey type on *P. aeruginosa* which showed 15.6 mm ZOI. The least ZOI was determined in *K. pneumonia,* being only 14.3 mm (Figure 4).

Table 2: ZOI for all tested strain. The values were found to be statistically significant (*p* <0.05).

	Different types of honey					
Microbial strain	MA20+	MA16+	MA5+	T.H	Z.H	B.F
G+ Bacteria	Zone of inhibition in mm (ZOI mm)					
MRSA	23	21	22	20.6	15.6	10
S. aureus	24.6	21.6	20.6	20.3	15	12.3
E. faecalis (B)	25	19.3	15	19	15.6	10
E. faecalis (A)	18.6	17.6	13	15.3	11.6	10
G– Bacteria						
P. aeruginosa	14	12	13.6	15.6	14.3	10
K. pneumoniae	19.6	20.3	16.3	14.3	12.6	12.3
E. coli	22.3	21	17.6	15.6	13.6	12

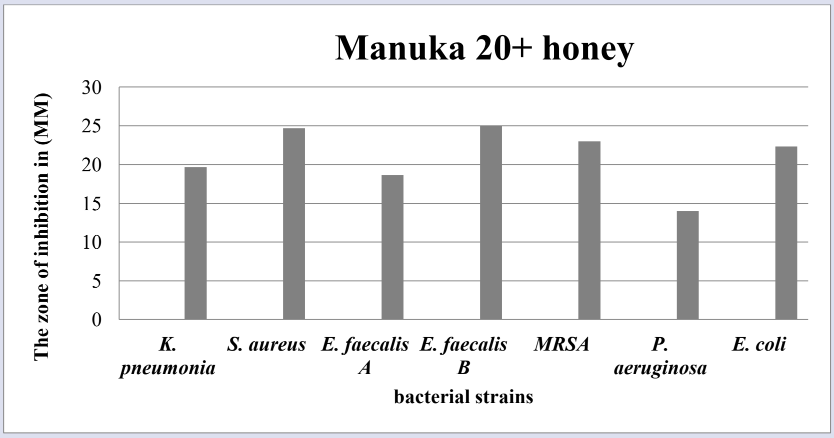


Figure 1: Antimicrobial activities of Manuka 20+ honey against tested bacterial strains. The largest three of ZOI E. *faecalis* (B), *S. aureus* and MRSA. Were the least one *P. aeruginosa*.

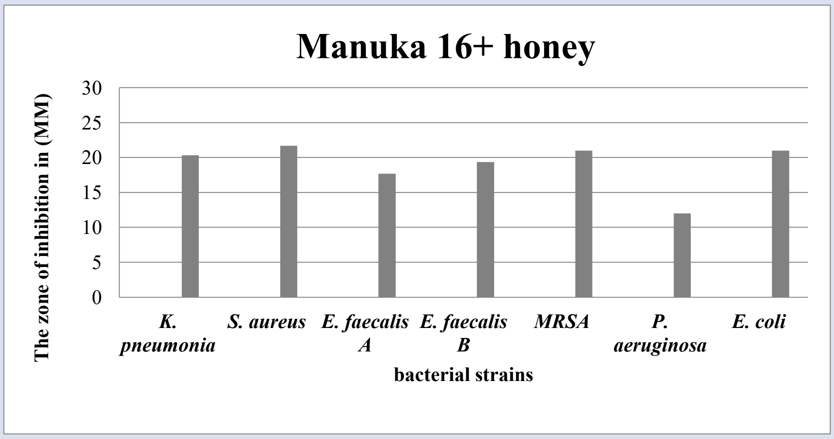


Figure 2: Antimicrobial activities of Manuka 16+ honey against tested bacterial strains. The largest four of ZOI *S. aureus*, *E. faecalis* (B), MRSA and *E. coli*. Were the least one *P. aeruginosa*.

The largest four of ZOI *S. aureus*, *E. faecalis* (B), MRSA & *P. aeruginosa*. Were the least one *K. pneumonia*.

Zahoor honey

The least effective honey type on the tested strains was ZH. MRSA and *E. faecalis* were found to be the most sensitive strains showing 15.6 mm, while *K. pneumonia* only showing 12.6 mm ZOI (Figure 5).

BF honey was found to be the least effective type among the tested honeys. Only small ZOI was found in *K. pneumonia* (12.3mm) and S. *aureus* (12.3 mm) (Figure 6).

DISCUSSION

All types of tested honey except BF honey displayed substantial antimicrobial activities against tested pathogenic bacterial strains. These primary findings may strengthen the idea that honeys can be used as potential alternative broad-spectrum strategy to treat bacterial infections. Many published studies determined the antimicrobial effects of various types of honeys, however, more extensive researches are necessary for conclusive declaration as substituting broad-spectrum antimicrobial drugs with different type's honey.

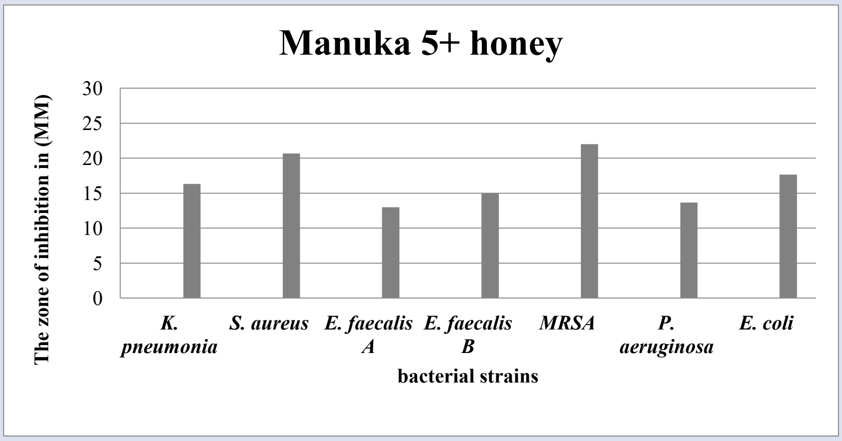


Figure 3: Antimicrobial activities of Manuka 5+ honey against tested bacterial strains. The largest two of ZOI *S. aureus* and MRSA. Were the least one *P. aeruginosa*.

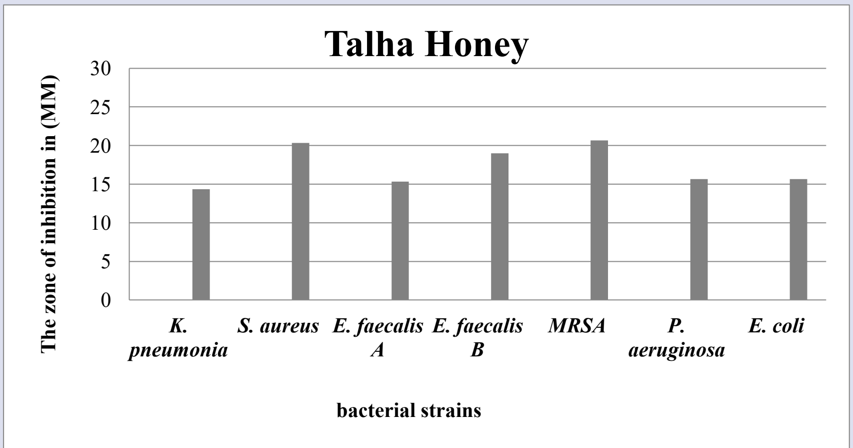


Figure 4: Antimicrobial activities of Talah honey against tested bacterial strains.

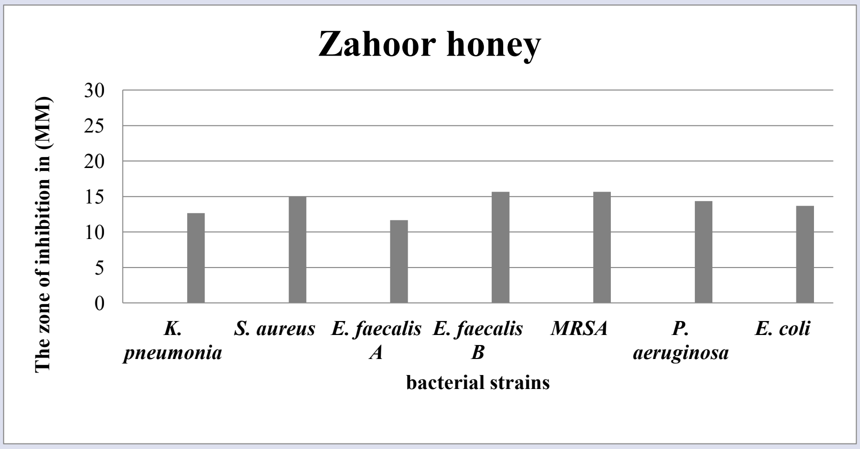


Figure 5: Antimicrobial activities of Zahoor honey against tested bacterial strains. The largest three of ZOI *E. faecalis* (B) &MRSA. Were the least one *K. pneumonia*.

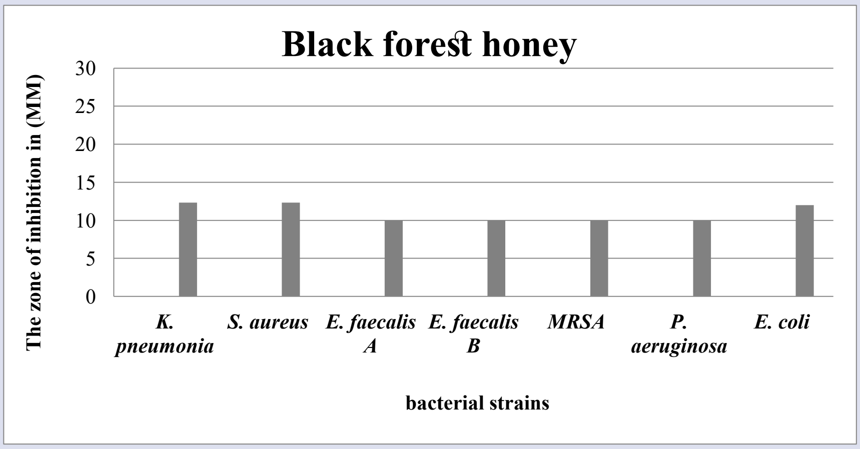
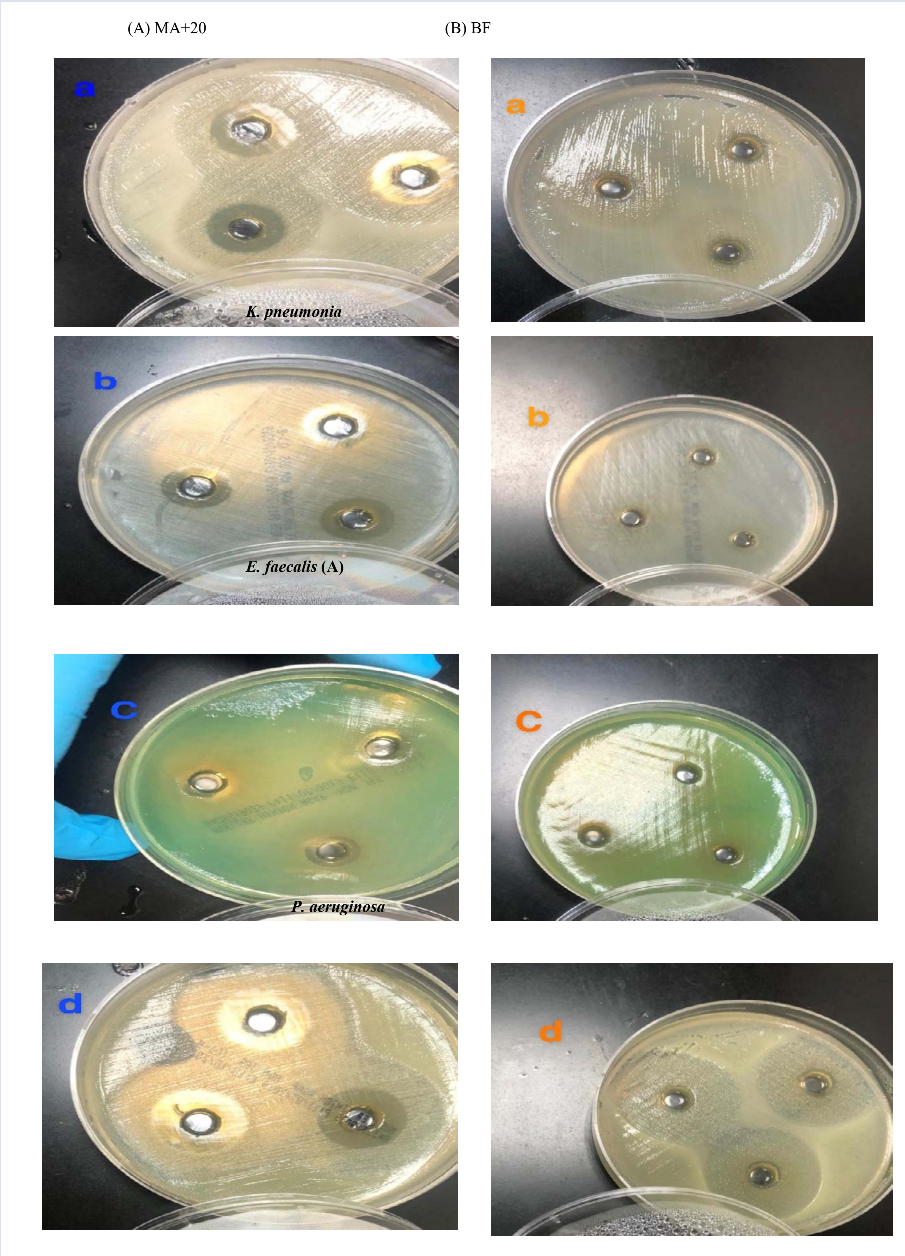


Figure 6: Antimicrobial activities of black forest honey against tested bacterial strains.



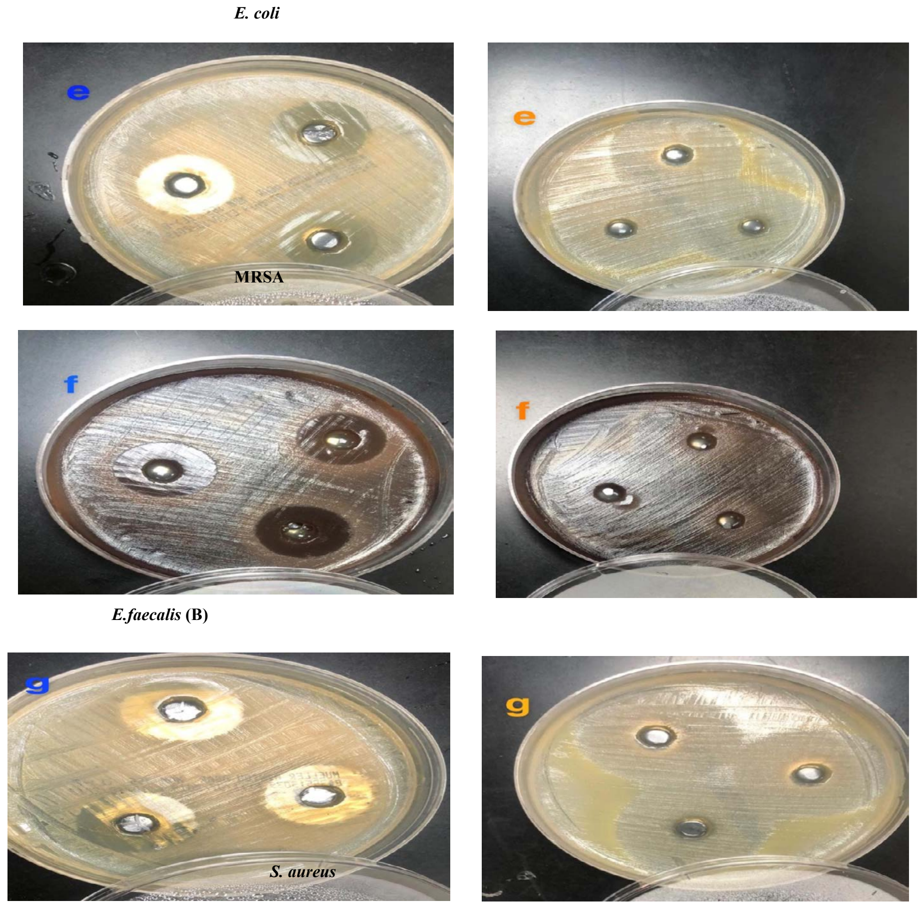


Figure 7: The zone of microbial growth inhibition on the cultures of bacteria obtained after adding natural honeys: (a) MA 20+ honey & (b) BF honey.
(A) MA+20

This study showed that all types of Manuka honey have strong antibacterial activities against all tested bacteria except *P. aeruginosa*. The most effected bacteria were *S. aureus, MRSA*, *E. coli* and *E. faecalis.* MH also showed smaller ZOI against *K. pneumoniae* and *P. aeruginosa*. This study is contradictory with other studies which showed that Manuka +20, Manuka +16 and Manuka +10 were found to have strong antibacterial activities against both sensitive and resistant *P. aeruginosa*. Another study showed that Manuka honey of lower UMF grade demonstrated equal to significantly increased antimicrobial activity compared to higher UMF grade honey for all tested organism.[10,11]Unlike to MH, Talha and Zahoor honey types showed strong antibacterial activities against *P. aeruginosa* and some of other gram+ and gram- bacteria. On the other hand, black forest honey showed the least inhibition (smallest ZOI). A very recent study demonstrated that Talha honey showed higher antimicrobial activities than Sidr honey. Both had strong antibacterial activities against pathogenic gram-positive bacteria including *Bacillus cereus* and *S. aureus,* and gram-negative bacteria including *E coli* and *Salmonella enteritidis*.[12] This study also showed that Talha honey had great antibacterial activities against all tested bacterial strains including gram-positive and gram-negative strains. Very recent studies reported that TH has higher antibacterial activities than Somur, Meria and sider honeys.[12] In contrast, this study illustrated another type of honey, MH exhibiting superior antibacterial activities against selected bacterial strains compared to TH. Black forest honey was previously found to have the highest antibacterial activities against *P. aeruginosa* ATCC 27853 and least against *E. coli* ATCC 25922.[13] This study was also found that black forest honey has the least inhibition zone (smallest ZOI). All recorded diameters of inhibition zones in the present study were greater than 11 mm except BF. This result well aligns with the declaration of Agbagwa and Frank-Peterside (2010) that "the diameter of inhibition zones less than 7 mm corresponds to resistant microorganisms and greater than 11 mm suggests that the microorganisms are sensitive to antimicrobial agent".[14] Therefore, our findings are consistent in that all tested microbial strains were sensitive to tested honeys, and these honeys might be proposed as prospective antimicrobial agents to benefit human health.

Physiochemical properties of honey

The high viscosity of honey was previously found to provide the barrier in order to protect against bacterial infection into the host.[15] Moreover, high sugar content of honey might affect the osmolarity which may lead to inhibit the microbial growth.[16] Antibacterial activities of manuka honey (Leptospermum scoparium) against *S. aureus*, MRSA, and *Pseudomonas* spp. were demonstrated. These activities of honey were suggested to be depend on various factors that function either singularly or synergistically. [17-19] The honey is well known to consists of hydrogen peroxide, phenolic compounds, lower pH, osmotic pressure, and other phytochemical content. Furthermore, honey has the ability to generate hydrogen peroxide related antimicrobial activity. The production of hydrogen peroxide by transforming glucose substrate with glucose oxidase of honey depends on enzyme level and floral sources of honey.[20] A study on Zahoor honey type (maltiflower honey) found that honey PH was ranged from 3.84 to 3.87, Phenolic content ranged from 674.81 mg GAE/kg to 674.81mg GAE/kg and flavonoid contents were in the range of 66.56 mg quercetin/kg to 79.75 mg quercetin/kg.[21] Therefore, all of these factors might have roles in the antimicrobial activities against tested pathogenic bacterial strains in the current study. The effect of honey on the development of bacterial resistance was determined and indicated that it might be very low duo to the variability in the composition among various types of honey which depends on some factors. These factors include types of nectar that the bees fed, the related weather conditions, storage time and conditions of preservation.[22,23]

CONCLUSION

All tested honey types except black forest, have exhibited antimicrobial activities on both gram-positive and gram-negative bacteria, especially *S. aureus* and MRSA. Manuka +20 and +16 were found to be more effective against all tested strain than other types, while black forest honey has no effectiveness. UMF was fond to be depend on MGO.

This study suggests that antimicrobial potential of honey types might be helpful in order to treat the pathogenic microorganisms threatening the public health and changing antibiotics into last-resort drugs. Further studies are required using different honey types on different bacterial strain in order to determine the exact composition of honey tested.

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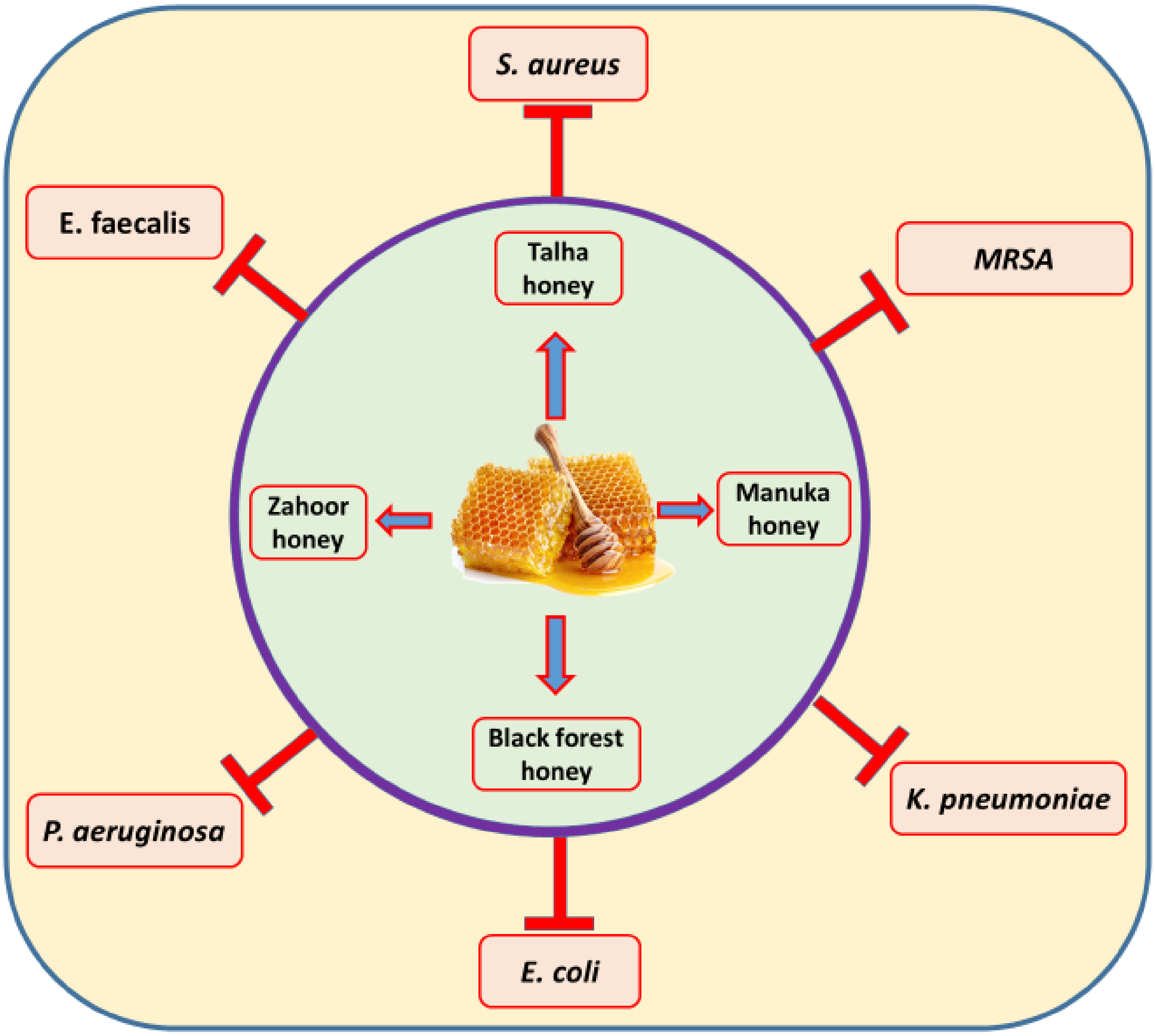
GRAPHICAL ABSTRACT



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Cite this article: Aljaghwani A, Allemailem KS, Aljaghwani LF, Alrumaihi F, Joseph RJ, Khan AA, *et al*. Antimicrobial Effect of Different Types of Honey on Selected ATCC Bacterial Strains. Pharmacog J. 2021;13(1): 217-25