ORIGINAL ARTICLE

Effect of Different Saudi Honey Types Mixed with Natural Substances on Some Bacterial Strains

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Abstract

To evaluate the antibacterial effects of three types of Saudi honey (Feghra, Sider and Natural honey) alone and mixed with ginger or lemon in comparison to Manuka honey as a potential natural antibacterial agent. Saudi honeys were evaluated against five types of bacterial strains; *Klebsiella pneumoniae, Staphylococcus aureus, Pseudomonas aeruginosa, Haemophilus influenzae* and *Streptococcus pneumoniae*. Chocolate agars were prepared first with different concentrations of each type of honey, and then with specific concentrations either of ginger or lemon added to honey. Bacterial species were inoculated on each agar and incubated at 37°C in a CO_2 incubator overnight. Significant differences were found between different types of honey and different concentrations of the same honey on bacterial growth. There are no significant differences and synergistic effects when adding ginger to different honey types. Addition of lemon show significant differences and good synergistic effects against all tested bacterial species except *Klebsiella pneumoniae* and *Staphylococcus aureus* at 15 and 20% honey concentration. In conclusion, antibacterial effects of different types of honey are type and concentration dependent. Adding lemon to the different types of honey changes the pH and acidity and increases the honey's antibacterial effect.

Keywords

Honey; Manuka; Feghra; Sider; Natural; Ginger; Lemon; Bacteria; Antibacterial

Introduction

The emergence of multi-drug resistant bacteria has developed into an international problem[1]. Based on extensive use and mistreatment of antibiotics, the number of diseases rises and the bacteria become more virulent with each generation[2]. This emerging trend in bacterial resistance to antibiotics is a major problem that needs a solution[3], and it is one of the most critical problems listed by the World Health Organization (WHO)[4,5]. Bacterial infection is a recurrent

problem in children that is typically connected with a poor prognosis[6]. *Staphylococcus aureus (S. aureus)* is a gram-positive, very frequent bacterium that lives on human skin and usually causes no problems; however, if it gets into the blood stream, from a cut or during an operation, it may very rapidly and toxically harm the heart, lungs, brain or blood circulating system[7]. *Pseudomonas aeruginosa (P. aeruginosa)*, a gram-negative organism, is a significant problem in hospital-acquired infections[8,9]. *Streptococcus pneumoniae (S. pneumoniae)*, is a microbe that can infect humans and is the major source of pneumonia, meningitis, and otitis media[10]. *Haemophilus influenzae* (*H. influenzae)* form b (Hib), is a significant source of meningitis, community-acquired pneumonia, septicaemia, morbidity and fatality in children during a five-year period[11,12]. *Klebsiella pneumoniae* (*K. pneumoniae)* category covers a broad spectrum of infections, including pneumonia, urinary tract disease, bacteraemia and liver swelling, and used to be a basic severe infection in immune-compromised individuals, but recent appearances of hyper-virulent strains have widened the number of people vulnerable to these infections, including healthy people who are immune-sufficient. Current studies prove that *K. pneumoniae* has developed virulent features, making it more resistant to antibiotics and adding further to the growth of this pathogen[13]. As an alternative treatment, honey has a broad spectrum of antibacterial activity and there are no forms of bacteria that honey cannot resist[2,14]. The antimicrobial features of honey come from its various components of high sugar concentration, low pH (ranging from 3.2–4.5), acidic conditions causing high osmolarity of hydrogen peroxide[15,16]. For thousands of years, the antimicrobial features of plant extracts have been used by humans[17]. Citric acid obtained from lemons works as an agent in the fermentation procedure that will cause antibacterial properties due to low pH levels found in lemons[18]. Squeezed lemon juice diluted with water or honey is reported to be a strong agent to fight against diseases that cause bacterial infection[19]. Thus, our aim was to evaluate the antibacterial effects of using honey only and mixtures of honey with fresh ginger and lemon juice on bacterial infections.

Materials and Methods

Manuka (*Leptospermum scoparium*) tree honey (imported, New Zealand) and three different types of Saudi Arabian honey: Feghra (after village name in Madinah), Sider (*Frangula alnus* known as Nabkh tree) and Natural honey were purchased from a local honey store in Jeddah, Saudi Arabia. Ginger and lemon were purchased from the local vegetable market.

The bacterial strains used in this study [*K. pneumoniae* (ATCC: 700613), *S. aureus* (ATCC: 29213), *P. aeruginosa* (ATCC: 27853), *H. influenzae* (ATCC: 9007), *S. pneumoniae* (ATCC: 49619)] were obtained from the Medical Microbiology Laboratory of King Abdulaziz University Hospital, Jeddah, Saudi Arabia. Chocolate agar media is obtained from (Oxoid, USA Oxoid Limited, Hampshire, U.K.).

Suspension of the organism was prepared with normal saline and the inoculums density was adjusted with turbidity of 0.5 McFarland standards[20]. Honey samples were first filtered with sterile mesh to remove debris, and then checked for microbial contamination on a chocolate agar plate[21]. Compared to Manuka honey, concentrations of honey used for testing for all types of honey were 10%, 15%, 20%, 30% and 40%[22]. Three grams of ginger, after being washed properly, peeled, dried, cut into pieces and put into a mixer to produce a grind, were added to each 100 ml of hot media[23] then filtered in a sterile muslin cloth[24]. Lemon was washed with tap water, and cut with a sterile knife so the juice drained into a sterile container. The juice was filtered (using MF-Millipore 0.45 micron pore size filter paper) and transferred to another sterile bottle. Two ml lemon juice was used fresh without being put into a refrigerator[25]. The pH of the media was measured using the pH meter machine (Hanna pH 211 Microprocessor pH Meter (Hanna Instruments Inc., Woonsocket, RI U.S.A.)) the pH changes, while the reference electrode remains stable[26]. Chocolate agar is appropriate media used for isolating species[27,28]. For preparation of agar media with honey, ginger and lemon: first, honey was added on the agar in the order of 10 ml, 15 ml, 20 ml, 30 ml and 40 ml. Then, honey was added on the agar in the same order and three grams of ground fresh ginger were added. After that, honey was added on the agar in the same order and two ml of the filtered lemon juice was added. In all steps, proper mixing was done by using a stirring tool and filtered into a sterile muslin cloth. All the above-prepared mixtures were poured on dishes. Two micro litres of each bacterial strain were inculcated on the surface of the media and then incubated in CO_2 overnight at 36–37°C and observe either growth or no growth of bacteria compared with control. All steps were repeated for each type of honey. Statistical analysis has been performed by using

IBM SPSS Statistics for Windows, Version 22 (IBM Corp., Armonk, NY USA).

Results

Tables 1, 2 and 3 show that there are no antibacterial effects of Saudi honey on all types of bacterial strains at the honey concentrations of 10, 15 and 20%, respectively. While the Manuka honey prevented the growth of all types of tested bacterial strains at 20%. Table 4 shows the effect of 30% honey concentration; Feghra honey showed the best result as it inhibited the growth of the five bacterial strains followed by Natural honey, which prevented the growth of all types of bacteria except *S. aureus*, while Sider honey prevented the growth of *P. aeruginosa* and *K. pneumonia*e. With the 40% honey concentration, all the tested honey types prevented the growth of all bacterial strains as seen in Table 5.

Adding ginger to Manuka honey did not change the Manuka antibacterial effect. Adding lemon improved the effect of Manuka honey by preventing the growth of all types of bacteria except *K. pneumoniae* at 10% and prevented all types of bacterial growth at 15% concentration (Table 6).

Table 7 shows that adding ginger did not change the effect of Feghra honey on bacterial growth. Adding lemon improved the effect of Feghra honey

Table 1. Antibacterial effect of different types of honey at 10% concentration among bacteria strains.

Factors		Bacteria					Statistics
Cons.	Types	*S. aureus*	*P. aeruginosa*	*K. pneumoniae*	*H. influenzae*	*S. pneumoniae*	Dead
10%	Manuka	Growth	Growth	Growth	NG	NG	2 (40.00%)
	Feghra	Growth	Growth	Growth	Growth	Growth	0 (0.00%)
	Sider	Growth	Growth	Growth	Growth	Growth	0 (0.00%)
	Natural	Growth	Growth	Growth	Growth	Growth	0 (0.00%)

Abbrv.: S. aureus: Staphylococcus aureus; P. aeruginosa: Pseudomonas aeruginosa; K. pneumoniae: Klebsiella pneumoniae; H. influenzae: Haemophilus influenzae; S. pneumoniae: Streptococcus pneumoniae; NG = No Growth.

Table 2. Antibacterial effects of different type of honeys at 15% percent concentration among bacteria strains.

Factors		Bacteria					Statistics
Cons.	Types	*S. aureus*	*P. aeruginosa*	*K. pneumoniae*	*H. influenzae*	*S. pneumoniae*	Dead
15%	Manuka	Growth	Growth	Growth	NG	NG	2 (40.00%)
	Feghra	Growth	Growth	Growth	Growth	Growth	0 (0.00%)
	Sider	Growth	Growth	Growth	Growth	Growth	0 (0.00%)
	Natural	Growth	Growth	Growth	Growth	Growth	0 (0.00%)

Abbrv.: S. aureus: Staphylococcus aureus; P. aeruginosa: Pseudomonas aeruginosa; K. pneumoniae: Klebsiella pneumoniae; H. influenzae: Haemophilus influenzae; S. pneumoniae: Streptococcus pneumoniae; NG = No Growth.

Table 3. Antibacterial effect of different types of honey at 20% concentration among bacteria strains.

Factors		Bacteria					Statistics
Cons.	Types	*S. aureus*	*P. aeruginosa*	*K. pneumoniae*	*H. influenzae*	*S. pneumoniae*	Dead
20%	Manuka	NG	NG	NG	NG	NG	5 (100.00%)
	Feghra	Growth	Growth	Growth	Growth	Growth	0 (0.00%)
	Sider	Growth	Growth	Growth	Growth	Growth	0 (0.00%)
	Natural	Growth	Growth	Growth	Growth	Growth	0 (0.00%)

Abbrv.: S. aureus: Staphylococcus aureus; P. aeruginosa: Pseudomonas aeruginosa; K. pneumoniae: Klebsiella pneumoniae; H. influenzae: Haemophilus influenzae; S. pneumoniae: Streptococcus pneumoniae; NG = No Growth.

Table 4. Antibacterial effect of different types of honey at 30% concentration among bacteria strains.

Factors		Bacteria					Statistics
Cons.	Types	*S. aureus*	*P. aeruginosa*	*K. pneumoniae*	*H. influenzae*	*S. pneumoniae*	Dead
30%	Manuka	NG	NG	NG	NG	NG	5 (100.00%)
	Feghra	NG	NG	NG	NG	NG	5 (100.00%)
	Sider	Growth	NG	NG	Growth	Growth	2 (40.00%)
	Natural	Growth	NG	NG	NG	NG	4 (80.00%)

Abbrv.: S. aureus: Staphylococcus aureus; P. aeruginosa: Pseudomonas aeruginosa; K. pneumoniae: Klebsiella pneumoniae; H. influenzae: Haemophilus influenzae; S. pneumoniae: Streptococcus pneumoniae; NG = No Growth.

Table 5. Antibacterial effect of different types of honey at 40% concentration among bacteria strains.

Factors		Bacteria					Statistics
Cons.	**Types**	***S. aureus***	***P. aeruginosa***	***K. pneumoniae***	***H. influenzae***	***S. pneumoniae***	**Dead**
40%	**Manuka**	NG	NG	NG	NG	NG	**5 (100.00%)**
	Feghra	NG	NG	NG	NG	NG	**5 (100.00%)**
	Sider	NG	NG	NG	NG	NG	**5 (100.00%)**
	Natural	NG	NG	NG	NG	NG	**5 (100.00%)**

Abbrv.: S. aureus: Staphylococcus aureus; P. aeruginosa: Pseudomonas aeruginosa; K. pneumoniae: Klebsiella pneumoniae; H. influenzae: Haemophilus influenzae; S. pneumoniae: Streptococcus pneumoniae; NG = No Growth.

Table 6. Effect of Manuka honey at different concentrations and with lemon or ginger among bacteria strains.

Factors		Bacteria					Statistics
Types	**Cons.**	***S. aureus***	***P. aeruginosa***	***K. pneumoniae***	***H. influenzae***	***S. pneumoniae***	**Dead**
Honey Only	**10%**	Growth	Growth	Growth	NG	NG	**2 (40.00%)**
	15%	Growth	Growth	Growth	NG	NG	**2 (40.00%)**
	20%	NG	NG	NG	NG	NG	**5 (100.00%)**
	30%	NG	NG	NG	NG	NG	**5 (100.00%)**
	40%	NG	NG	NG	NG	NG	**5 (100.00%)**
	10%	Growth	Growth	Growth	NG	NG	**2 (40.00%)**
	15%	Growth	Growth	Growth	NG	NG	**2 (40.00%)**
	20%	NG	NG	NG	NG	NG	**5 (100.00%)**
	30%	NG	NG	NG	NG	NG	**5 (100.00%)**
	40%	NG	NG	NG	NG	NG	**5 (100.00%)**
Honey + Lemon	**10%**	NG	NG	Growth	NG	NG	**4 (80.00%)**
	15%	NG	NG	NG	NG	NG	**5 (100.00%)**
	20%	NG	NG	NG	NG	NG	**5 (100.00%)**
	30%	NG	NG	NG	NG	NG	**5 (100.00%)**
	40%	NG	NG	NG	NG	NG	**5 (100.00%)**

Abbrv.: S. aureus: Staphylococcus aureus; P. aeruginosa: Pseudomonas aeruginosa; K. pneumoniae: Klebsiella pneumoniae; H. influenzae: Haemophilus influenzae; S. pneumoniae: Streptococcus pneumoniae; NG = No Growth.

Table 7. Effect of Feghra honey at different concentrations and with lemon or ginger among bacteria strains.

Factors		Bacteria					Statistics
Types	**Cons.**	***S. aureus***	***P. aeruginosa***	***K. pneumoniae***	***H. influenzae***	***S. pneumoniae***	**Dead**
Honey Only	**10%**	Growth	Growth	Growth	Growth	Growth	**0 (0.00%)**
	15%	Growth	Growth	Growth	Growth	Growth	**0 (0.00%)**
	20%	Growth	Growth	Growth	Growth	Growth	**0 (0.00%)**
	30%	NG	NG	NG	NG	NG	**5 (100.00%)**
	40%	NG	NG	NG	NG	NG	**5 (100.00%)**
Honey + Ginger	**10%**	Growth	Growth	Growth	Growth	Growth	**0 (0.00%)**
	15%	Growth	Growth	Growth	Growth	Growth	**0 (0.00%)**
	20%	Growth	Growth	Growth	Growth	Growth	**0 (0.00%)**
	30%	NG	NG	NG	NG	NG	**5 (100.00%)**
	40%	NG	NG	NG	NG	NG	**5 (100.00%)**
Honey + Lemon	**10%**	Growth	NG	Growth	NG	NG	**3(60.00%)**
	15%	Growth	NG	Growth	NG	NG	**3(60.00%)**
	20%	NG	NG	NG	NG	NG	**4 (80.00%)**
	30%	NG	NG	NG	NG	NG	**5 (100.00%)**
	40%	NG	NG	NG	NG	NG	**5 (100.00%)**

Abbrv.: S. aureus: Staphylococcus aureus; P. aeruginosa: Pseudomonas aeruginosa; K. pneumoniae: Klebsiella pneumoniae; H. influenzae: Haemophilus influenzae; S. pneumoniae: Streptococcus pneumoniae; NG = No Growth.

Table 8. Effect of Sider honey at different concentrations and with lemon or ginger among bacteria strains.

Factors		Bacteria					Statistics
Types	**Cons.**	***S. aureus***	***P. aeruginosa***	***K. pneumoniae***	***H. influenzae***	***S. pneumoniae***	**Dead**
Honey Only	**10%**	Growth	Growth	Growth	Growth	Growth	**0 (0.00%)**
	15%	Growth	Growth	Growth	Growth	Growth	**0 (0.00%)**
	20%	Growth	Growth	Growth	Growth	Growth	**0 (0.00%)**
	30%	Growth	NG	NG	Growth	Growth	**2 (40.00%)**
	40%	NG	NG	NG	NG	NG	**5 (100.00%)**
Honey + Ginger	**10%**	Growth	Growth	Growth	Growth	Growth	**0 (0.00%)**
	15%	Growth	Growth	Growth	Growth	Growth	**0 (0.00%)**
	20%	Growth	Growth	Growth	Growth	Growth	**0 (0.00%)**
	30%	Growth	NG	NG	Growth	Growth	**2 (40.00%)**
	40%	NG	NG	NG	NG	NG	**5 (100.00%)**
Honey + Lemon	**10%**	Growth	NG	Growth	NG	NG	**3 (60.00%)**
	15%	Growth	NG	Growth	NG	NG	**3 (60.00%)**
	20%	NG	NG	NG	NG	NG	**5 (100.00%)**
	30%	NG	NG	NG	NG	NG	**5 (100.00%)**
	40%	NG	NG	NG	NG	NG	**5 (100.00%)**

Abbrv.: S. aureus: Staphylococcus aureus; P. aeruginosa: Pseudomonas aeruginosa; K. pneumoniae: Klebsiella pneumoniae; H. influenzae: Haemophilus influenzae; S. pneumoniae: Streptococcus pneumoniae; NG = No Growth.

Table 9. The Effect of Natural honey at different concentrations and with lemon or ginger among bacteria strains.

Factors		Bacteria					Statistics
Types	**Cons.**	***S. aureus***	***P. aeruginosa***	***K. pneumoniae***	***H. influenzae***	***S. pneumoniae***	**Dead**
Honey Only	**10%**	Growth	Growth	Growth	Growth	Growth	**0 (0.00%)**
	15%	Growth	Growth	Growth	Growth	Growth	**0 (0.00%)**
	20%	Growth	Growth	Growth	Growth	Growth	**0 (0.00%)**
	30%	Growth	NG	NG	NG	NG	**4 (80.00%)**
	40%	NG	NG	NG	NG	NG	**5 (100.00%)**
Honey + Ginger	**10%**	Growth	Growth	Growth	Growth	Growth	**0 (0.00%)**
	15%	Growth	Growth	Growth	Growth	Growth	**0 (0.00%)**
	20%	Growth	Growth	Growth	Growth	Growth	**0 (0.00%)**
	30%	Growth	NG	NG	NG	NG	**4 (80.00%)**
	40%	NG	NG	NG	NG	NG	**5 (100.00%)**
Honey + Lemon	**10%**	Growth	NG	Growth	NG	NG	**3(60.00%)**
	15%	Growth	NG	Growth	NG	NG	**3(60.00%)**
	20%	Growth	NG	Growth	NG	NG	**3 (60.00%)**
	30%	NG	NG	NG	NG	NG	**5 (100.00%)**
	40%	NG	NG	NG	NG	NG	**5 (100.00%)**

Abbrv.: S. aureus: Staphylococcus aureus; P. aeruginosa: Pseudomonas aeruginosa; K. pneumoniae: Klebsiella pneumoniae; H. influenzae: Haemophilus influenzae; S. pneumoniae: Streptococcus pneumoniae; NG = No Growth.

by preventing the growth of three types of bacteria (*P. aeruginosa, H. influenzae* and *S. pneumoniae*) at concentrations of 10% and 15% and prevented the growth of all types of bacteria at 20% concentration.

Adding ginger had no visible effect of Sider honey on bacterial growth. Adding lemon improved the effect of Sider honey as no growth was observed for three types of bacteria (*P. aeruginosa, H. influenzae* and *S. pneumoniae)* at concentrations of 10% and 15%; however, the growth of all types of bacteria was prevented at 20% (Table 8).

Table 9 shows that bacterial growth was not affected by adding ginger to Natural honey. Adding lemon improved the effect of Natural honey by preventing the growth of three types of bacteria (*P. aeruginosa, H. influenzae,* and *S. pneumoniae)* at concentrations of 10%, 15% and 20%, but prevented the growth of *S. aureus* at 30%.

Discussion

The emergence of bacterial multi-drug resistance in hospitals around the world raises great concern for many researchers and encourages them to search out alternative approaches, such as herbal and alternative medicine. Literature from the past indicates that honey is one alternative remedy that has antibacterial effects attributed to a broad spectrum of powers to fight most gram-negative and gram-positive bacteria for the relief of many infections[29-31]. The present study showed that there are different antibacterial effects among different honey types and concentrations. It has also illustrated that adding fresh ginger to all types of honey did not change the antibacterial effect of honey even with the standard honey (Manuka). Adding lemon increased the antibacterial effects of all types of honey. The efficacy of lemon depends on the type of honey and bacteria. The antimicrobial potential of honey, which is confirmed by this study, are in agreement with another study shown by Al-Nahari and others[32] which concluded that the antibacterial effects of honey is type and concentration dependent. In this study, mixing honey with ginger did not improve the antimicrobial effect. The same observations were documented in Adeshina *et al.*[33] who reported that *P. aeruginosa, S. aureus*, *Escherichia coli* and *Salmonella typhi* bacteria were not susceptible to fresh ginger when isolated in the test. Also our results were in accordance with a study by Isiaka *et al.*[34] who concluded that agents (ginger) may not be useful when added to honey, the only impact of ginger when added to honey was to give it a different flavour. In comparison, Ewnetu *et al.*[35] found higher inhibition results when using ginger extract, either ethanol or methanol, considering that honey-ginger powder extract mixtures were found to have more antimicrobial outcomes than the use of honey or ginger extracts independently. The results of our study agree with the results of lemon research documented by Hindi and Chabuck[25] who reported that lemon extracts, using the juice of lemon or other citrus, have an important role as antimicrobial agents against microorganisms by inhibiting the growth of *P. aeruginosa*. The results of honey after adding lemon are also similar to Isiaka *et al*.[34] findings who revealed that honey fortified with lemon has improved antibacterial activity because of the presence of fortifying agents more than just the honey alone. It can be noted that honey fortified with lemon shows higher antibacterial activity against *P. aeruginosa*, *H. influenzae* and *S. pneumoniae* than honey alone. This is in agreement with the findings of Adeshina *et al.*,[36] who studied the vulnerability of bacteria to honey and lemon and concluded that the impact of honey and lemon together is higher than the individual effect. A study by Gattuso *et al.*[37] indicates that lemon has significant antibacterial features and is active against bacteria. Our results are matched with Hayes and Markovic[38] who investigated the antimicrobial features of lemon and reported that it has significant antimicrobial activity against *S. aureus*, *Klebsiella, E. coli*, *P. aeruginosa* and *Candida albicans*.

Conclusion

The antibacterial effects of different type of honey are dependent on type and concentration. Manuka honey shows antibacterial effect starting from 10%, and inhibited all the tested bacterial strains at 20%, while all tested honey types (Feghra, Sider and Natural honey) with the concentrations 10, 15 and 20% had no antibacterial effect on any of the five-tested bacterial strains. At the 30% concentration Feghra honey had the strongest antibacterial effect as it prevented the growth of all the bacterial strains followed by Natural honey which showed no growth for all bacterial strains except *S. aureus*. It is imperative to note that fortified honey samples with lemon and ginger show relatively dissimilar antibacterial activity when compared in the tests using pathogenic bacteria. No synergistic effects when adding fresh ginger, the reason for the uselessness of fresh ginger against the test bacteria has not yet been determined. Lemon increased the antibacterial effect for all honey types and shows therapeutic value as an antibacterial agent against different bacterial strains.

Recommendation

We recommend further studies on animal or human subjects using more pathogens.

Conflict of Interests

The authors declare that there is no conflict of interest regarding the publication of this paper.

Disclosure

None of the authors received any type of commercial support either in forms of compensation or financial for this study. They have no financial interest in any of the products or devices, or drugs mentioned in this article.

Ethical Approval

Obtained.

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تأثير أنواع مختلفة من العسل السعودي مخلوط مع المواد الطبيعية على بعض أنواع البكتيريا

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المستخلص. لمعرفة الآثار المضادة للبكتيريا لثلاثة أنواع مختلفة من العسل السعودي (السدر والفقرة وعسل طبيعي) ومخلوطين مع الزنجبيل أو الليمون بالمقارنة مع عسل المانوكا كعسل فعال كعوامل مضادة للبكتيريا. تم تقييم أثر عسل الفقرة والسدر والعسل الطبيعي ضد خمسة أنواع من البكتيريا: كلبسيلا الرئوية، المكورات العنقودية الذهبية، الزائفة الزنجارية، المستدمية النزلية، العقدية الرئوية وتقييم تأثير أنواع العسل الثلاثة مخلوطين مع الزنجبيل أو الليمون. تم إعداد أجار الشوكولاته مع تركيز مختلف من كل نوع من أنواع العسل ثم مع تركيز معين من الزنجبيل أو الليمون مع العسل. تم إضافة السلالات البكتيرية لاختبارها على كل أجار. ووضعت في درجة ٣٧ درجة مئوية في حاضنة CO_2 لمدة يوم كامل.

هنالك فروق معنوية بين التركيزات المختلفة لأنواع العسل علي نمو البكتيريا. لا توجد فروق معنوية ولا تأثيرات داعمة عند إضافة الزنجبيل لأنواع العسل المختلفة ولكن هنالك فروق معنوية وتأثيرات داعمة عند إضافة الليمون على نمو البكتيريا. خلط العسل مع الليمون زاد من التأثير المضاد للجراثيم ضد جميع الأنواع البكتيرية باستثناء كلبسيلا الرئوية والمكورات العنقودية الذهبية، الزائفة عند تركيزي ١٥ و٢٠٪ للعسل. خلصنا إلى أن التأثير المضاد للأنواع المختلفة من العسل على البكتيريا يعتمد على نوع العسل وتركيزه وإضافة الليمون تدعم تأثير العسل المضاد للبكتيريا بزيادة الوسط الحمضي.