Study Antibacterial Activity of Libyan Honey Against Some Common Pathogenic Bacterial Species

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دراسة مضاد البكتيريا الفعال للعسل الليبي ضد بعض الأنواع البكتيرية المسببة للأمراض الشائعة

الملخص:

العسل مركب طبيعي ينتجه نحل العسل ومن أنواع مختلفة من الرحيق من مختلف الأزهار والحبوب اللقاح، وقد استخدم العسل كدواء منذ القدم، بشكل رئيسي لعلاج الجروح الجلدية، والحروق، والقروح، والتهابات العين، الحلق، والتهاب الجلد، من بين أمور أخرى. يمكن للعسل أن يزيل العدوى بعدة طرق – بما في ذلك تعزيز جهاز المناعة من خلال وجود مضادات للالتهابات ومضادات الأكسدة ومن خلال تحفيز نمو الخلايا.

الأهداف: عزل وتشخيص البكتيريا المسببة للأمراض.

تقييم تأثير أنواع مختلفة من العسل الليبي على البكتيريا المعزولة بتراكيز مختلفة.

الطريقة: أجريت هذه التجربة في مختبرات الأحياء الدقيقة السريرية بمستشفى الجلاء للأطفال (طرابلس – ليبيا) في الفترة من أبريل إلى يوليو 2022، وتم جمع عينة العسل المستخدمة في هذه الدراسة من مناطق مختلفة من ليبيا، وتم تخفيف العسل في أربعة تراكيز مختلفة (25%، 50%، 75%، 100%) لتقييم النشاط المضاد للبكتيريا للعسل الليبي ضد عدة أنواع من البكتيريا المسببة للأمراض (Escherichia coli، Klebsiella pneunomoniae، Pseudomonas aeruginosa، Staphylococcus aureus، Staphylococcus epidermidis) باستخدام آجار الانتشار والآجار وطرق انتشار القرص.

النتائج: أظهرت النتائج التي تم الحصول عليها أن العسل الليبي يمتلك نشاط مضاد للبكتيريا في دراستنا. ويختلف التأثير حسب العينة ونوع البكتيريا والتركيز. تعتمد نتائج العسل عبر الأنواع البكتيرية على أربعة أنواع من العسل وتأثيرها على خمسة أنواع من حساء البكتيريا. وتمت مضاعفة الاختبارات لتأكيد النتائج على أربعة تخفيفات (25%، 50%، 75%، و100%). ويمكن الحصول على القيمة الكبيرة للتأثير المضاد للبكتيريا عندما يكون العسل بتركيز 100%. أظهر عسل السدر أهمية أكبر في البكتيريا P. aeruginosa، في حين كانت أهمية متوسطة على البكتيريا S. aureus وغير معنوية على البكتيريا. فيما يتعلق بالمتوسط ± SD لكل من SEDER وCOMMERCIAL وSPRING، هناك اختلافات كبيرة(p= .001). في هذا العمل نركز على نتائج تأثير العسل على البكتيريا من خلال قياس منطقة التثبيط.

الخلاصة: النشاط المضاد للبكتيريا الممتاز للعسل ضد العزلات البكتيرية السريرية يشير إلى فائدة العسل في الممارسة السريرية ضد العدوى البكتيرية.

الكلمات المفتاحية: *العسل، البكتيريا المسببة للأمراض، عينة البول.*

Abstract:

Background: Honey is a natural complex produced by honey bees and from different types of nectar from various flowers and grains the vaccine, Honey has been used as a medicine since ancient times, mainly for the treatment of skin wounds, burns, ulcers, ocular infections, sore throat, and digital dermatitis, among others. Honey can clear infection in several ways _including boosting the immune system by having anti-inflammatory and antioxidant activists and via stimulation of cell growth.

Objectives: Isolation and identification of pathogenic bacteria.

Evaluation of the effect of different types of Libyan honey on isolated bacteria at different concentrations.

Method: This experiment was conducted in the clinical microbiology laboratories at AL-Gala children's Hospital (Tripoli– Libya) from April to July 2022, the honey sample used in this study was collected from different regions of Libya, and the honey was diluted in four different concentrations (25%, 50%,75%, and 100%) to evaluate the antibacterial activity of Libyan honey against several pathogenic bacteria (*Escherichia coli, Klebsiella pneunomoniae*, *Pseudomonas aeruginosa*, *Staphylococcus aureus, Staphylococcus epidermidis*) using agar well diffusion and agar disk diffusion methods.

Results: Our results obtained showed that Libyan honey possesses anti-bacterial activity in our study. The effect varies according to the sample, bacteria spp., and concentration. The results of the honey across bacterial species are based on four types of honey and their effect on five types of bacteria soup. The tests were doubled to confirm the results on four dilutions (25%, 50%, 75%, and 100%). The great value of the antibacterial effect can be obtained when honey is at a concentration100%. Seder honey showed more significance in *P. aeruginosa*, while moderate significance was on *S. aureus* and non-significant was on the bacterial spp. Concerning the mean ± SD for SEDER, COMMERCIAL, and SPRING there are significant differences (p= <.001). In this work, we focus on the results of the affectivity of honey across bacteria by measuring the Inhibition zone.

Conclusion: The excellent antibacterial activity of honey against clinical bacterial isolates indicates the usefulness of honey in clinical practice against bacterial infection.

Keywords: *honey, pathogenic bacteria and urine sample.*

Introduction:

honey is a by- product of flower nectar and the upper aero-digestive tract of honey bee, which is concentrated through a dehydration process inside the bee. The collected substances are mixed with other specific compounds from the honeybees and are then deposited by the honeybees in the wax honeycomb and allowed to mature over time. The composition of honey depends on the source of the plants that the bees feed on (Eteraf-Oskouei and Najafi, 2013; Schneider et al., 2013). However, Honey of all origins is composed mainly of sugars glucose sucrose, and fructose, which constitute_80% of its weight, with water composing the remaining 20%. In addition, vitamins, flavonoids, amino acids, enzymes, minerals, and phenolic acids are also present in honey. Honey has anti-inflammatory (Tonks et al., 2003), healing (Bergman et al., 1983), antioxidant (Jaganathan et al., 2010), and anti-neoplastic effects (Swellam et al., 2003). Honey is a natural complex produced by honey bees and from different types of nectar from various flowers and grains the vaccine, the worker bee collects it and combines it with special

materials, turning it into a sweet syrup that varies according to plants in the pasture (Wang and li, 2011). Honey is an example of a naturally available product and is the only concentrated sweetener that can be found in nature (Mandal, 2011). Honey has been used as a medicine since ancient times, mainly for the treatment of skin wounds, burns, ulcers, ocular infections, sore throat, and digital dermatitis, among others (The national honey Board, 2002), (Molan, 1999), (Oelschlaegel et al., 2012), The healing capacity of honey is strongly influenced by both physical and chemical properties of this food (Basuoldo et al., 2007) which are also related to the botanical source, honey bee's metabolism as well as environmental, seasonal and climatic conditions. Apart from healing, honey has been also employed as an excellent preservative for other food commodities, due to its antimicrobial activity (Krushna et al., 2007), (Malik, 2010). Honey can clear infection in several ways _including boosting the immune system by having anti-inflammatory and antioxidant activists and stimulating cell growth (Jabri, 2005). According to the world health care organization (WHO) statistics, up to 80% of the population in some developed countries used natural products in their primary health care (World Health Organization (WHO, 2014). Honey has been reported to have an inhibitory effect on around 60 species of bacteria, including aerobes and anaerobes gram-positive and gram-negative bacteria (Hannan et al., 2004). Many reports of bactericidal as well as bacteriostatic activity of honey may be particularly useful against bacteria that have developed resistance to many antibiotics (Patton et al., 2006). Various parameters affect the antibacterial potential of honey, including its low water content (low water activity), high viscosity, acidity, and H2O2 content. In addition, many compounds are associated with honey and provide its antibacterial potential, including phytochemicals, peptides non-peroxidase glycopeptides and proteins that are notable features of honey and are associated with its antimicrobial effects (Molan, 1992). A study in Libya showed that several concentrations (25%, 50%, 75%, 90 %) of camphor honey to know the extent of its inhibition of bacteria resistant to many antibiotics Amikacin, Cloisom, Sulfate, Amoxycillin, Gentamycin, on bacteria *E. coli, Enterococcus faecalis, S.aureus, Klebsiella pnemoniae*, *Acinetobacter* spp. The study confirmed that the higher the concentration of honey, the higher the inhibitory diameter, and at 90% concentration, the inhibitory diameter was for *Enterococcus faecalis* bacteria (27 mm) and (29 mm) on *K. pnemoniae* while (31 mm) on both *S. aureus, E. coli* and (20 mm) on *Acinetobacter spp* (Maraia, 2016). Al-Jabri, 2003 studied the antibacterial activity of 24 samples of honey (16 from Oman and eight from Africa) against three bacteria, namely, *S. aureus, E. coli*, and *P. aeruginosa*. They found that 81% of the Omani honey samples and 88% of the African honey samples assessed in the study had ant staphylococcal activity, but only 63% of the Omani honeys and 62% of the African honeys showed any activity against E. coli. Activity against *P. aeruginosa* was less common in Omani honey (38%) but more common in African honey (75%).

Aims of the study:

1. Isolation and identification of pathogenic bacteria.
2. Evaluation of the effect of different types of Libyan honey on isolated bacteria at different concentrations.

Material and methods:

1. Study population:

This experiment was conducted in the clinical microbiology laboratories at Al-Gala Children's Hospital (Tripoli– Libya) from April to July 2022.

2. Material:

2.1. Media:

Its environment contains all the components necessary for bacterial growth, its either commercially prepared or with the addition of distilled water.

The type of media used in this study:-

Table 1. Media used.

Type of media	Manufacture of company
Blood agar	Oxoid
MacConkey agar	Oxoid
Nutrient agar	Oxoid
Mueller Hinton agar	Oxoid
Cystine-lactose-electrolyte-deficient agar (CLED agar)	Oxoid

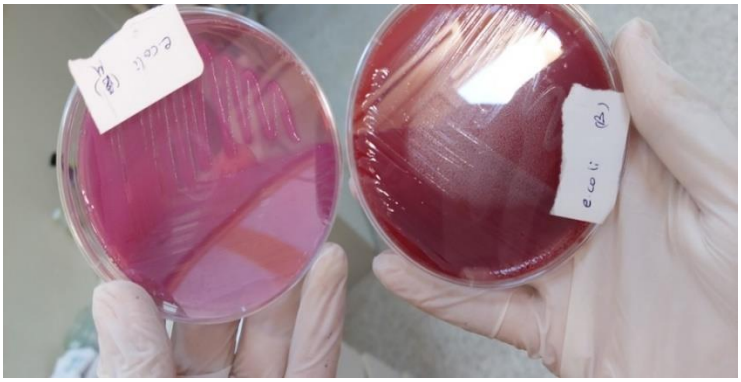


Figure 1. Inoculated Media

2.2. Type of bacteria:

A total of 5 bacterial isolates were used (*Escherichia coli, Staphylococcus epidermidis, Staphylococcus aureus, Pseudomonas aeruginosa, klebsiella. pnemoniae*).

2.3. Types of honey used in the in study:

Four honey samples were used (Seder, Thyme, Spring and Commercial).

2.4. The devices used in this study:

Table 2. Devices used.

Memment-Germany	Incubator
Micros-Australia	Microscope
Cluss- China	Autoclave
Rotofix 32A-Germany	Centrifuge

3. Method: -

3.1. Bacterial isolates

The samples were obtained from urinary tract infection cases in the microbiology department at AL-Gala

Children's Hospital (Tripoli– Libya). A swab of urine sample was taken and lines were created by a sterile ring on the

Blood agar, MacConkey agar, and Cystinelactose Electrolyte Deficient (CLED) agar and then incubated 37°C for 24h.

A total of 5 bacterial isolates were included (*Escherichia coli* – *Staphylococcus epidermidis* - *Staphylococcus aureus* – *Pseudomonas aeruginosa* – *Klebsiella pnemoniae*).



Figure2 . Bacterial isolated.

3.2. Gram stain: -

The process included five basic steps: -

1- bacterial sample was applied onto a clean slide then air dried and heat fixed.
2- A primary stain (crystal violet) was applied onto the slide.
3- A mordant (Gram's iodine) was applied onto the slide.
4- A decolorizer (ethanol, acetone or a mixture of both) was applied onto the slide.
5- Counter stain (Safranin) was applied onto the slide.

3.2.1. Coagulase test: -

Used to differentiate *Staphylococcus aureus* (giving positive **coagulase test,** thus, produces the enzyme coagulase, from *S. epidermidis* and *S. saprophyticus* (giving negative **coagulase test**, thus, do not produce coagulase.

Procedure:

1- The tube slide coagulase test was performed by mixing the suspension of bacterial cells on a clean slide and mixed into a drop of EDTA-treated rabbit plasma.
2- If bound coagulase is present in the bacterial cells, then the presence of plasma will cause the bacterial cells to clump, meaning a positive coagulase, otherwise it is a negative coagulase test.

3.2.2. Analytic profile index (API): -

The API is a rapid test for bacterial identification based on their biochemical properties.

The Analytical Profile Index (API) Strips - Rapid Test:

The API 20E, is a standardized micro plate method used for the Identification of Enterobacteriaceae. The positive and negative reactions are shown by color reactions.



Figure -3. Diagnose is of bacteria with a API20E system.

Method:

1- A single isolated colony (from a pure culture) was picked and a suspension of it was added to a tube of sterile distilled water.

2-The API20E Biochemical Test Strip which contains dehydrated bacterial media/bio-chemical reagents in 20 separate compartments was opened.

3- The compartments with the bacterial suspension were filled up (up to the brim) using a Pasteur pipette.

4- A sterile oil was added to the ADH, LDC, ODC, H2S, and URE compartments.

5- Some drops of water were added to the tray, and the API Test strip was placed in the tray and closed.

6-The tray was marked with the identification number (Patient ID or Organism ID).

7- The tray was incubated at 37oC for 18 to 24 hours.

8-Reaents are added and the results are recorded according to

Steps and Result Interpretations:

1. For some of the compartments, the color change can be read straightway after 24 hours but for some reagents were added to them before the result interpretation.

2. The following reagents were added to these specific compartments:

· TDA: Put one drop of Ferric Chloride-

· IND: Put one drop of Kovacs reagent

· VP: Put one drop of 40 % KOH (VP reagent 1) & One drop of VP Reagent 2 (α-Naphthol).

3. Each test was marked as positive or negative according to the API Reading Scale (color chart). The wells are marked off into triplets by black triangles, for which scores are allocated.

4. The scores for the positive wells only in each triplet were added up.

5. Three test reactions were added together at a time to give a 7-digit number, which can then be looked up in the codebook. The highest score possible for a triplet is 7 (the sum of 1, 2, and 4) and the lowest is 0.

6. The organism was identified by using an API catalog.

3.3. Bacteria preservation: -

Bacterial isolates were not preserved, but used immediately after culturing for 24hr at 37°C.

3.4. Collection and preparation of honey: -

Three honey samples were collected from several cities in Libya the Seder honey

Obtained from southeast (Tarhona-Libya), Thyme honey was obtained from west region (Tripoli), Spring honey was obtained from the east region (AL-Gable AL-akhdar-Libya) and **Commercial honey was** obtained from local markets in Tripoli, called AL-Shefaa Company. All honey samples were collected in screwed bottles and kept in a cool and dry place and labelled with the time of collection and type of honey**.**

3.5. Honey components analysis:-

The components of honey were examined in two different ways: -

First: - Four different types of Libyan honey were tested streaked on blood agar, nutrient agar, and Muller Hinton agar in duplicate and incubated for 24 to 48h at 37°C to check microbial purity.

Second: - By microscopic examination.

3.6. Evaluation of the effect of honey concentration on bacteria resistance at two methods: -

3.6.1. Agar well diffusion method: -

Agar well diffusion method is widely used to evaluate the antimicrobial activity of plants or microbial extracts (Magaldi, 2004; Valgas, 2007).

3.6.2. Agar disk-diffusion method:-

is the official method used in many clinical microbiology laboratories for routine antimicrobial susceptibility testing. Nowadays, many accepted and approved standards are published by the Clinical and Laboratory Standards Institute (CLSI) for bacteria and yeast testing (CLSI, 2012; CLSI, 2004).

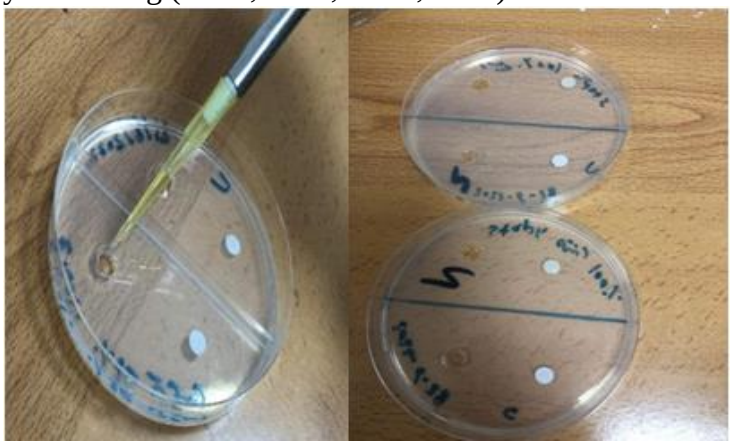


Figure 4. Working method.

The honey was diluted this way:-

Volume/volume percentage concentration calculation.

V/V % (V/V %) =ml/100ml

25%(V/V)=25ml/75ml

50 % (V/V) =50ml/50ml

75% (V/V) =75ml/25ml

3.4. Statistical analysis

-The experiment was designed according to a completely randomized design (CRD) and the significant differences between treatment means were find out by using Duncan Multiple Range Test (Duncan, 1955)**.** Statistical Analysis System (SAS, 2002) was used to apply analysis of variance.

-The data were analyzed using IBM SPSS 20.0 (Armonk, NY USA). Quantitative variables are expressed as mean ± standard deviation (SD). All reported values were 2-tailed, and those <0.05 were considered to be statistically significant. Graph pad prism 8.0 was used for plotting graphs.

Results:

1. Sample characteristics.

● This study evaluated the effect of three types of Libyan natural honey (Seder, Thyme, and spring) and one type of commercial honey on five types of bacterial species.

2. Laboratory results for the effect of honey on bacterial species: -

2.1. The effect of different Seder honey concentrations on the selected bacteria.

The results showed the effect of Seder honey on most of the bacteria spp. with significant differences at 100 % concentration where the average diameters of the inhibition zones were recorded for *P. aeruginosa* (37.5 ± 1), on the other hand, significant differences were recorded for S. *aureus* with an intermediate zone of Inhibition (41.0 ± 6), at 100% concentration. (Table 3 and Figure 5).

Table (3) Effect of different concentrations of Seder honey on bacterial spp.

Concentration %	*E. coli*	*S. aureus*	*k. pneumoniae*	*P. aeruginosa*	*S. epidermidis*
25	2 6 ± 1	23 ± 0	NE	31.0 ± 1	NE
50	30.5 ± 1	32.0 ± 0	11.5 ± 16	32.0 ± 3	NE
75	30.0 ± 0	32.5 ± 4	22.0 ± 0	35.0 ± 0	NE
100	34.5 ± 1	41.0 ± 6	27.0 ± 0	37.5 ± 1	NE

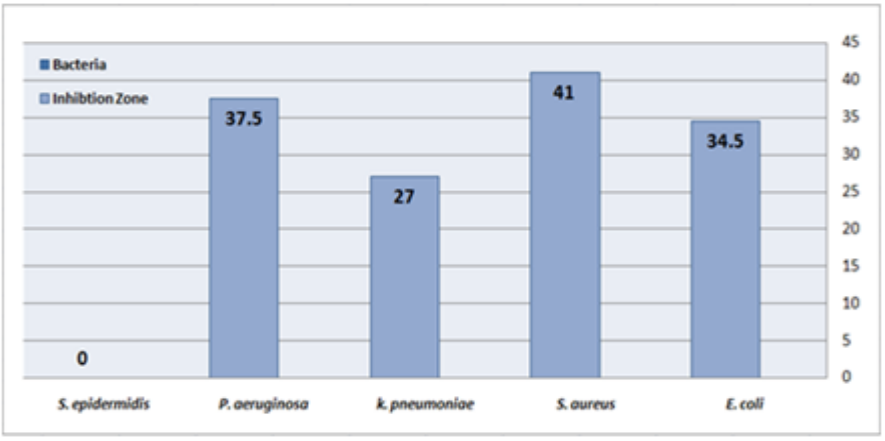


Figure 5: The mean diameters of the inhibition zones of Seder honey on different types of bacterial spp. at 100%concentration.

2.2. The effect of different Thyme honey concentrations on the selected bacteria.

The results showed the effect of Thyme honey on most of the bacteria spp. with significant differences at 100 % concentration where the average diameters of the inhibition zones were recorded for *P. aeruginosa (*42.0 ± 0), on the other hand, (41.0 ± 5) significant differences were recorded for *S. aureus* with an intermediate zone of Inhibition. At 100% concentration. (Table 4 and Figure 6).

Table (4). Effect of different concentrations of Thyme honey on bacteria spp.

Concentration %	*E. coli*	*S.aureus*	*k. pneumoniae*	*P. aeruginosa*	*S. epidermidis*
25	31.0 ± 1	27.5 ± 3	5.5 ± 7	24.5 ± 1	NE
50	33.5 ± 1	30.5 ± 5	12.5 ± 1	31.0 ± 1	NE
75	35.5 ± 1	35.5 ± 1	20.5 ± 1	26.5 ± 1	NE
100	36.0 ± 1	41± 5	29.5 ± 1	42.0 ± 0	NE

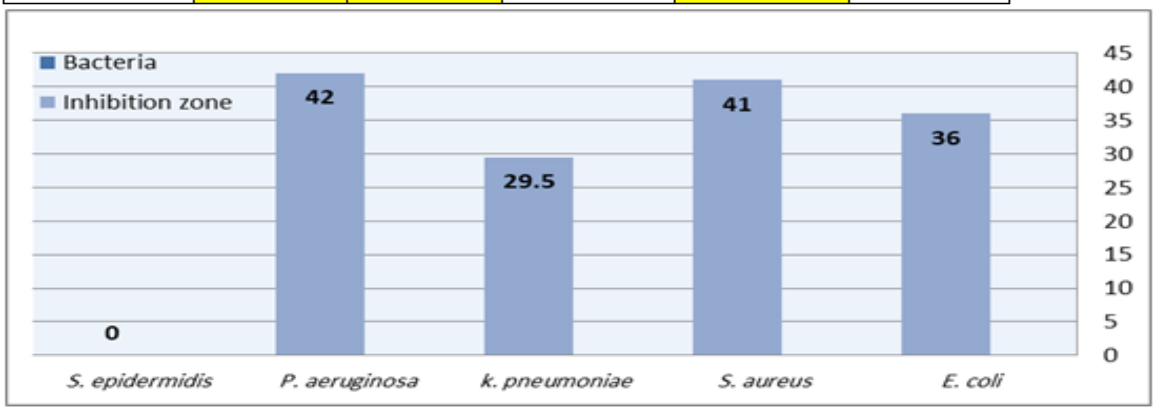


Figure 6: The mean diameters of the inhibition zones of Thyme honey on types of bacterial spp. at 100% concentration different.

2.3. The effect of different Spring honey concentrations on the selected bacteria.

The results showed the effect of Spring honey on most of the bacteria spp. with significant differences at 100 % concentration where the average diameters of the inhibition zones were recorded for *S. aureus* (38.0 ±3), on the other hand, significant differences were recorded for *E. coli* with an intermediate zone of Inhibition (36.5 ±1). At 100% concentration. (Table 5 and Figure 7)

Table (5). Effect of different concentrations of Spring honey on bacteria spp.

Concentration %	*E. coli*	*S. aureus*	*k. pneumonia*	*P. aeruginosa*	*S. epidermidis*
25	29.5 ± 2	25.0 ± 0	6.0 ± 9	NE	NE
50	35.5 ± 1	27.5 ± 1	23.0 ± 0	NE	NE
75	34.5 ± 1	37.0 ± 3	20.5 ± 1	NE	NE
100	36.5 ± 1	38.0 ± 3	32.5 ± 4	19.5 ± 1	NE

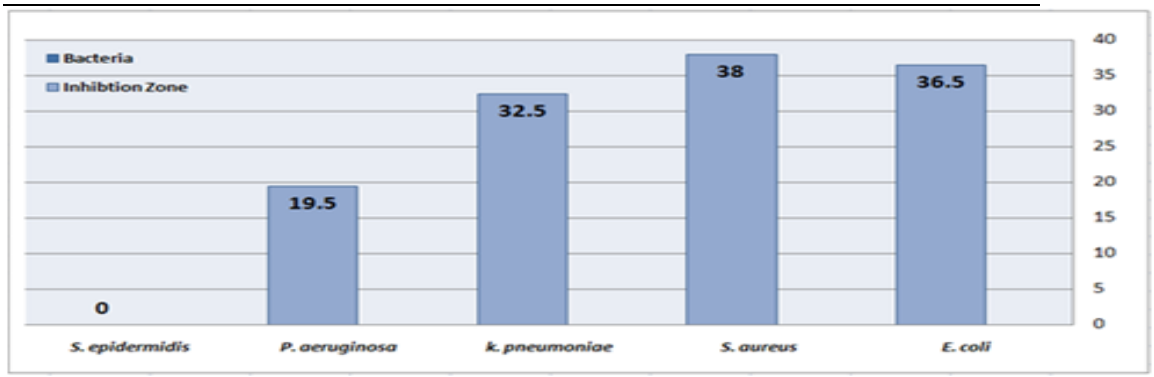


Figure 7: The mean diameters of the inhibition zones of Spring honey on types of bacterial spp. at 100% concentration different.

2.4. The effect of different Commercial honey concentrations on the selected bacteria.

The results showed the effect of Commercial honey on most of the bacteria spp. with significant differences at 100 % concentration where the average diameters of the inhibition zones were recorded for *E.coli* (37.5 ± 2), on the other hand, significant differences were recorded for *S.aureus* with an intermediate zone of Inhibition (35.0 ± 1). At 75% concentration. (Table 6 and Figure 8).

Table (6) Effect of different concentrations of Commercial honey on bacteria spp.

Concentration %	*E. coli*	*S. aureus*	*k. pneumoniae*	*P. aeruginosa*	*S. epidermidis*
25	27.0 ± 0	31.0 ± 1	0	24.0 ± 1	NE
50	28.0 ± 0	34.5 ± 2	22.5 ± 4	28.0 ± 0	NE
75	30.5 ± 2	35.0 ± 1	28.5 ± 2	28.5 ± 1	NE
100	37.5 ± 2	33.5 ± 2	33.0 ± 1	33.5 ± 2	NE

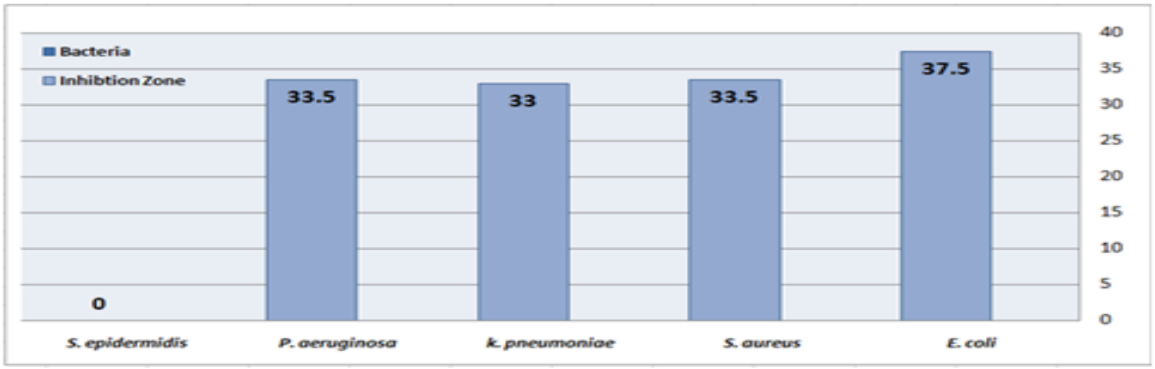


Figure 8: The mean diameters of the inhibition zones of honey Commercial on types of bacterial spp. at 100% concentration different.

3. Statistical data analysis:

Four types of honey were used and their effect on 5 types of bacteria. The tests were doubled to confirm the results on four dilutions (25% - 50% - 75% and 100%) in addition to the control (a lunch medium with bacteria without concentration of honey), Agar Disk and Agar Well diffusion have been the two techniques used to confirm our research results statistical results using the Duncan multiple range test and Statistical Analysis System was used to apply analysis of variance, significantly different to ($P<0.001$).

3.1. The Effect of Seder honey concentration on the five types of bacteria.

Figure 9.a, 9.b, 9.c shows how the difference and effect of honey on different isolated bacteria by using agar disk diffusion and agar well diffusion methods.

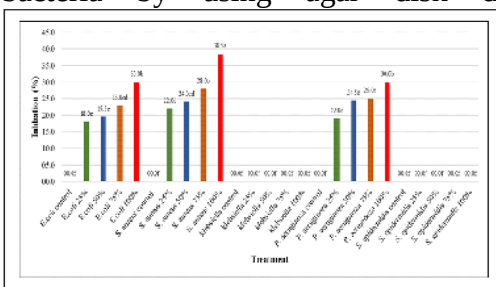


Figure 9a: Effect by using Agar disk diffusion method.

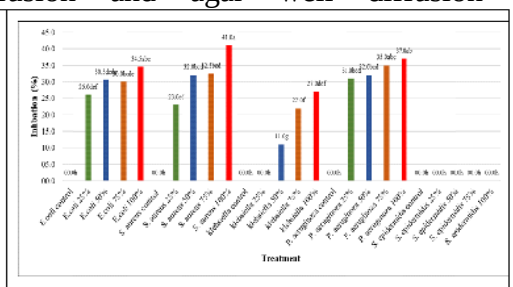


Fig 9b: Effect by Agar well diffusion method

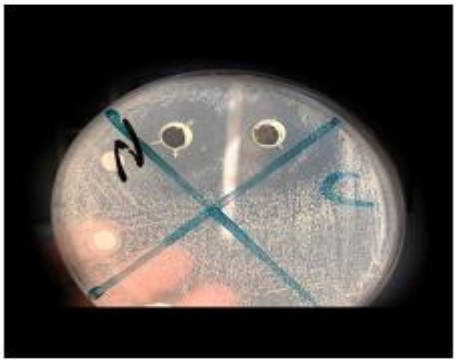


Figure 9c: The effect sider honey on bacteria *S. aureus.*

3.2. The Effect of thyme honey concentration on the five types of bacteria, Figure 10.a, 10.b, 10. c shows how the difference and effect of honey on different isolated bacteria by using agar disk diffusion and agar well diffusion methods.

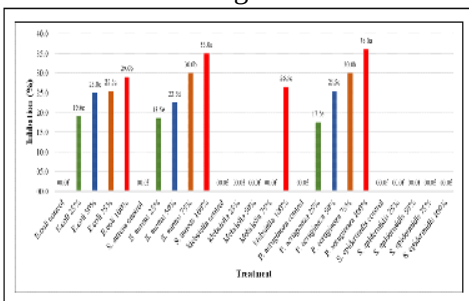


Figure 10.a Effect by using Agar disk diffusion method.

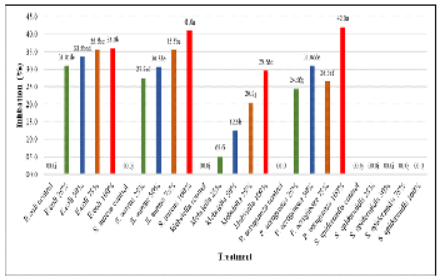


Figure 10.b Effect by using Agar well diffusion method.



Figure 10c: The effect of thyme honey on bacteria *S. aureus.*

3.3. The Effect of spring honey concentration on the five types of bacteria Figures 11.a, 11.b, 11. c shows how the difference and effect of honey on different isolated bacteria by using agar disk diffusion and agar well diffusion methods.

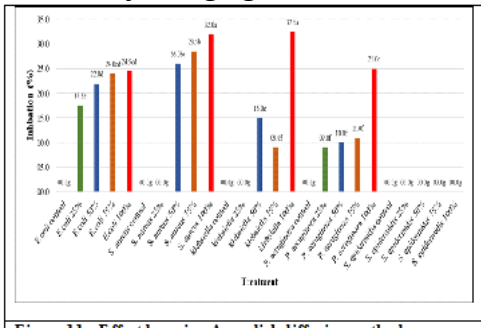


Figure 11.a Effect by using Agar disk diffusion method.

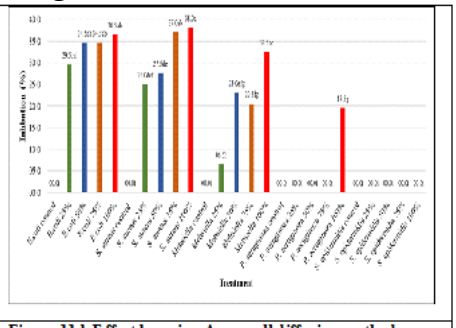


Figure 11.b Effect by using Agar well diffusion method.

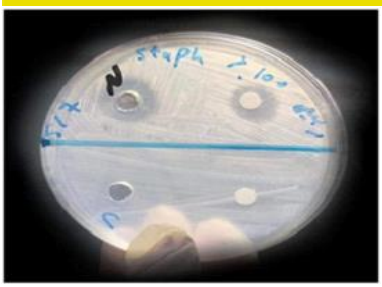


Figure 11.c The effect of spring honey on bacteria *S. aureus*.

3.4. The Effect of commercial honey concentration on the five types of bacteria Figures 12.a, 12.b, 12.c shows how the difference and effect of honey on different isolated bacteria by using Agar disk diffusion and Agar well diffusion methods.

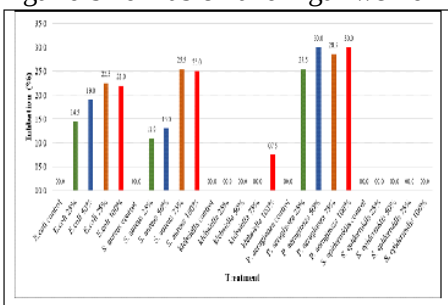


Figure 12. a Effect by using Agar disk diffusion method

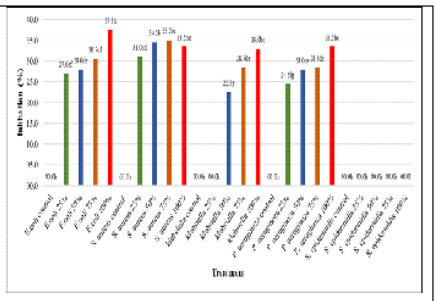


Figure 12.b Effect by using Agar well diffusion method.

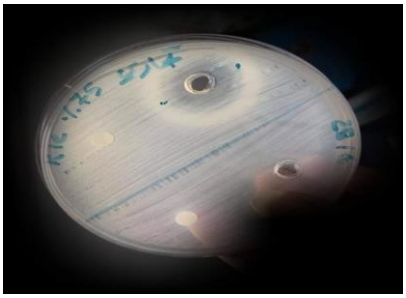


Figure 12.c Effect of commercial honey on bacteria *klebsiella*.

3.5. The honey effect was observed among bacterial species using the well method:

The well Method showed concerning the mean ± SD for Seder, Commercial, and SPRING there are significant differences (p= <.001). The mean ± SD in Seder was (21.71 ± 14.88), in Spring (17.78 ± 15.47),), Thyme (22.54 ± 14.839), and Commercial (22.22 ± 14.10). (Table 7).

Table 7. Mean ± SD and significant level of the honey and bacterial spp. (Well Method).

	N	Minimum	Maximum	Mean	Std. Deviation	*P-Value*
SEDER	41	0	45	21.71	14.878	<.001 [a]
THYME	41	0	42	22.54	14.839	<.001 [a]
SPRING	41	0	40	17.78	15.467	<.001 [a]
COMMERICAL	41	0	39	22.22	14.101	<.001 [a]
Valid N (listwise)	41					

A: Kruskal-Wallis Test

Discussion:

Natural Libyan honey is characterized by an antimicrobial effect and the results were presented in these Figures (9,10,11,12) for the average diameters of the inhibition zones for honey varieties, with different concentrations, it was confirmed that there are significant differences between the average of the inhibition areas for honey types, the probability value was < 0.001, which is less than the moral level specified for the study, which confirms the existence of a highly significant difference for this study.

The Seder honey showed more significance in *P. aeruginosa)* while *S. aureus* moderate significant, non-significant in *S. epidermidis*, *E. coli, k. pnemoniae*, this is in agreement with the study conducted by Kimoto et al., 2008. Our study also showed that thyme honey was more significant in *E. coli* and moderate significant in *S. aureus* and had non-significant on *k. pnemoniae, P. aeruginozsa*, and *S. epidermidis*, this study is similar to the study conducted by Abu Annizah, K et al, 2017 while thyme honey had a similar effect on *S. aureus* at concentrations of 75%, 100%. In this study, spring honey concentrations, had more significant effect on *E. coli* and moderate effect *S. aureus* and non-significant effect on *K. pnemoniae, P. aeruginosa*, and *S. epidermidis* this finding is similar with the study conducted by Abu Annizah, K et al, 2017 while this honey had an effect on *E. coli*), finally the commercial honey showed more significant effect on *S. aureus* and non-significant effect on *P. aeruginoza*, *S. epidermidis*, *K. pnemoniae,* and *E. coli*, this finding is not in agreement with the study conducted by Sheriff, et al, 2011 whereas the commercial honey was more significant in his study on *E. coli*. As Showed in the appendix.

The Statistical results in this study indicated that the fact that types of honey used in our study were effective in inhibiting or killing the studied bacterial species. Estimates were not calculated accurately due to the time factor that requires studying each type of honey and the variables from the nutritional environment or bacterial species.

Conclusion:

Our results suggest that honey can be used as an antibacterial agent against bacterial species regardless if the bacteria were gram positive or gram-negative group.

The great value of antibacterial effect can be obtained when honey was used at concentration of 100%. The inhibition effect of honey decreased as the honey is diluted.

Additionally, the antibacterial activity of the honey tested depended on the type of bacterial species, which means that the honey antibacterial effect can also vary from one bacterial strain to another. The excellent antibacterial activity of honey against clinical bacterial isolates indicates the usefulness of honey in clinical practice against bacterial infections.

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Declaration of competing interest

None.

References:

Abu Annizah, K., & Al-khayali, Y. (2017). Antimicrobial effect of natural Libyan honey & propolis extract against some human pathogens, *Journal of pure & Applied Sciences*, 18(4), 27-34.

Al-Jabri, A. A. (2005). Honey, milk and antibiotics. *African Journal of Biotechnology*, 4(13).

Al-Jabri, A. A., Nzeako, B., Al Mahrooqi, Z., Al Naqdy, A., & Nsanze, H. (2003). In vitro antibacterial activity of Omani and African honey. *British journal of biomedical science*, *60*(1), 1-4.

Almasaudi, S. (2021). The antibacterial activities of honey. *Saudi Journal of Biological Sciences*, *28*(4), 2188-2196.

Basualdo, C., Sgroy, V., Finola, M. S., & Marioli, J. M. (2007). Comparison of the antibacterial activity of honey from different provenance against bacteria usually isolated from skin wounds. *Veterinary microbiology*, 124(**3-4**), 375–381.

Board, N. H. (2002). Honey-health and therapeutic qualities. *Natl Honey Board*, 1-28.

CLSI, C. (2012)**.** Performance Standards for Antimicrobial Disk Susceptibility Tests**..** Approved Standard, 7th ed., CLSI document M02-A11. Clinical and Laboratory Standards Institute, 950 West Valley Road, Suite 2500, Wayne, Pennsylvania 19087, USA.

CLSI, C., & Wayne, P. (2004). Method for antifungal disk diffusion susceptibility testing of yeasts, approved guideline. *CLSI Doc. M44-A*, *24*, 1-36.

Dash, N., Panigrahi, D. & Al-Zarouni, M. (2016) Antimicrobial Effect of Honey from the Arabian Gulf Region against Bacterial Isolates from Pus and Wound Swabs. *Advances in Microbiology*, 6**(10)**, 745-752.

Dustmann, J. H. (1989). "Antibacterial effect of honey," *Apiacta*, 14(**1**), pp. 7–11.

Duncan, D. B. (1955). Multiple range and multiple F tests. *Biometrics*, 11(**1**), 1–42.

Eteraf-Oskouei, T., & Najafi, M. (2013). Traditional and modern uses of natural honey in human diseases: a review. *Iranian journal of basic medical sciences*, *16*(**6**), 731-742.

Hannan, A., Barkaat, M., Saleem, S., Usman, M., & Gilani, W. A. (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**.**

Hamza, A. S., Aliyu, A., & Ibrahim, F. M. (2015). Evaluation of Antibacterial Activity of Sudanese Bee honey Against Four Species of Bacteria. *American Journal of Research Communication*, 3(**4**): 132- 143.

Heatley N. G. (1944). A method for the assay of penicillin. *The Biochemical journal*, *38*(**1**), 61–65.

Jaganathan, S. K., Mandal, S. M., Jana, S. K., Das, S., & Mandal, M. (2010). Studies on the phenolic profiling, anti-oxidant and cytotoxic activity of Indian honey: in vitro evaluation. *Natural product research*, 24(**14**),1295–1306.

Krushna, N. S., Kowsalya, A., Radha, S., & Narayanan, R. B. (2007). Honey as a natural preservative of milk. *Indian journal of experimental biology*, 45(**5**), 459–464.

Kimoto-Nira, H. & Amano, K. (2008). Antimicrobial activity of honey produced by stingless honey bees, *Journal of Apicultural Research*, 47**(4)**, 325–327.

Malik, A. H., & Sharma, B. D. (2010).Comparison of hurdle treatments for buffalo meat. *International Journal of Food Science & Technology*, 45 (**8**), 1552-1563.

Magaldi, S., Mata-Essayag, S., Hartung de Capriles, C., Perez, C., Colella, M. T., Olaizola, C., & Ontiveros, Y. (2004). Well diffusion for antifungal susceptibility testing. *International journal of infectious diseases : IJID : official publication of the*

International Society for Infectious Diseases, *8*(**1**), 39–45.

Mandal, M. D., & Mandal, S. (2011). Honey: its medicinal property and antibacterial activity. *Asian Pacific journal of tropical biomedicine*, *1*(**2**), 154–160.

Molan, P. C. (1992). "The antibacterial activity of honey." *Bee World 73*(**1**), 5-28

Maraia, F. (2016). Antibacterial Activity of Eucalyptus Honey Of Libyan Against Multidrug Resistant Bacteria (MDR). EC Bacteriol-ogy and Virology Research, **2**, 115-120.

Molan, P. C. (1999). The role of honey in the management of wounds. *Journal of Wound Care*, 8 (8), 415-418.

Oelschlaegel, S., Pieper L, Staufenbiel R, Gruner, M., Zeippert, L., Pieper, B., Koelling-Speer, I., & Speer, K. (2012) Floral markers of cornflower (Centaurea cyanus) honey and its peroxide antibacterial activity for an alternative treatment of digital dermatitis. *Journal of Agricultural and Food Chemistry*, 60 (47),11811-11820.

Patton, T., Barrett, J., Brennan, J., & Moran, N. (2006) "Use of a spectrophotometric bioassay for determination of microbial sensitivity to manuka honey," *Journal of Microbiological Methods*, vol. 64 (1), pp. 84–95.

Schneider, M., Coyle, S., Warnock, M., Gow, I., & Fyfe, L. (2013). Anti-microbial activity and composition of manuka and portobello honey. *Phytotherapy Research*, 27(8), 1162-1168**.**

Sanz, M. L., Gonzalez, M., de Lorenzo, C., Sanz, J., and Martínez-Castro, I, (2005). "A contribution to the differentiation between nectar honey and honeydew honey," *Food Chemistry*, vol. 91, (2): 313–317.

SAS. (2002). Statistical Analysis System (SAS) 9.00. SAS Institute In, Cary, NC, USA.

Swellamm, T., Miyanaga, N., Onozawa, M., Hattori, K., Kawai, K., Shimazui, T., &Akaza, H. (2003). Antineoplastic**,** activity of honey in an experimental bladder cancer implantation model: In vivo and in vitro studies. *Interantional journal of urology: official journal of the Japanese urological Association*, 10 (4), pp. 213-219.

Sheriff, A.,Y., Younis, K., M., Mohammed, R., I. (2011). A comparative study of the effect of natural and trade honey in the growth of some bacterial species. *Tikrit journal of pure stientes*, 17(**1**), 101:103.

Tonks, A. J., R. A. Cooper, K. P. Jones, S. Blair, J. Parton & A. Tonks (2003). Honey stimulates inflammatory cytokine production from monocytes. *Cytokine* 21 (5): 242-247.

Valgas, C., De Souza, S.M., Smânia, E.F., & Smania, J. A. (2007) Screening methods to determine antibacterial activity of natural products. *Brazilizn Journal of Microbiology.* 38:369–380.

Wang, J., & Li, Q. X. (2011). Chemical composition ،characterization ،and differentiation of honey botanical and geographical origins. *Advances in food and nutrition research*, **62**, 89-137.

World Health Organization (WHO). (2014).Traditional Medicine Strategy. Geneva, Switzerland.