

## Study of some physical and chemical properties of local honey and antimicrobial activity against pathogenic bacteria

### دراسة بعض الخصائص الفيزيائية والكيميائية للعسل المحلي والفعالية ضد ميكروبية ضد الجراثيم الممرضة

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#### Abstract :

The indiscriminate use of antibiotics made many microorganism develop resistance to them. This created immense clinical problems in the treatment of infectious disease, therefore ; there is need to develop alternative antimicrobial agents for the treatment of infectious disease. The purpose of this study was to investigate the physical and chemical properties of some different types of honey in Kerbala city (*Helianthus L.*, *Zizyphus*, *Eucalyptus glodulus*, *Medicago satival*) such as water content, pH value, color, refractive index and electrical conductivity and detect the antimicrobial activity against some pathogenic bacteria (*Staphylococcus epidermidis*, *Streptococcus spp.*, *Pseudomonas spp.* and *Proteus spp.*). The results showed that honey exhibited different antimicrobial activity against all bacterial species according to the type of plant where the bee feeded , which the MIC of the four samples was all up to (11.25%) against all tested bacteria ,also there is a correlation between the previous physical and chemical properties and the antimicrobial activity, on which the sample B showed a best antimicrobial activity due to the affect of pH value (4.88) and also because the enrichment of this sample with glucose, while sample A showed lowest results due to the prsence of metal ion that inhibite the production of toxic microbial compound hydrogen peroxide .

#### الخلاصة

ان الاستخدام الخاطئ للمضادات الحياتية جعل الكثير من الاحياء المجهرية تظهر مقاومة لهذه المضادات وأدى الى حدوث مشاكل سريرية عديدة عند معالجة الأمراض المختلفة, لذا دعت الحاجة الى البحث عن مصادر جديدة ذات فعالية ضد ميكروبية لمعالجة الإصابة بالأمراض. الغرض من هذه الدراسة هو التحري بعض الخواص الفيزيائية والكيميائية لبعض أنواع العسل في محافظة كربلاء (*Helianthus L.*, *Eucalyptus*, *Zizyphus glodulus*, *Medicago satival*) مثل المحتوى المائي, الأس الهيدروجيني, الكثافة الضوئية, معامل الانكسار (RI) والتوصيل الكهربائي والكشف عن الفعالية التثبيطية ضد بعض أنواع البكتيريا المرضية (*Staphylococcus epidermidis*, *Streptococcus spp.*, *Pseudomonas spp.* and *Proteus spp.*). لقد أظهرت النتائج ان الأنواع المختلفة من العسل لها تأثير تثبيطي ضد كل أنواع بكتيريا قيد الدراسة والذي يختلف باختلاف مكان ونوع النبات التي تتغذى عليه النحلة , حيث كانت قيم MIC للنماذج الأربعة من العسل أكثر من (11.25%) ضد كل أنواع البكتيريا قيد الدراسة . كذلك وجد ان هناك علاقة تربط الخواص الفيزيائية والكيميائية الأنفة الذكر مع الفعالية التثبيطية حيث أظهرت النتائج ان نموذج العسل B كان له أقوى فعالية تثبيطية بسبب الأس الهيدروجيني (4.88) و لانه غني بالكلوكوز ,بينما نموذج العسل A اظهر أسوأ فعالية تثبيطية بسبب احتوائه على الايونات المعدنية التي تعمل على تثبيط انتاج بيروكسيد الهيدروجيني الذي يعتبر من المواد السامة لبعض الاحياء المجهرية.

#### Introduction

The need to develop alternative antimicrobial agents for the treatment of infectious diseases was evokes to the association adverse effect of antibiotic ( which is a molecules that stop microbes from growing or killing them outright) on host which include hypersensitivity , depletion of beneficial gut and mucosal microorganisms ,immunosuppresion and allergic reaction (1). Honey is acceptable in the medical profession as an antibacterial agent for the treatment of some diseases and infections resulting from wound and burns(2),which is a substances made when the nectar and sweet deposits from plants are gathered , modified and stored in the honeycomb by honey bees(3).and also useful in the treatment of burns, ulcers and acute diarrhea (4).

For centuries it has been known that different types of honey exhibit different antibacterial activity, so Aristotle, in 350 BC , and Discorides recommended that honey collected in specific

regions and seasons could be used for the treatment of different ailments. Manuka honey gathered from the Manuka tree *Leptospermum scoparium* has high antibacterial activity due to the osmotic effects of their high sugar contents against *Helicobacter pylori* , *Staphylococcus aureus*, *Staphylococcus epidermidis* (5,6) while the honey of Egyptian clove has antibacterial activity against *Escherichia coli* and *Salmonella typhimurium* which isolated from stool of children (7) .There are many features in the composition of honey that combine together to give its antimicrobial properties, these includes hydrogen peroxide which is produced by glucose oxidase enzyme cause a shrinkage disruption of bacterial cell wall , low pH of 3.6 and the fermentation of honey (3,4)

This study was undertaken in order to detect the chemical and physical prosperities and determined the minimum inhibitory concentration (MIC) of Kerballa honeys against bacteria isolated from different clinical samples.

## Materials and methods

### Honey samples

Four samples of honey (A,B,C and D) were collected from Kerballa City. All samples were Kerballa honey, as shown in table- 1.

Table 1: The four samples of honey

samples	Sources
A	<i>Zizyphus</i>
B	<i>Helianthus L.</i>
C	<i>Eucalyptus glodulus L.</i>
D	<i>Medicago satival L.</i>

### Bacterial isolates

The following isolates which isolated from different clinical samples were obtained from biology department/ Kerballa University ,as shown in table- 2.

Table 2: The clinical isolates of bacteria

Bacterial species	Gram stain reaction	Sources
<i>Staphylococcus epidermidis</i>	+ ve	Wounds
<i>Streptococcus spp.</i>	+ve	Throat
<i>Pseudomonas spp.</i>	-ve	Burns
<i>Proteus spp.</i>	-ve	Urine

### Antibacterial susceptibility test

#### Preparation of honey suspensions for MIC test

The four samples of honey (A,B,C and D)were each diluted in sterile distilled water to obtain 3.75%, 7.5%, 11.25%, 15%, 18.75%, 22.75% and 26.75% ( v/v) concentration.(3)

#### Preparation of bacterial suspension for MIC test

Each 5 ml of nutrient broth was inoculated with a test microorganism and incubated at 37°C for 18 hours

### Physical and chemical analysis of the honey samples

half gram of each of the four samples (A,B,C and D) was dissolved in 100 ml of distilled water and analyzed for the following parameters:

#### Water content

The samples was put in an oven at (115 °C) for 30 minute , then the water content was measured as recommended by William,1980 (8).

### **Measurement the pH value**

The pH value was measured by using the pH meter type OAKLON / 1200 (Germany ) at room temperature.(9)

### **Measurement of the color density**

Ten grams of each honey sample were diluted with 100 ml of distilled water and centrifuged for 10 minutes; 3,000 xg. The absorbency of the filtrate supernatant was measured on 350 nm by using distilled water as a blank (10 ).

### **Measurement of refractive index (RI°)**

The refractive index of the samples was measured by using a refractometer ATAGO/1T (6100-EU) and the value was determined according to Al-gamali,1987 (11).

### **Measurement of specific conductivity**

Electrical conductivity was measured as described by Vorwohl.,*et al* 1988 (12). The reading was taken at a standard temperature of 23°C.

## **Minimum inhibitory concentration of honey against pathogenic bacteria**

The broth dilution technique was used to ascertain the MIC of the honey samples. The test was carried out as described by Heunvelink., *et al* 1998 (13). The different concentration of honey (v/v) were prepared in a Muller-Hinton broth (MHB) at 56°C to give final concentration 3.75%, 7.5%, 11.25%, 15%, 18.75%, 22.75% and 26.75% ,then the tubes were inoculated with each tested bacteria and incubated at 37°C for 18- 24 hours. The MHB without honeys was inoculated with tested bacteria to serve as a control. MIC is the lowest concentration of honey that yield no growth.

## **Results and discussion**

### **Physical and chemical analysis of the honey samples**

#### **Measurement of Water content**

The water content of samples was shown in table -1 . which demonstrated that sample A had a higher percentage of water content 11.25%, while sample B had a lowest percentage 8.698%,the C and D had a similar range of percentage which were 9.17% and 9.155% respectively.

The highest the water content, the increase the inhibition activity against microbes due to the activation of glucose oxidase an enzyme which enhanced the production of H<sub>2</sub>O<sub>2</sub> which is toxic to bacteria(14,15,16)

#### **Measurement of pH value**

The pH value of the samples was at range of acidity (4.88-5.06) as shown in table -1 , the highest pH was shown in sample D (5.06) and lowest pH in sample B (4.88) ,where pH value of sample A and C was (5.00) and (4.97) respectively.

The acidity was due to the content of gluconolactone/gluconic acid present as a result of enzyme action in the ripening nectar(17).

#### **Measurement of Color density**

The color of honey which measured in optical density at 350nm are shown in table- 1 , the sample B and D show the higher value while sample A and C had the lowest value of optical density.

There was a correlation between pH and color density, on which acidity of honey is affected by the color ,when the transparency of the color increases this indicates that honey rich in potassium and glucose which part of it converted to gluconic acid and so decreases the pH value(18,19)

### **Measurement of Refractive index (RI)**

The refractive index of all samples had a slightly differences in their value table -1. The sample C had a highest value of RI (1.349042) followed by sample A,D and B which the RI were 1.347042, 1.342042 and 1.341242 respectively.

The reason of this similarity may be due to the presence of hanging particle waxes which mixed with the honey which led to increase the ratio of RI value. Also it may be due to the difference of sugar component and type of amino acid which react with sugar to give the specific color of honey (20)

### **Measurement of Electrical conductivity**

Sample A had a high level of electrical conductivity (789) while samples D, B and C had a lower level which were 209, 188, and 181 respectively.

Conductivity is a good criterion of the botanical origin of honey and today it is determined in routine honey control instead of the ash content. This measurement depends on the ash, metal ions and acid content of honey; the higher their content , the higher the resulting conductivity (21,22) .

### **Minimum inhibitory concentration of honey against pathogenic bacteria**

The minimum inhibitory concentration of the four natural honeys against gram +ve and gram – ve bacteria, are shown in (figure 1) , there was a striking similarity between the four honeys concentration up to 11.25% , at these concentrations , the *Proteus spp.* showed a similar inhibitory at concentration (15%) toward all types of honey samples except sample A (18.75%) . In *Pseudomonas spp.* the MIC of the four samples of honey against all tested bacteria was at concentration (18.75% ), while *Staphylococcus epidermidis* showed a variable results on which MIC of sample A was (22.75%) while sample B and C were (11.25%) and sample D was (15 %). Finally the MIC of honey against *Streptococcus spp.* was (22.75%) for sample A , (11.25%) for sample B and D and (15%) for sample C.

The honey which tested in this study had antimicrobial activity in the range between 11.25% - 22.75% against the tested microorganisms. The variability among these results may be due to the different source of honey (23). Pogdanow and Champman *et al.* revealed that the antimicrobial activity of honey may range from concentration lower than 3% to concentration of 50% (24,25) .

From these results it can be concluded that sample B had a best antibacterial activity compared with the other samples, this may attributed to many factors such as acidity pH (4.88) which is affected by the color of honey ( optical density), when the optical density increase, this indicate that honey is rich with glucose which part of it converted to gluconic acid(18). The gluconic acid led to decrease the pH value from one side ,and from the other side it responsible for the formation of hydrogen peroxide( $H_2O_2$ ) an enzymatic by- product (glucose oxidase) which is toxic to bacteria(17). The water content considered another factor that increase the inhibition activity against microbes through its activation of glucose oxidase production, but the water content antagonized with electrical conductivity i.e. when electrical conductivity increase this mean that honey is rich with metal ions  $Ag^+$ ,  $Hg^{++}$ ,  $Mn^+$  ,these metal ions considered as an inhibitors for the enzyme glucose oxidase and finally led to prevent the production of hydrogen peroxide which contributes to the antibacterial prosperities of honey(26,27). This explain why sample A in spite of its high water content but it showed a lower antibacterial activity.

In general view not all physical and chemical prosperities of honey had an effect on the killing of microorganism , because there are an important role other than those agent which are the virulence factor of microorganism itself and proteineuos compounds that play an important role in its susceptibility to honey as an antimicrobial agents(4,24).

**Table-3: the physical and chemical prosperities of the four samples of honey**

<b>TYPE OF HONEY</b>	<b>water content</b>	<b>pH value</b>	<b>Color as optical density</b>	<b>Electrical conductivity</b>	<b>Refractive index</b>
<b>A</b>	11.56%	5.00	0.84236	789	1.347042
<b>B</b>	8.698%	4.88	0.92832	188	1.341242
<b>C</b>	9.17%	4.97	0.8121	181	1.349042
<b>D</b>	9.15%	5.06	0.94288	209	1.342042

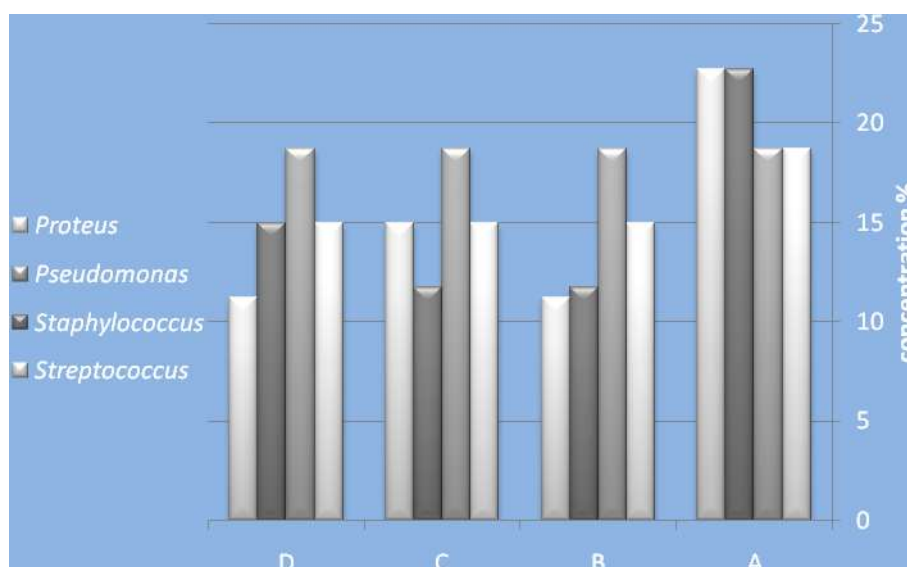
A= *Zizyphus*

C= *Eucalyptus glodulus L.*

B= *Helianthus L.*

D= *Medicago satival L.*

**Figure-1: The correlation between the concentration of the four samples of honey and pathogenic bacteria**



A= *Zizyphus*

C= *Eucalyptus glodulus L.*

B= *Helianthus L.*

D= *Medicago satival*

**References**

1. Al-Jabri,A.A.(2005). Honey , milk and antibiotics. African J. of Biotechnology 4(13) :1580-1587.
2. Zulma,A. ; Lulat,A.(1989). Honey – a remedy rediscovered .J.Roy. soci. Med. 83:384-385.
3. Molan , P.C.(1992 ). The antibacterial activity of honey .2. variation in the potency of the antibacterial activity. Bee world 73: 59-76.
4. Subrohmanyam, M. ; Hemmady; Pawar, S.G.(2001). Antibacterial activity of honey on bacterial isolated from wounds. Annuals of burns and fire disasters. Vol.xiv.
5. Al-Somal, N. ; Coley, K.E. ; Molan, P.C. ; Hancock, B.M.(1994) . Suseptibilty of *Helicobacter pylori* to the antibacterial activity of Manuka honey. J.Royal soc. Med .87:644.
6. French, V.M. ; Cooper, R.A. and Molan , P.C.(2005). The antibacterial activity of honey against coagulase negative *Streptococcus*. J. antimicrobial chemotherapy.56:228-231.

7. Badawy, O.F.H. ; Shafi, S.S.A ; Tharwat, E.E. and Kamal, A.(2004). Antibacterial activity of bee honey and its therapeutic usefulness against *Escherichia coli* O157:H7 and *Salmonella typhimurium* infection. Rev .sci. ther. Off.int .Epiz. 23(3), 1011-1022.
8. William H. (1980). Official methods of analysis of the association of official analytical chemists,13<sup>th</sup> ed . association of official analytical chemists,Washington,DC,520 pp
9. Egan H. Ronald K. and Ronald S.(1987). Pearsons chemical analysis of food , 8<sup>th</sup> ed. \longman Group , London
10. White , J.W.Jr(1992). Quality evaluation of honey:role of HMF and diastase assays .Am.Bee.J.132(11,12): 737-742.
11. Al –gamali, J.S.(1987) . fuel technology. University of technonolgy , Baghdad (arabic).
12. Vorwohl G. ; Salem M.S. and Nour M.E(1988).chemical and physical prosperities of Egyptian honey. In proc.4<sup>th</sup> international conference on apiculture in Tropical Climates, 6-10, November,Cairo,Government of Egypt, Cairo and the international Bee research association, Carriff, 240-244.
13. Heunvelink, A.E. ; Van den Biggelaar, F.L. ; Zwartkruis,N. ;Melchers, W.J. ; Monnens,L.A. ;de Boer,E. (1998). Occurrence of verocytotoxine- producing *E.coli* o157 on Dutch dairy farms. J.Clin.Microbial .36(12),3480-3487.
14. Stinson E.E. , Subers, M.H. ; Petty J. and White,J. W.Jr. (1960). The composition of honey. Separation and identification of the organic acids. Arch.Biochem.Biophys. ,89, 6-12.
15. White J.W.Jr. and Subers M.H.(1963). Studies on honey inhibine 4. Destruction of the peroxide accumulation system by light.J.food sci., 29(6), 819-828.
16. Dustmann J.H.(1979). Antibacterial effect of honey. Acta,14(1), 7-11.
17. Thiamann, K.V.(1963). The life of bacteria, 2<sup>nd</sup> ed . Macmillan,New Yourk,909 pp.
18. Wooton M.; Edwards R.A.; Faraji-Haremi R. and Jonson A.T(1976). Effect of accelerated storage conditions on the chemical composition and prosperities of Australian honeys. 1: color, acidity and total nitrogen content.J.Apic.Res. 15(1), 23-28.
19. Nour M.E. , Abou zaid M.N. and Selim(1991). Effect of long term storage on Egyptian honey quality. J.agri.Sci. ,16 (11), 2691-2698.
20. Luma M.A. , Burhan R.N. and Zeina, M.K.(2007). Study of some physical and chemical prosperities to some types of explored and local honeys. J .Al-Qadisiya. 4(12)1-17.
21. Bogdanov, S. Honey Quality and International Regulatory Standards: Review of the Work of the International Honey Commission. Mitt. Gebiete Lebensm. Hyg., 90, 108-125
22. Piazza, M.G., Accorti, M. e Persano Oddo, L.(1991): Electrical conductivity, ash, color and specific rotatory power in Italian unifloral honeys. Apicoltura 7, 51-63.
23. Allen K.L. , Molan P.C. and Raid .(1991). A survy of the antibacterial activity of some new Zealand honeys. J. Pharm.Pharmacol. , 43(12), 817-822.
24. Bogdanow, S.(1997). Non-peroxide antibacterial activity of honey. In Bee-products: properties applications and apitherapy(A .Mizrahi and Y. Lensky eds). Plenum publishing corporation, New Yourk, 39 -47.
25. Champman P.A., Siddons C.A. , Wright D.J., Norman P. and Crick, E. (1993). Cattle as a possible source of verocytotoxine-producing *Escherichia coli* 0157 infections in man . Epidmiol . Infect.. 111(3), 439-447.
26. Terrab.A. ; Gonzalea,A. ; Diez,N. and Heredia, F.J.(2003).mineral content and electrical conductivity of the honey produced in Northwest Morocco and their contribution to the characterization of unifloral. J. sci food and agricul. 83(7):634-643.
27. Nakamura,S. and Ogura,Y. (1968). Mode of inhibition of glucose oxidase by metal ion. biochem. 64(4): 439-4