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Comparison of the effect of natural and commercial honey on the growth and Antibiotic sensitivity of *Escherichia coli* and *Pseudomonas aeruginosa*

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Abstract

This study was conducted to evaluate the inhibitory activity of two types of natural honey (citrus flowers honey and eucalyptus honey) and two types of commercial honey available in the market (sinbola honey and shafi honey), on growth of *Escherichia coli*, *Pseudomonas aeruginosa*. and their sensitivity to antibiotics Amikacine (AK) 30µg, Ampicillin / Sulbactum (SAM) 20µg, Augmenten (AUG) 30µg, Chloramphenicol (C) 30µg and Gentamicin (GM) 10µg. The results showed that the natural honey significantly superiority compared to commercial honey on inhibiting bacterial growth for isolates. The diameter of inhibition zone of citrus flowers honey and eucalyptus honey against *E. coli* growth was 20 and 21 mm, respectively and *P. aeruginosa* was 19 and 14 mm, respectively compared to commercial sinbola honey which gave an inhibition diameter of *E. coli* 8 mm while not affected on *P. aeruginosa* , as for shafy honey it not affected in bacterial isolates growth. Also the results showed that the citrus flowers honey exceeded significantly compared to eucalyptus honey for *P. aeruginosa* the inhibition diameters of citrus flowers honey and eucalyptus honey were *19* and 14 mm respectively. On the other hand, the results showed that the inhibitory effect of natural honey was closely related to antibiotics, and it gave a positive result when compared to the standard tables of the inhibition of antibiotics. *P. aeruginosa* was resistant to both chloramphenicol, Augmenten and Ampicillin, while sensitive for two types of natural honey. The results indicated that the combination of natural and commercial honey with antibiotics was increased the efficiency of antimicrobial activity of antibiotics by increasing the diameters of bacterial growth inhibition compared with the diameters which given by antibiotics. The citrus flower honey had the greatest inhibitory effect on bacterial isolates when it was mixed with antibiotics followed by Eucalyptus honey, while commercial sinbola honey was the least impact.

Key words: Honey, antibiotics, *Escherichia coli*, *Pseudomonas aeruginosa*

Introduction

The excessive use of drugs led to the emergence of resistant bacterial isolates to antibiotics as well as harmful side effects as chemicals or chemical substances, which made scientists and researchers are turning to the use of natural materials, especially honey as an alternative to medicines or supplement to reduce the dose of medicines. The oldest use of honey in the cleansing and healing of wounds, and the history of the use of honey is back to 2000 – 2100 BC. is characterized by being widespread in most cities of the world, if not all (1).

Honey has an antimicrobial effect against many species of bacteria (both positive and negative), as well as viruses and fungi, this is due to the composition of it which contains a number of different component (2) include phenolic acids and hydrogen peroxide, as well as the osmotic effect of honey caused by its sugary components (1) and (3) in addition to low acidity ranging from 3.6 to 4.0 and the high honey viscosity prevents penetration of bacteria and formation of colonies (4).

Honey has an antibiotic effect on bacterial species that have the ability to form biofilm especially *P. aeruginosa* which cause many diseases such as sinusitis, wound inflammation and burns, and other Gram-negative species as aquired Hospitalized diseases (5), it has become multidrug resistant because of their biofilm composition, which does not allow the antibiotics penetration that used in treatment, but when shed honey on these types of bacteria found that it is more effective than antibiotics in killing these bacteria.(6), (7) and (8).

The honey consists of 38% fructose, 31% glucose, 10% other sugars, 17% water and a high percentage of nutrients, amino acids, vitamins and minerals as well as some enzymes added by bees during the manufacture and the enzyme Invertase, which converts sucrose to glucose and fructose therefore the content of the honey is only 1% sucrose (9). our prophet Mohammed (peace be upon him) said: "Healing in three honey drink, cupping and burning with fire but I do not recommend fire.

As a result of the resistance mechanisms development of microbes against many antibiotics, we considered this study, which aims to use honey as an alternative antibiotic, increase the efficiency of antibiotics and make a comparison in the inhibitory effect between natural honey and commercial honey against bacterial growth.

Materials and methods:

- Types of honey:

Four types of honey were used, two types of natural honey (Citrus flower honey, eucalyptus and clover honey) and two types of commercial honey were available in the market as (Sinbola and Shafi)..

Antibiotics discs: -

1- Amikacine (AK) 30 µg
2- Ampicillin / sulbactum (SAM) 20µg
3- Augmenten(Amoxillin /clavulanic acid)30µg
4 Chloramphenicol (C) 30 µg
5- Gentamicin (GM)10µ

- Culture media

1- nutrient agar
2- macConkey agar
3- muller hinton agar

Bacterial isolates -

Two isolates were taken from Microbiology Laboratory at the college of Science / University of Tikrit isolated from urinary tract infections and intestinal inflammation and were confirmed to return to *Pseudomonas aeruginosa* and *Escherichia coli* following a number of tests involving growth on the MacConkey Agar , gram stain, as well as a number of chemical tests, such as the indole test, the methyl red test and the citrate utilization test (10) and reactivated on nutrient agar medium then incubated at 37°C (11).

- Antibiotic sensitivity test:

The sensitivity test for a number of antibiotics was conducted by using the Kirby-baure method as described by (12). A suspension of bacterial isolates was carried out by transferring a number of pure colonies to tubes containing the nutrient broth and incubated in 37 °C for 18-24 hours and then compared with macfarl and standared Solution which is equal 1.5 x 10^8 cell/ml (13). In the case of unequal tubes turbidity, the normal slain solution add until the turbidity is equal to the mcfarl and tube, the sterile cotton swab is submerged in the growth and spread on the culture media surface and left to dry for 15 minutes and then distributed the antibiotic discs by sterile forceps and incubated dishes at 37 °C for 18-24 hours, after which the diameters of the inhibition area were measured for each disk and compared with the standard tables of the WHO.

Effect of honey on bacterial growth:-

The sensitivity test was conducted by using disc diffusion method according to the method described by (14). Prepare the discs of the filter paper saturated with each type of honey under study after confirmation of honey from microbes by filtering in special filters, bacterial inoculums for each of *E. coli*, *P. aeruginosa*, were transfer by sterile cotton swab to the surface of the muller hinton agar after comparing it with the mcfarl and tube. The honey-saturated discs were then placed on the surface of the cultivated dishes and incubated at 37 ° C for 18-24 hours and diameter of inhibition zones were measured by millimeters.

- The sensitivity test of bacterial growth affected by combination of honey with some antibiotics:

The bacterial inoculums was transferred from each of bacterial isolates studied by sterile cotton swab to the muller hinton surface by using disc diffusion method, these antibiotic discs for the five studied species after they were saturated with 50 µl of each of the four honey types were placed on the surface of the cultivated dishes and incubated at (37 ̊c) for the period (18-24) hours after which the regions of the inhibition zones were measured for each disc by millimeters (12).

Statistical analysis

The experiment was statistically analyzed using ANOVA, and the averages was compared with Duncans values at the level of 0.05 based on the program (15).

Results and discussion:

Data in table (1) shows the sensitivity of bacterial isolates to some antibiotics. The table shows that *P. aeruginosa* resistant to Ampicillin , Amoxillin and clavulanic acid (Augmenten) while, *E. coli* was sensitive to Ampicillin, the bacteria resistance to these antibiotics due to its possession β-lactamase enzymes, which altered the structure of the antibiotic by breaking the beta-lactam ring(16). The results show that all bacterial isolates were sensitive to Amikacine and Gentamicin. As for chloramphenicol, the bacterial isolates showed resistance to them. The causes of bacterial resistance to antibiotics are due to several factors including the modulation of the target site of antibiotic binding (17), as well as the possession of bacteria to the active stream mechanism which reduces the antibiotic accumulation within the bacterial cell (18).

Table 1. Bacterial isolates Sensitivity to certain some antibiotics (inhibition zone by mm)

Bacterial isolates	Antibiotics				
	C	AK	GM	AUG	SAM
E.coli	18 (R)	21 (S)	19 (S)	18 (R)	17 (S)
P.aeruginosa	0 (R)	26 (S)	23 (S)	0 (R)	0 (R)

S: Sensetive R: Resistance
C: Chloramphenicol AK: Amikacine GM: Gintamicin
AUG: Augmenten (Amoxillin / Clavulanic acid) SAM: Ampicillin / Sulbactam

Data in table 2 shows the sensitivity of bacterial isolates to the natural and commercial honey species. Two types of natural honey were used: (citrus flowers honey and eucalyptus honey) as well as two types of commercial honey available in the market (sinbola honey and shafi honey). The results show that natural honey achieved significant superiority on commercial honey in inhibiting bacterial growth for all bacterial isolates, the diameter of inhibition of citrus flower honey and eucalyptus honey of the *E.coli* growth 20, 21 mm respectively and 19 mm, 14 mm respectively for *P. aeruginosa* compared to commercial honey sinbola which gave an inhibition diameter of E. coli 8 mm while not affected in *P. aeruginosa*. These results may be due to the difference in the sources of the bees feeding in being sources abnormal for honey commercial as a sugar and water. This is agreed with the findings of (19) and (20) who found that the natural honey superiority on commercial honey . As for the two types of natural honey, the results show that the citrus flower honey exceeded significantly on eucalyptus honey for *P. aeruginosa* were 19 mm and 14 mm , as for *E. coli* there was no significant difference between the two types of natural honey, these results are in agreement with those obtained by Alqurashi *et al.*(21). When we observed the inhibitory effect of natural honey is closely related to antibiotics (Table, 1), and it gave a positive result when compared with the standard tables of the diameters of inhibition of antibiotics. *P. aeruginosa* was resistant to chloramphenicol , Augmenten and Ampicillin, while sensitive for two types of natural honey as shown in Table (2). The diameter of the inhibition area is 19 mm for citrus flower honey and 14 mm for eucalyptus honey, this result may be due to the type and natural of the composition of the nectar of flowers and also, the weather conditions where the bees were reared (22).

Table 2. Bacterial isolates sensitivity to some antibiotic (inhibition zone by mm)

Bacterial isolates	**Natural honey**		**Commercial honey**	
	Citrus flower honey	Eucalyptus honey	Sinbola honey	Shafi honey
E.coli	20	21	8	0
	a	a	b	c
P.aeruginosa	19	14	0	0
	a	b	c	c

a, b, c Duncans values Similar letters indicate no significant differences, but different letters indicate significant differences.

Data in table (3) shows the sensitivity of *E. coli* to the interaction of honey with antibiotics. The table shows that the natural honey of citrus flower is significantly higher than the other types of it followed by eucalyptus honey, the rate of their impact is 25.2 mm and 24.2 mm respectively. While, there is no significant difference Between the rate effect for sinbola and shafi honey, and the results show that the Amikacine was the most influential on *E. coli* with an influence rate of 25.25 mm followed by both the Gentamicin and Chloramphenicol with an influence rate of 23.5 and 23.25 mm respectively. These result are in agreement with those obtained by Hijwal (23). The effect of interaction between honey and antibiotics show that the treatment citrus flower honey with the amikacine was significantly higher than other treatment with diameter of inhibition 29 mm followed by the interaction citrus flower honey with the gentamicine with diameter of inhibition 28 mm, then interaction between eucalyptus honey with amikacine with diameter of inhibition 28 mm. The lower diameter of inhibition was recorded of the interaction between sinbola honey with the Ampicillin was 17mm.

Table 3. Effect of the interaction between honey and antibiotic on inhibition zone(mm) of E. coli

Honey types	**antibiotic**					**Rate of honey effect**
	C	**AK**	**AK**	**AUG**	**SAM**	
Citrus flower	**27**	**29**	**29**	**19**	**23**	**25.2**
	b	a	a	gh	cd	A
Eucalyptus	**27**	**28**	**28**	**18**	**22**	**24.2**
	b	ab	ab	hi	de	B
Sinbola	**19**	**23**	**23**	**20**	**17**	**19.6**
	gh	d	d	gf	i	C
Shafi	**20**	**21**	**21**	**20**	**18**	**20**
	fg	ef	ef	gf	hi	C
Rate of antibiotic effect	**23.25**	**25.25**	**25.25**	**19.25**	**20**	
	B	A	A	D	C	

A, B, C Duncans values Similar letters indicate no significant differences, but different letters indicate significant differences.

Data in table (4) shows the sensitivity of *P. aeruginosa* isolates to honey interaction with antibiotics. The results shows that the natural citrus flower honey a significant superior on other honey types, followed by eucalyptus honey, with an effect rate of 25.2 and 20.2 mm respectively. The results showed that Amikacine was more inhibited for bacterial growth followed by Gentamicine, with a 30.00 and 26.75 mm inhibitor rate, respectively. The effect of Interaction between citrus flower honey and amikacine was significantly superior on other interaction treatments with inhibition diameter 36 mm, followed by eucalyptus honey, with Amikacine with inhibition diameter 35 mm ,while not giving each of sinbola and shafi honey interaction with Chloramphenicol, Augmenten, and Ampicillin any result, there was no inhibition of bacterial growth.

Table 4. Effect of the interaction between honey and antibiotic on inhibition zone(mm) of *P. aeruginosa*

Honey types	antibiotic					Rate of honey effect
	C	AK	AK	AUG	SAM	
Citrus flower	18 j	36 a	32 c	20 h	20 h	25.3 A
Eucalyptus	0 k	35 b	30 d	20 h	19 i	20.8 B
Sinbola	0 k	23 f	22 g	0 k	0 k	9 D
Shafi	0 k	26 e	23 f	0 k	0 k	9.8 C
Rate of antibiotic effect	4.5 D	30 A	75.26 B	10 C	9.75 C	

A, B, C Duncan values Similar letters indicate no significant differences, but different letters indicate significant differences.

The results of tables (3) and (4) show that honey and antibiotic interaction were increased the efficiency of antibiotic activity by increasing the diameters of bacterial growth inhibition compared with the results in Table (1), As honey possesses inhibitors of bacterial growth, which include hydrogen peroxide and phenolic acids, as well as the osmotic effect of honey caused by sugary component, which causes the breakdown of cellular walls as well as the high honey viscosity, which prevent microbes from penetration and the formation of colonies as well as low acidity of honey ranging from 3.6 to 4.0 (2) and (24) . This study confirmed that the use of honey led to increase the efficiency of antibiotics and reduce their dosage and thus reduce the side effects, as we also find from the results that citrus flower honey had the greatest impact on the bacterial isolates followed by eucalyptus and citrus flower honey, while commercial honey was less impact on bacterial isolates.

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مقارنة تأثير العسل الطبيعي والتجاري على نمو بكتريا *Escherichia coli* و *Pseudomonas aeruginosa* وحساسيتهما للمضادات الحيوية

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الملخص :

أجريت هذه الدراسة لتقييم الفعالية التثبيطية لنوعين من العسل الطبيعي (عسل القداح وعسل اليوكالبتوس) ونوعين من العسل التجاري المتوفر في الأسواق (عسل السنبلة وعسل الشافي) في نمو العزلات البكتيرية *Escherichia coli* , *Pseudomonas aeruginosa* وحساسيتها للمضادات وهي Amikacine (AK) 30µg , Ampicillin/sulbactum(SAM) 20µg , Augmenten (AUG)30µg , Chloramphenicol (C)30µg , Gentamicin(GM)10µg . وأظهرت النتائج تفوق العسل الطبيعي معنويا على العسل التجاري في تثبيطه للنمو البكتيري لجميع العزلات اذ بلغ قطر تثبيط عسل القداح وعسل اليوكالبتوس لنمو *E. coli* 20 و21ملم على التتابع و *P. aeruginosa* 19 و 14ملم على التتابع مقارنة بالعسل التجاري السنبلة اذ أعطى قطر تثبيط لبكتريا *E. coli* 8 ملم بينما لم يؤثر في *P. aeruginosa*, أما بالنسبة لعسل الشافي فلم يظهر أي تأثير على نمو العزلات البكتيرية. كذلك أظهرت النتائج تفوق عسل القداح معنويا على عسل اليوكالبتوس , اذ بلغ قطر تثبيط عسل القداح لبكتريا *P. aeruginosa* 19 ملم مقارنة بعسل اليوكالبتوس والذي بلغ 14 ملم. كما أظهرت النتائج أيضا بأن التأثير التثبيطي للعسل الطبيعي مقارب لتأثير المضادات الحيوية وان العسل الطبيعي أعطى نتيجة ايجابية عند مقارنته مع الجدأول القياسية لأقطار تثبيط المضادات الحيوية ولقد أظهرت بكتريا *P. aeruginosa* مقاومة لكل من المضادات Chloramphenicol و Augmenten و Ampicillin في حين كانت حساسة لنوعي العسل الطبيعي . وكذلك أظهرت النتائج ان تداخل أنواع العسل الطبيعي والتجاري مع المضادات الحيوية قد زاد من كفاءة عمل المضادات من خلال زيادة أقطار التثبيط للنمو البكتيري اذا ما قورنت مع أقطار التثبيط التي اعطتها المضادات الحيوية وقد كان لعسل القداح التأثير التثبيطي الأكبر في العزلات البكتيرية عند تداخله مع المضادات الحيوية يليه عسل اليوكالبتوس بينما كان عسل السنبلة التجاري الأقل تأثيرا .

الكلمات المفتاحية : العسل ، المضادات الحيوية , *Escherichia coli* ، *Pseudomonas aeruginosa*