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Antibacterial Activities of Natural Iraqi and Commercial Honey Against *Klebsiella pneumoniae*

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ABSTRACT

This study was to compare the antibacterial activity of two types of localy natural honey (citrus flowers honey, eucalyptus honey) and two types of the commercial honey (sinbola honey and shafi honey) against *Klebsiella pneumonia* were evaluated, and testing the effect of their in increasing the efficiency of antibiotics Ampicillin / sulbactum (SAM) 20µg, Amikacine (AK) 30µg, Augmenten (AUG) 30µg, Chloramphenicol (C) 30µg ,Gentamicin (GM) 10µg in inhibiting *K. pneumoniae*. The results indicated that the natural honey significantly superiority on commercial honey in inhibiting growth for *K. pneumoniae*, the diameter of inhibition zone of citrus flowers honey and eucalyptus honey was 23 , 19 mm respectively while for sinbola and shafi honey were 8 and 9 mm respectively. Also the results showed that the Citrus Flowers honey exceeded significantly on eucalyptus honey , the inhibition diameters of their were 23 mm and 19 mm respectively. The results indicated 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. The combination of natural and commercial honey with antibiotics increased the efficiency of antimicrobial activity of antibiotics by increasing the diameters of bacterial growth inhibition if compared with the diameters given by antibiotics.

الفعالية المضادة للبكتريا للعسل العراقي الطبيعي والتجاري ضد بكتريا *Klebsiella pneumoniae*

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الخلاصـــة

هدفت هذه الدراسة مقارنة الفعالية المضادة للبكتريا لنوعين من العسل الطبيعي (عسل القداح وعسل اليوكالبتوس) ونوعين من العسل التجاري (عسل السنبلة وعسل الشافي) ضد بكتريا *Klebsiella pneumoniae* واختبار تأثير العسل في زيادة كفاءة المضادات الحياتية وهي Augmenten ، Amikacine (AK) 30µg ، Ampicillin /sulbactum(SAM) 20µg في Gentamicin(GM)10µg ، Chloramphenicol (C)30µg ، (AUG)30µg تثبيط *K. pneumonia*. وبينت النتائج تفوق العسل الطبيعي معنويا على العسل التجاري في تثبيطه لنمو *K. pneumoniae* اذ بلغ قطر تثبيط عسل القداح وعسل اليوكالبتوس 23 ملم ، 19 ملم على التتابع بينما كان 8 و 9 ملم لعسل السنبلة والشافي على التتابع. ويتبين من النتائج ايضا تفوق عسل القداح على عسل اليوكالبتوس، اذ بلغ قطر تثبيط عسل القداح 23 ملم مقارنة بعسل اليوكالبتوس والذي بلغ 19 ملم. وقد اظهرت النتائج بأن التأثير التثبيطي للعسل الطبيعي مقارب لتأثير المضادات الحيوية وقد أعطى نتيجة ايجابية عند مقارنته مع الجداول القياسية لأقطار تثبيط المضادات الحيوية. ان تداخل انواع 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.

الكلمات المفتاحية:
العسل ، المضادات الحيوية ، *Klebsiella pneumoniae*
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Introduction:

The excessive use of drugs led to the emergence of resistant bacterial isolates to antibiotics as well as harmful side effects as 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 about 400 years old 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 fact that honey contains many inhibitors agent that include phenolic acids and hydrogen peroxide, as well as the osmotic effect of honey caused by its sugary components [2], [3] in addition to pH ranging from 3.6 to 4 and the high honey viscosity prevents penetration of bacteria and formation of colonies [4],[5].

Other studies by [6] suggested that it has an antibiotic effect on bacterial species that have the ability to form biofilm especially *P. aeruginosa* and *Staph. aureus*, which cause many diseases such as sinusitis, wound inflammation and burns, and other Gram-negative species as aquired Hospitalized diseases, which began to show high resistance to antibiotics 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.

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 [7]. The other enzyme is glucooxidase, which analyzes glucose to gluconic acid and hydrogen peroxide, this enzyme is not effective in honey but is reactivated when honey is diluted with water[8] 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:

Honey used:

Two types of Iraqi natural honey(Citrus flower honey, eucalyptus honey) and two types of commercial honey (Sinbola and Shafi).

Antibiotics discs:

Ampicillin / sulbactum (SAM) 20µg ,Amikacine (AK) 30 µg , Augmenten(Amoxillin /clavulanic acid)30µg , Gentamicin (GM)10µ, Chloramphenicol (C) 30 µg .

Test of Bacterial isolate:

The isolate was taken from Microbiology Laboratory at the college of Science/ University of Tikrit isolated from urinary tract infections and was confirmed to return to *Klebsiella pneumonia* by following a number of tests involving gram stain, growth on the MacConkey agar , as well as a number of biochemical tests, such as the the methyl red test , indole test and the citrate utilization test [9] and reactivated on Nutrient Agar medium [10].

Sensitivity of antibiotic:

The sensitivity test for a number of antibiotics was conducted using the Kirby-baure method described by [11]. A suspension of bacterial isolate 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 Macfarland standared Solution which is equal 1.5 x 10^8 cell/ml [12]. In the case of unequal tubes turbidity, the normal slain solution add until the turbidity is equal to the McFarland 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

Honey effect:

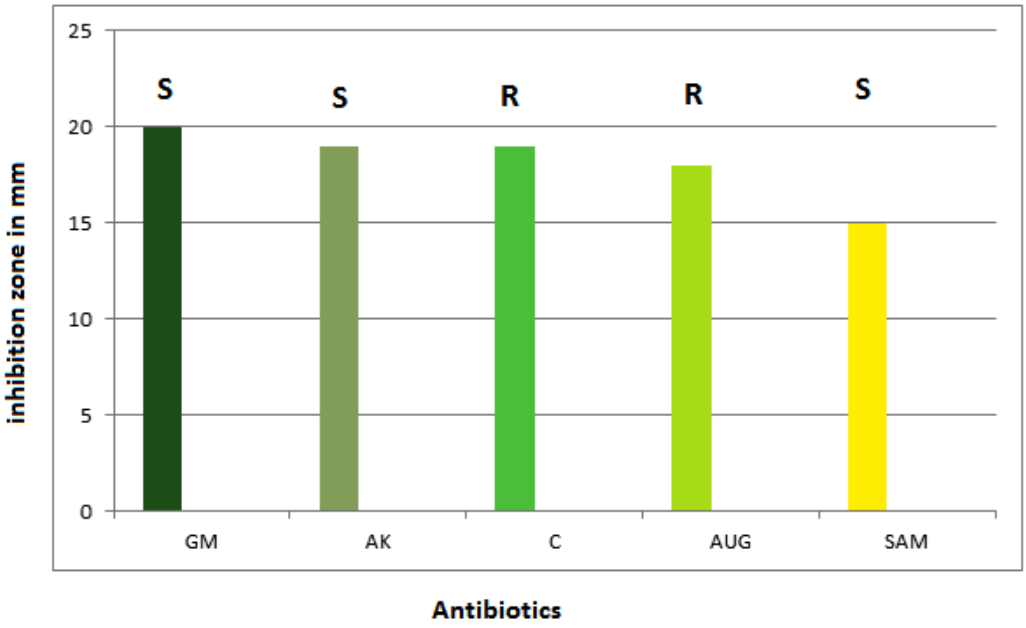
The sensitivity test was conducted by using disc diffusion method according to the method described by [13] prepare the discs of the filter paper saturated with each type of honey under study after confirmation of honey from microbes by filtering in special filters, bacterial inoculums for *K. pneumonia* was transfer by sterile cotton swab to the surface of the Muller Henton Agar after comparing it with the McFarland tube. The honey-saturated disks 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 in millimeters

Interaction of honey and antibiotics:

The bacterial inoculum was transferred from the bacterial isolate studied by sterile cotton swab to the Meuller Hinton surface by using disc diffusion method, these antibiotics 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℃) for the period (18-24) hours after which the regions of the inhibition zones were measured for each disc in millimeters [11],[14

Results and discussion:

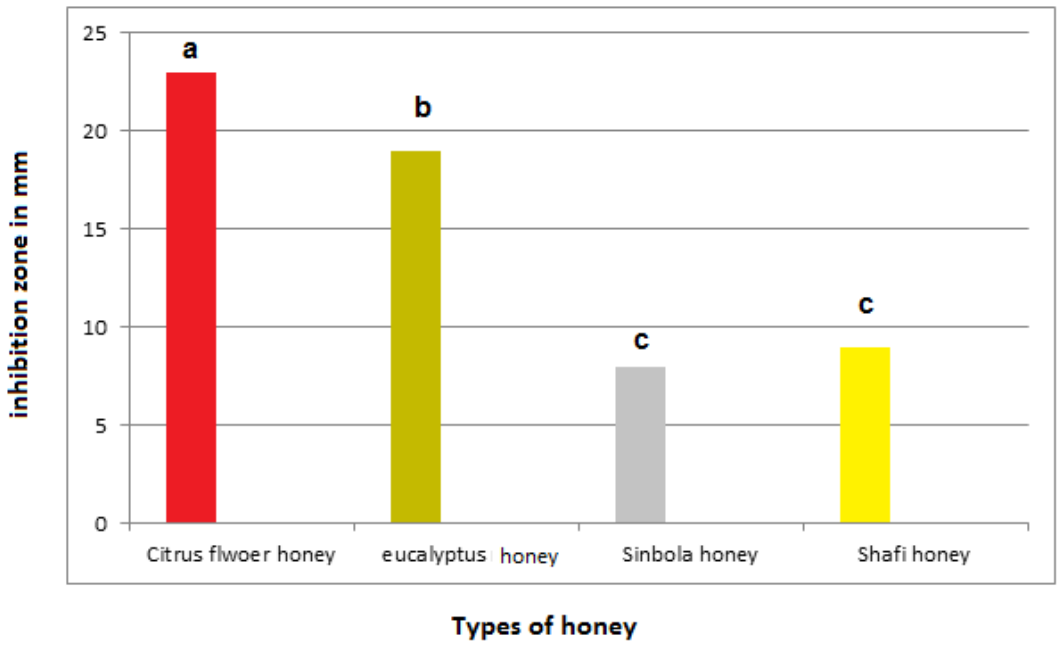
Figure 1 shows the sensitivity of K. pnuemoniae to some antibiotics. The figur shows that bacterial isolate is resistant to Ampicillin and Amoxillin and clavulanic acid (Augmenten), the bacteria resistance to these antibiotics due to its possession β-lactamase enzymes, which altered the structure of the antibiotic by breaking the beta-lactam ring [15]. The results show that bacterial isolate was sensitive to Amikacine and Gentamicin. As for chloramphenicol, the bacterial isolate 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 [16], as well as the possession of bacteria to the active stream mechanism which reduces the antibiotic accumulation within the bacterial cell [17], as well as the causes of bacterial resistance due to mutations such as mutagenesis lead to modulation In the DNA gyrase [18



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

Figure 1: ***k. pneumoniae*** **Sensitivity to certain some antibiotics (inhibition zone in mm)**

Figure 2 shows the sensitivity of K. pnuemoniae to the natural and commercial honey species. Two types of natural honey were used: (citrus flowers honey, 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 , diameter of inhibition of citrus flower honey and eucalyptus honey were 23 mm, 19 mm respectivly compared to commercial honey sinbola which gave an inhibition diameter 8 mm , as for shafi honey it affected with a diameter of 9 mm inhibition, 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] 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 *K.pneumoniae* was 14 mm , 23 mm and 19 mm respectively , 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 [20]. When we observed the inhibitory effect of natural honey is closely related to antibiotics(Figure 1), and it gave a positive result when compared with the standard tables of the diameters of inhibition of antibiotics



a, b, c Duncan's values Similar letters indicate no significant differences, but different letters indicate significant differences

Figure 2 : *K. pneumoniae* sensitivity to some antibiotic(inhibition zone in mm)

Table 1 shows the sensitivity of the isolation of *K. pneumoniae* towards the interaction of honey with antibiotics. The table shows significantly effect of the citruse flower honey which is superior on other honey type followed by eucalyptus honey, with an effect rate 25.4 mm and 22 mm respectivly. The results also show that Chloramphenicol was the most significant effect on K.pneumoniae, followed by Gentamicin and Amikacine, with an effect rate of 20.5 mm and 20.25 mm respectively. The effect of interaction between honey and antibiotics shows that the citrus flower honey is significantly superior with both Chloramphenicol and Amikacine, with 28 mm inhibition diameter followed by interaction citrus flower honey with Gentamicin and Eucalyptus honey with Chloramphenicol with 26 mm inhibition diameter While the lowest diameter inhibition of the result of the interaction of sinbola honey with both Augmenten and Ampicillin with a diameter of inhibition 10 mm for each.

Table 1 : Effect of the interaction between honey and antibiotic on inhibition zone(mm) of K. pneumonia

Honey types	antibiotic					Rate of honey effect
	C	AK	AK	AUG	SAM	
Citrus flower	28 a	28 a	26 b	24 C	21 d	25.4 a
Eucalyptus	26 b	23 c	23 c	18 f	20 ed	22 b
Sinbola	21 d	14 h	15 hg	10 i	10 i	14 d
Shafi	19 ed	16 g	18 f	18 f	16 g	17.4 c
Rate of antibiotic effect	23.5 a	20.25 b	20.5 b	17.5 c	16.75 d	

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

The results of table (1) show that honey and antibiotic interaction increased the efficiency of antibiotic activity by increasing the diameters of bacterial growth inhibition if compared with the results in figure (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 [2],[5],[21] . 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 clover honey while commercial honey was less impact on Bacterial isolates

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