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ANTIMICROBIAL ACTIVITY OF IRAQI NATURAL HONEY AGAINST SOME MICROBIAL PATHOGENS OF HUMAN

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ABSTRACT : The current study aims to evaluate the antimicrobial activity of Iraqi natural honey against a number of pathogens of humans, as follows: *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli* and *Bacillus cereus*, in addition to *Candida albicans* using the well diffusion method. Eight samples of natural honey were collected from several governorates for the period from February 2019 to September 2019. After the results showed that the honey samples were not contaminated with microbes as well as no fermentation and that density, viscosity, crystallization and pH came within the recommended international standard specifications, several concentrations of natural honey samples were performed, represented by concentrations (100%, 75%, 50% and 25%). The results revealed that the Iraqi natural honey possessing an antimicrobial activity under study, this efficacy act varies according to the sample, the microbe and the concentration, where the best was Sidr honey with an average diameter of the inhibition zone 35 (mm) followed by Caleptose honey (34 mm) followed by Sesame honey and Olive while the lowest effect was Pear honey, the concentration was 100%, the most effective, followed by the two concentrations 75% and 50% and the concentration 25%, it had no inhibiting activity towards all types of microbes studied.By comparison between the effectiveness of Iraqi honey and commonly used commercial antibiotics, it was found that the effect of honey exceeded that of commercial antibiotics used in the study.

Key words : Antimicrobial, honey, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli* and *Bacillus cereus.*

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INTRODUCTION

The therapeutic resistance to microbes is one of the major and important problems facing our societies, as the widespread random and irregular use of antibiotics leads to the development and spread of the characteristic of resistance, which is a problem that has existed for a long time, due to the lack of the discovery of new antibiotics over the past few years and the high cost of production and its side effects increased one of the threats to public health is that it limits the effectiveness of treatment, so patients remain infected for a longer period of time, which increases the risk of spreading pathogenic microorganisms that are resistant to antibiotics to other healthy people (Tenover, 2006).

This led to the interest of the researchers in the natural materials that were previously used as a treatment, in addition to the increase in the number of researches proving the effectiveness of these substances and their healing ability to control the resistance of microbes, and to obtain a natural and tonic treatment for the immune system of the body and the integration between traditional medicine such as treatment using herbs can be Bee products, and modern medicine is the best option for patient treatment (World Health Organization, 2016).

Honey and propolis are among the oldest natural materials that humans have used since ancient times as lunch and medicine as the inscriptions on the walls of the Pharaonic temples made clear that they used honey and propolis in mummification to preserve the mummy tissues and in health and beauty and to treat mouth ulcers and as a means to accelerate wound healing (Zainol *et al*, 2013). Treatment with bee products has gained interest as a form of folk preventive medicine for the treatment of many diseases and the promotion of public health. Healing with bees and its products is a new science called Apitherapia

and treatment with its products has become one of the pillars Alternative medicine (Kwakman *et al*, 2011).

Honey is a sweet, dense, liquid with high viscosity, which differs in its natural characteristics (color, smell, flavor, density, viscosity, moisture and its ability to crystallize) and in its chemical composition according to each of the flowers that are derived from the nectar and pollen and the type of worker that collected all of this and the time of collection It is the natural food for honeybees and is nothing but nectar after the female workers of the genus (Apis) prepare it and digest it to turn into mature honey stored in wax disks and the nectar is converted to honey by several enzymes including: an enzyme envertase that converts disaccharides into monosaccharide's and an amylase enzyme that converts substances Starchy matter into simpler materials, while at the same time moisture in honey decreases by evaporating a large percentage of the water in it to prevent fermentation (Codex, 2001).

MATERIALS AND METHODS

Sample collections

Honey samples

Eight samples of Iraqi natural honey were collected from several apiaries in a number of governorates,for the period from February 2019 to September 2019, which include different regions and are classified with the help of beekeepers by traditional methods, according to the type of plants from which the nectar was collected (diversity of vegetation cover) and pastures used for beekeeping, and preserved in glass bottles of capacity for one and tightly closed It was kept at room temperature away from light until used, and all information related to samples, which includes the type of honey, the date of collection and the location of the collection of samples, was collected as shown in Table 1.

Table 1 : Types of honey samples and the location and date of collection.

No	Honey type	The scientific name of the plant	Date of collection sample	Sample collection site
1	Sidr honey	Zizyphus vulgaris	5 /3/ 2019	Najaf governorate
2	Eucalyptus honey	Eucalyptus melifera	5 / 5/2019	Babylon / Jableh region
3	Pear Honey	Pyrus communis	5 / 4 /2019	Diala governorate
4	Clover honey	Medicago sativsa	5 / 6/2019	Baghdad / Al-Latifiya region
5	Sunflower honey	Helianthus annuus	7/7/2019	Babel / Al-Qasim region
6	Cotton honey	Gossypium barbadense	12/9/2019	Al-Qadisiyah governorate
7	Sesame honey	Sesamum indicum	7/9/2019	Babel / Al-Mahawil region
8	Olive honey	Olea europaea	5/4/2019	Karbala governorate

Analysis of honey samples and studying some properties

In order to ensure the quality of honey and not to be subjected to any treatment, whether by heating or adding any other substance to it, the Contamination tests was done to ensure that the honey is sterile and free from microbial contamination, solutions of honey samples were prepared using sterile distilled water by mixing 10 g of honey with 10 ml of water and taken with a quantity of cotton swab and planted in Petri dishes container on the media Nutrient agar, MacConkey's agar and Mannitol salt agar, and incubated at 37°C for 48 hours to ensure that honey is not contaminated with bacteria and Sabouraud Dextrose agar medium at 37°C for three weeks to ensure there are no fungi.

Other test also included like pH test to estimated acidity in honey, the Crystallization test, Viscosity test, color and fermentation tests were made according to (ElSohaimy *et al*, 2015 and Boussaid *et al*, 2013).

Microbial isolates

Microbial isolates were obtained as shown in Table 2.

Table 2 : The type of microbial isolates and where to obtain them.

Isolation	Where to get isolation
Staphylococcus aureus	Biology department, Baghdad University
Escherichia coli	Biology department, Baghdad University
Pseudomonas aeruginosa	Biology department, Baghdad University
***Bacillus cere*s**	Agriculture College, Baghdad University
Candida albicans	Agriculture College, Baghdad University

Microbial identification

The identification of microorganisms depends mostly on the cultural and biochemical characteristics and also microscopic patterns. morphologically typical colonies were verified by Gram staining.

MacConky agar, Mannitol salt agar, Eosin methylin blue (EMB) and Saboroud Dextrose medium Agar were used for cultivation of microorganism.

Formation of germination tube is a test also included carried out by taking a volume of 2 mL of *Candida* fungus and placing it in sterile test tubes. After inoculated and incubated, Then took one drop and placed it on a glass slide and examined under a light microscope to observe the formation of the germination tube.

On the other hands, biochemical tests were carried out according to Maina *et al* (2014). The API 20 system for Enterobacteriaceae bacteria was selected according to the method of Dortet *et al* (2012).

RESULTS AND DISCUSSION

Diagnosis of microbial isolates

The results of the morphological and biochemical tests of microbial isolates showed that the results matched with the characteristics of the microbes tested in this study as well as the results of bacterial diagnosis after matching the data obtained from API 20 test and refer to the sources used to confirm the quality of the studied bacteria and fungi of *C. albicans.*

Microbiological tests and physical properties of honey samples

The results showed that the samples were not contaminated with microbes, as well as non fermentation, as well as that the density, viscosity, crystallization and pH came within the recommended international standard specifications and Table 3 clarifies some properties of the honey samples under study.

Microbial sensitivity to commercial antibiotics

The results showed that *E. coli* and *B.cereus* bacteria were the most sensitive to Chloramphenicol and Gentamicin antibiotics, while *P. aeruginosa* is resistant to this antibiotics followed by*S. aureus*. Gentamicin and Tetracycline were also found to be the most effective on the studied bacteria, followed by the antibiotic Chloramphenicol, while the two antibiotics Vancomycin and Penicillin had a weak effect on the tested bacteria. As for *C. albicans*, it showed resistance to Miconazole (50mg) and the mean diameter of the area differed. Inhibition from one antagonist to another and from one bacterium to another, as shown in Table 4. The study showed that the effect of honey often exceeded the effect of commercial antibiotics.

Evaluating the anti-honey effect against the tested microbes

The results of the study showed that the possession of Iraqi natural honey samples used in this study was anti-growth activity of microbes pathogenic to humans, which was based on the average diameters of the inhibition zone, where this effect differed from a microbicidal and killer in areas near the filled pits, inhibitory and non-lethal effect in relatively faraway areas. That is in the first case, there was no growth,

Table 3 : Physical properties of honey samples under study.

Honey type	pH value	Color (O.D)	Density g/ml	Viscosity g/cm.s	Crystallization	Fermentation	Color appearance	
Sidr honey	4.43	1.45	1.28	18.7	N	-		L
Eucalyptus honey	4.44	1.62	1.26	18.5	N	-	D	
Pear honey	4.32	1.88	1.17	20.5	N	-		L
Clover honey	4.14	1.36	1.55	25.8	C	-		L
Sunflower honey	3.64	1.69	1.35	26.3	N	-	D	
Cotton honey	4.57	1.27	1.46	17.9	N	-		L
Sesame honey	4.9	1.10	1.59	28.8	N	-	D	
Olive honey	4.5	1.32	1.48	18.5	P	-		L

N = Not crystallization, C = Complete crystallization, P = Partial crystallization, L= Light, D = Dark.

Table 4 : The effect of antibiotics on bacterial growth.

Antibiotics	Diameter of Inhibition zone (mm)			
	Escherichia coli	*Pseudomonas aeruginosa*	*Bacillus cereus*	*Staphylococcus aureus*
Vancomycin 5 mg	Ne	Ne	13	10
Amoxicillin 30 mg	Ne	Ne	14	9
Gentamicin 10 mg	15	9	27	21
Penicillin 10 mg	Ne	Ne	9	Ne
Tetracycline 30 mg	18	10	22	24
Chloramphenicol 30 mg	21	Ne	23	12

Ne = no effect

Table 5 : Average diameters of inhibition areas for honey samples under study.

Honey type	Concentration	Inhibition zone (mm)				
		C. albicans	*P. aeruginosa*	*E. coli*	*S. aureus*	*B. cereus*
Sidr honey	25%	0	0	0	0	0
	50%	15	0	14	11	12
	75%	20	13	15	18	16
	100%	30	28	35	32	30
Eucalyptus honey	35%	0	0	0	0	0
	50%	0	0	0	10	0
	75%	6	0	8	13	6
	100%	16	18	26	34	16
Sesame honey	25%	0	0	0	0	0
	50%	0	0	0	0	0
	75%	0	19	18	0	0
	100%	14	20	22	28	22
Olive honey	25%	0	0	0	0	0
	50%	0	0	0	0	0
	75%	10	0	12	0	0
	100%	22	26	20	18	16
Sunflower honey	25%	0	0	0	0	0
	50%	0	0	0	0	0
	75%	0	0	0	0	0
	100%	4	4	6	12	6
Cotton honey	25%	0	0	0	0	0
	50%	0	0	0	0	0
	75%	0	0	0	0	4
	100%	0	4	8	6	8
Clover honey	25%	0	0	0	0	0
	50%	0	0	0	0	0
	75%	0	8	0	8	0
	100%	0	10	0	14	12
Pear honey	25%	0	0	0	0	0
	50%	0	0	0	0	0
	75%	0	0	0	0	0
	100%	0	8	0	10	6

because microbes did not grow even after culturing from the inhibition zone, and this indicates that the effect is lethal, while in the second case, bacterial colonies emerged after a period of incubation, as well as after culturing, because the microbes are not dead and are evidence that it inhibits growth and accordingly the results differed due to the different effect. From a honey sample to another and from a microbe to another, as the concentration factor has an important role in the inhibitory activity, it has shown resistance and dense growth that increases as the concentration of honey decreases. This indicates a clear indication that honey at high concentrations has a high effect in stopping bacterial growth while its ability to inhibit growth bacteria at low concentrations.

The results also showed that Sidr honey is the best, followed by Eucalyptus, Sesame and Olives as shown in Table 5, which shows the average diameters of the

inhibition zone of the Iraqi natural honey varieties.

The results of the arithmetic average for the diameters of the areas of inhibition shown in Table 5 show the superiority of the Sidr honey sample, as it showed the highest inhibition area on *E. coli* bacteria (35 mm) at a concentration of 100 mg / ml without dilution followed by *S. aureus* with a diameter (32 mm). Followed by the fungus Candidiasis *C. albicans*, as the area of inhibition reached (30 mm). As for the bacteria *P. aeruginosa* and *B. cereus*, the area of inhibition was equal for each of them, reaching (28 mm), followed by Eucalyptus honey, then Sesame and Olives honey. The results also showed that Sidr honey unlike other varieties, it had an even effect at concentrations 50% and 75% on all microbes except for *P. aeruginosa*. It did not appear inhibition zone at concentrations of 50% or less, while the concentration of 25% had no effect on all honey varieties under study. Statistically, when determining the lowest significant differences LSD = 0.00, it was found that there are significant differences between the microbes under study and the honey concentrations at a significant level of 0.05 α = where the probability value reached (0.00), which is less than the level of significance specified in the study.

DISCUSSION

The results obtained in this study for microbiological tests of honey samples to detect microbial contamination showed that samples were not contaminated and fermented. This is evidence that the humidity of the honey samples was at the normal and appropriate level, as its percentage ranged between 11.59-17%. It is an important factor in the long shelf life of honey during storage, as increased moisture and water content can lead to the growth of yeasts in honey. Thus, the production of ethyl alcohol and carbon dioxide is consistent with the results (Khalil *et al,* 2014). As for crystallization, it is considered one of the indications of the quality of honey, and the percentage and speed of crystallization varies from one type to another depending on the maturity of the honey and the type of nectar, as well as the ratio of glucose sugar to fructose, colloids, moisture and glucose sugar only crystallizes and this explains the existence of a liquid layer and another crystallized in honey as the glucose sugar. It is in a state of melting at moderate and high temperatures, but once the temperature drops, the glucose is separated from the solution in the form of granular crystals, as the solution becomes above the degree of saturation of glucose (Almeida *et al*, 2013). As for the density of honey on average, it reaches 1.4 g/ml at a temperature of 20°C and it changes with the change of moisture content in it and with regard to the color of honey, it ranges from light yellow to dark brown, as the honey color is affected by its content of pollen, phenolic compounds, minerals and dyes from the derivatives of some substances such as chlorophyll, isnzophyll, and carotene, as well as the air temperature, the lower the color of honey becomes when exposed to a high temperature or when stored for a long time and it is consistent with the results (Moniruzzaman *et al*, 2017).

While, viscosity is different depending on several factors, including: temperature, humidity and chemical composition of honey, such as the ratio of fructose to glucose, the ratio of dextrin, protein and colloids, as it increases as the temperature rises and when the ratio of dextrin, proteins, and colloids increases, the percentage of moisture in it decreases and decreases as its concentration increases and increases fructose-to-glucose ratio. As for the acidity of honey, it came within the normal range of acidity of honey, which ranges from (3.4 - 4.9), which varies according to the plant source of honey and its composition. And acids such as malic acid and succinic acid and cloconic acid is the most important organic acid in honey and it results from the enzymatic activity of the enzyme glucose oxidase on the glucose sugar as it is formed in addition to cloconic acid and oxygenated water and although honey has an acidic effect, but it is considered to have an alkaline taste in principle, as the acidity of the food depends on the type. Predominant from metal materials. The different pH values for all samples are due to the difference in the types of honey and the difference in bee feeding in terms of the prevailing plant type in the region, where the bees graze, as well as the difference in the chemical composition of honey in terms of the ratio of minerals and acids found in honey and the results of this study came identical to what he mentioned (Ahmida *et al*, 2013). The study of the physical and chemical properties of some types of Iraqi honey is also in agreement with study conducted by Khalil *et al* (2011), Brudzynski and Kim (2011).

The current study showed that natural Iraqi honey is characterized by an antimicrobial action and the results shown in Table 5 for the averages of the inhibition areas' diameters for honey varieties at different concentrations confirmed the presence of statistically significant differences between the averages of the areas of inhibition areas for honey species, as the probability value reached (0.00), which is a value Less than the level of significance specified for the study, α = 0.05, which confirms the existence of significant differences and also showed that the best honey is Sidr honey, which had a pesticide effect and lethal all the microbes under test, as the most sensitive to honey was *E. coli*, *S. aureus* and

C. albicans, while *B. cereus* and *P. aeruginosa* were considered moderately sensitive, which is also consistent with what was stated (Kimoto *et al*, 2008). As for the effect of the different concentrations of honey samples, the study showed that the microbial growth in the solid food medium was nonexistent in some species at concentrations of 75% and 100% of honey, while the growth was weak in the concentration of 50%, with the exception of Sidr honey, it showed a superior ability to inhibit as it had an effect. It fought even at this concentration, but at the concentration of 25%, the tested microbes showed resistance and intense growth that increases as the concentration of honey samples decreases and they are consistent with a study conducted by the researchers (Ceyhan and Ugur, 2001).

This indicates that honey at high concentrations has a strong effect in preventing Microbial growth, while its ability to inhibit growth in low concentrations decreases, and this is due to the high concentrations of sugars leading to the occurrence of the binding process and another important reason confirmed by several studies is the presence of anti-bacteria and fungi substances in natural honey that helps inhibit the growth of microbes (Elbanna *et al*, 2014). Also the composition of honey has an important role in reduction microbial growth, because it is mainly a sugar solution and water with other components but in small proportions such as amino acids, organic acids, some fats and minerals (Zamora *et al*, 2013). The reason for microbial growth in low concentrations of honey is due to the degree. The main reason is the loss of honey for high acidity and low concentration of sugars in it, which causes honey to lose its natural components, and this leads to bacterial growth that is almost natural in the low concentrations of honey (Ng *et al*, 2014). The results of the statistical analysis for comparing the average diameters of areas of inhibition of honey varieties at different concentrations and the inhibitory effect of natural honey were also identical with other results conducted in this regard (Bogdanov, 2014). The ability of honey to inhibit microbes is due to the high acidity that is produced by the enzymes secreted by bee workers on the nectar when it is converted into mature honey such as Glucosoxidase in addition to the action of other enzymes during the ripening process and as a result of activating glucose oxidase, which leads to the production of oxygenated water (Brudzynski, 2006).

Also, the vegetable source of honey and its containment of pollen from different plants give it a high potential for inhibition, as well as the presence of some anti-bacterial and antiseptic agents such as hydrogen peroxide, antioxidants, lysozyme, polyphenols, phenolic acids, flavonoids, methylglyoxal and bee peptide and the presence of oxidation and reduction enzymes in honey such as the enzyme peroxidase, catalase and phosphatase and their killer effect of bacteria in addition to the lipolytic enzyme and these factors all have a role in this inhibition (Allen *et al*, 2015).

As for the resistance of microbes to commercial antibiotics, the results showed that the bacterial of *E. coli* and *B. cereus* were the most sensitive to the antibiotics Chloramphenicol and Gentamicin, while *P. aeruginosa*, *S. aureus* and *C. albicans* showed resistance to the antibiotics used and this is consistent with the findings of Nishio *et al* (2016). The reason for this resistance may be due to the frequent and irregular use of antibiotics, as the sources indicated that the use of doses under treatment leads to the emergence of spontaneous mutations (Poole, 2014). Also, the widespread use and repeated use of the same antibiotic for a long period of time to treat some diseases may lead to the spread of the phenomenon of resistance to these antibiotics by microorganisms and the emergence of strains with high resistance to it (WHO, 2016).

CONCLUSION

We conclude through this study that Iraqi natural honey contains inhibitory and lethal substances for human pathogenic microbes and that the concentration of 100% for all types of Iraqi honey was the most effective on all types of microbes studied. The study also proved that Sidr honey was the most inhibitor of microbes under investigation, especially 100% concentration.

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