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Microbiological-Antibacterial Activities and Antioxidant Potential of Some Honey Libyan Types

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Abstract: In this study the concentration of antioxidant were studied in two types of Libyan honey samples which collected from the green mountain region during spring 2012. The honey samples including; *Ziziphus lotus* and *Arbutus pavarii*. Antioxidant activity of different type were screened using the ferric reducing power and 1, 1-diphenyl-2-picrylhydrazyl (DPPH•) radical scavenging. Result showed that the high value in sample *Ziziphus lotus* at 10 mg/m l(97.3mg/ml), While in *Arbutus pavarii* (93.0mg/ml). The high Phenolic compound content were recorded in *Ziziphus lotus* sample (2.879µg/ml) at concentration (500µg/ml). The antibacterial activity of the two type of honye was evaluated against five bacterial strains. The results showed that two type of *honye* at concentration of 90%, 80%, 75%, 50% and 25% were effective against both Gram-positive and Gram-negative bacteria. We suggest the two type of honey were rich in phenolic constituents.

Key words: Honey · 1, 1-diphenyl-2-picrylhdrazyl (DPPH) · Antioxidant and anti-bacterial

INTRODUCTION

Honey contains several compounds which function as antioxidants compounds that may help delay the oxidative damage to cells or tissues in our bodies. Known antioxidant compounds in honey are chrysin, pinobanksin, vitamin C, catalase and pinocembrin [1]. Honey contains a variety of phytochemicals (as well as other substances such as organic acids, vitamins and enzymes) that may serve as sources of dietary antioxidants. The amount and type of these antioxidant compounds depends largely upon the floral source variety of the honey. In general, darker honeys have been shown to be higher in antioxidant content than lighter honeys [2]. Researchers at the University of Illinois Champaign Urbana examined the antioxidant content (using an assessment technique known as Oxygen Radical Absorbance Capacity or ORAC) of 14 unifloral honeys compared to a sugar analogue. ORAC values for the honeys ranged from 3.0 µ mol TE/g for acacia honey to 17.0 µ mol TE/g for Illinois buckwheat honey. The sugar analogue displayed no antioxidant activity. Honey volatiles are the substances responsible for the honey aroma. Research on honey volatiles started in the early 1960s. Recently, by studying volatiles isolated from the blossom and from the respective unifloral honey, it was found that most volatile compounds originate probably from the plant, but some of them are added by bees. Until the present time about 600 compounds have been characterized in different honeys, many of them being unifloral. As unifloral honeys differ in respect of their sensory properties, it is probable that analysis of volatile compounds will allow classification of unifloral honeys. Indeed, typical volatile substances have been found in many unifloral honey and analysis of volatiles substances can be used for the authentification of the botanical origin of honey [3-6]. Phenolic acids and polyphenols are plant-derived secondary metabolites. These compounds have been used as chemotaxonomic markers in plant systematics. They have been suggested as possible markers for the determination of botanical origin of honey. Considerable differences in composition and content of phenolic compounds between different unifloral honeys were found. Dark coloured honeys are reported to contain more phenolic acid derivatives but less flavonoids than light coloured ones. It was shown

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that most of the studied 9 European unifloral honeys can be distinguished by their typical flavonoid profile. Honey samples contain also variable amounts of propolis-derived phenolic compounds that were not helpful for the determination of botanical origin. On the whole, the determination of the flavonoid patterns is useful for the classification of some but not all unifloral honeys. For a more in depth analysis of the flavonoid spectra of unifloral honeys [7, 8]. The purpose of this study is to evaluate the antioxidant properties of the two types of Libyan honey (*Ziziphus lotus* honey and *Arbutus pavarii* honey).

MATERIALS AND METHODS

Material

Honey Material: Three types of Libyan honey (*Ziziphus lotus* honey, *Arbutus pavarii* and *ceratania siliqua* honey) were collected from the green mountain (during spring season, 2012).

Bacteria Used: Bacteria were taken from the laboratory of microbiology in Banghazi medical center, which know as multi drag resistant bacteria. The bacteria used were *Escherichia coli* (MDR)ATCC, *Staphylococcus aureus* (MDR) ATCC, *Pseudomonas aeruginosa* (MDR) ATCC, *Klebsiella pneumonia* (MDR) and *Acinetobacter* sp (MDR). Other bacteria were not multi drag resistant such as *Escherichia coli*, *Staphylococcus aureus* and *Pseudomonas aeruginosa*. The organisms were isolated and identified by standard methods and identification confirmed by using phonex. The organisms were then subcultured and maintained on nutrient agar slants.

Chemicals: 1, 1-Diphenylpicrylhydrazyl (DPPH•) and Ethanol alcohol were supplied from Sigma and Merck company. Ascorbic acid, Folin-Ciocalteu reagent, ferric chloride, potassium ferricyanide, monobasic dihydrogen phosphate, dibasic monohydrogen phosphate, trichloro acetic acid, sodium carbonate, petroleum ether, anhydrous sodium sulfate and pyrogallol were obtained from the biochemistry laboratory of chemistry department-Benghazi University.

Methods:

Antioxidant Activities Assays and Quantitative Analysis: In this study diluted all types of honey were used. The diluted all types of honey were prepared by using distal water to obtain 100 µg/ml, 200 µg/ml, 300 µg/ml, 400 µg/ml and 500 µg/ml concentrations [9].

Total Phenolic Content (TPC): Phenolic compound concentration in the two types of Libyan honey were estimated using the colorimetric method based on Folin-Ciocalteu reagent [10]. Quantification was done with respect to stander calibration curve of Pyrogallol the results were expressed as pyrogallol "µg/ml". Estimation of the phenolic compounds was carried out in triplicate.

Reducing Power Assay (RPA): The reducing power was determined according to the [11]. Quantification was done with respect to stander calibration curve of ascorbic acid the results were expressed as ascorbic acid "µg/ml".

DPPH free Radical Scavenging Activity (RSA): The antioxidant activity of the honey varieties were measured in terms of hydrogen donating or radical-scavenging ability using the stable DPPH method as modified by [12]. Radical scavenging activity was expressed as percent of inhibition and was calculated using the following formula:

%DPPH "RSA"= [Abs. of Control – Abs. of Sample / Abs of Control]x 100

Antibacterial Activities of Some Honey Libyan Types: In this study diluted all types of honey were used. The diluted all types of honey were prepared by using Dimethyl Sulfoxide (DMSO) to obtain 25%(v/v), 50%(v/v), 75%(v/v), 80%(v/v) and 90%(v/v) concentrations. DMSO was used as negative control. A screening assay using well diffusion [13]. Muller Hinton agar plates were inoculated by rubbing sterile cotton swabs after immerse 100µ l bacterial suspensions on plates (overnight cultures grown at 37°C on nutrient agar and adjusted to 0.5 McFarland in sterile saline) over the entire surface of the plate. After inoculation 9 mm diameter wells were cut into the surface of the agar using a sterile cork borer. Different concentrations (25, 50, 75, 80 and 90%) were added to the wells. Plates were incubated at 37°C for 24 h. Control wells contained solvent DMSO. Zones of inhibition were measured by using ruler. The diameter of zones was recorded. Each assay was carried out in triplicate. The antibacterial assay plates were incubated at 37°C for 24hr. The effect of fixed and volatile oils on the tested bacteria was compared with the sensitivity of the same bacteria to five antibiotics Colisti sulphate, Amicacin, Amoxycillin, gentamicin and sulphamethoxazole trimethoprim (60µg/ml) [14, 15].

RESULTS AND DISCUSSION

Antioxidant Activities of Honey Varieties: The antioxidant capacity of all samples were determined using Total phenolic content (Table 1), Reducing power (Table 2) and DPPH (Table 3). The reducing power activity of bioactive compounds is associated with antioxidant activity. Thus, it is necessary to determine the reducing power of phenolic constituents to elucidate the relationship between their antioxidant effects and there reducing power [16]. The DPPHÿ test is the oldest indirect method for determining the antioxidant activity, which is based on the ability of the stable free radical 2, 2-diphenyl-1- picrylhydrazyl to react with hydrogen donors including phenols. Radical scavengers may directly react and quench with peroxide radicals to terminate the peroxidation chain reaction and improve the quality and stability of food product. The stable DPPH• radical has

Table 1: Total phenolic content of water extract of Honey varieties and Total phenolic content of pyrogallol (standard)

			Mean ± Standard Deviation	
Concentration of Pyrogallol "µg/ml"	Mean ± Standard Deviation	Concentration of Honey varieties "µg/ml"	Ziziphus lotus	Arbutus pavarii
100	0.481 ± 0.0036	100	0.585 ± 0.03	0.522± 0.001
200	0.718 ± 0.0085	200	0.775 ± 0.001	0.725 ± 0.007
300	0.977 ± 0.011	300	0.937 ± 0.004	0.842 ± 0.001
400	1.283 ± 0.0194	400	1.882 ± 0.006	1.655 ± 0.006
500	1.462 ± 0.0693	500	2.879 ± 0.001	2.421 ± 0.004

Table 2: Reducing power assay of water extract of honey varieties and Vitamin C (standard).

			Mean ± Standard Deviation	
Concentration of Vitamin C "µg/ml"	Mean ± Standard Deviation	Concentration of Honey varieties "µg/ml"	Ziziphus lotus	Arbutus pavarii
100	0.466 ± 0.0217	100	0.666 ± 0.006	0.654± 0.018
200	0.884 ± 0.0173	200	0.923 ± 0.002	0.943 ± 0.002
300	1.315 ± 0.0045	300	1.899 ± 0.005	1.766 ± 0.003
400	1.738 ± 0.0162	400	2.265 ± 0.016	1.999± 0.015
500	2.194 ± 0.0198	500	0.052 ± 3.103	.987±0.0012

Table 3: DPPH- radical scavenging activity of water extract from (honey varieties) and quercetin (as a reference free radical scavenger) according to% of inhibition

		% DPPH de coloration mg\ml		
Honey varieties and quercetin		1mg\ml	5mg\ml	10mg\ml
Honey varieties	*Arbutus pavarii*	81.5	87.3	93.8
Ziziphus lotus	90.9	94.5	97.3	
Quercetin	91.1	91.3	92.3	

Table 4: Screening of Antibacterial activity of honey varieties from Libyan

			Zone of Inhibition (mm)± Standard deviation				
S/No.	Treatment	Concentration (%)	EC	PA	SA	KP	AS
1.	*C*	-	-	-	-	-	-
2.	*Ziziphus lotus*	25	11±0.208	2±0.03	16±0.03	12±0.07	18±0.11
		50	17±0.041	9±0.09	20±0.01	17±0.21	20±0.34
		75	±0.1320	14±0.23	34±0.25	22±0.34	25±0.09
		80	23±0.23	17±0.11	28±0.17	28±0.29	28±0.15
		90	25±0.61	19±0.06	32±0.33	31±0.11	30±0.02
3.	*Arbutus pavarii*	25	10±0.3 2	1 ±0.02	14±0.09	10 ±0.12	18±0.11
		50	16 ±0.17	6 ±0.14	21±0.41	18 ±0.09	20±0.23
		75	20±0.04	11 ±0.21	32±0.11	22 ±0.15	25±0.10
		80	24±0.23	17 ±0.20	28±0.23	27 ±0.22	27±0.23
		90	25±0.11	18 ±0.11	31±0.37	29 ±0.16	30±0.41

Values are expressed as Mean (X)+ SD, n=3 Abbr. EC= *Escherichia coli,* PA = *Pseudomonas aeruginosa,* KP = *Klebsiella pneumonia,* SA= *Staphylococcus aureus,* AS= A*cinetobacter sp*., C: Control (DMSO).

Table 5: Antibiotic activity of different type of bacteria

	Zone of Inhibition (mm) ± Standard deviation				
Antibiotic	EC	SA	PA	AS	KP
Colisti sulphate	-	2±0.01	3±0.01	±0.036	4±0.01
Amicacin	15±0.02	0.02±13	9±0.01	-	12±0.04
Amoxycillin	-	3±0.01	-	-	2±0.01
Gentamycin	10±0.03	6±0.01	5±0.02	3±0.03	1±0.01
Sulphmethoxazole	3±0.12	19±0.03	-	4±0.08	-

Values are expressed as Mean (X)+SD, n=3. EC= *Escherichia coli,* PA = *Pseudomonas aeruginosa,* KP = Klebsiella pneumonia, SA= *Staphylococcus aureus,* AS= A*cinetobacter sp*

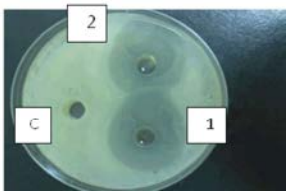

Fig. 1: Antibacterial activity of honey varieties from Libyan against *E.coli* bacteria at 90% concentration. 1= *Ziziphus lotus,* 2= *Arbutus pavarii,* C= DMSO

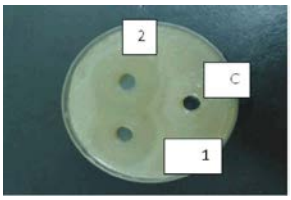

Fig. 2: Antibacterial activity of honey varieties from Libyan against *E.coli* bacteria at 80% concentration. 1= *Ziziphus lotus,* 2= *Arbutus pavarii,* C=DMSO

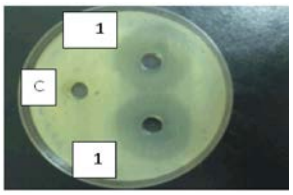

Fig. 3: Antibacterial activity of honey varieties from Libyan against *E.coli* bacteria at 75% concentration. 1= *Ziziphus lotus,* 2= *Arbutus pavarii,* C=DMSO

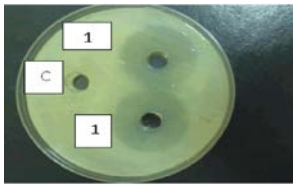

Fig. 4: Antibacterial activity of honey varieties from Libyan against *E.coli* bacteria at 50% concentration. 1= *Ziziphus lotus,* 2= *Arbutus pavarii,* C=DMSO

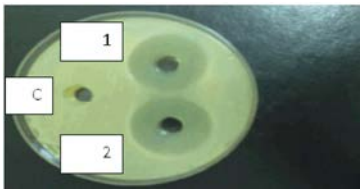

Fig. 5: Antibacterial activity of honey varieties from Libyan against *E.coli* bacteria at 25% concentration. 1= *Ziziphus lotus,* 2= *Arbutus pavarii,* C=DMSO

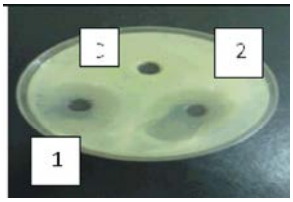

Fig. 6: Antibacterial activity of honey varieties from Libyan against *Klebsiella pneumonia* bacteria at 90% concentration. 1= *Ziziphus lotus,* 2= *Arbutus pavarii,* C= DMSO

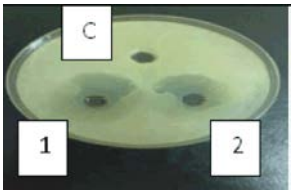

Fig. 7: Antibacterial activity of honey varieties from Libyan against *Klebsiella pneumonia* bacteria at 80% concentration. 1= *Ziziphus lotus,* 2= *Arbutus pavarii,* C=DMSO

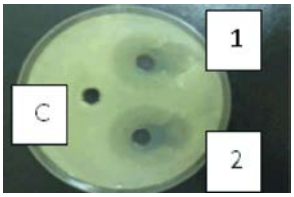

Fig. 8: Antibacterial activity of honey varieties from Libyan against *Klebsiella pneumonia* bacteria at 75% concentration. 1= *Ziziphus lotus,* 2= *Arbutus pavarii,* C=DMSO

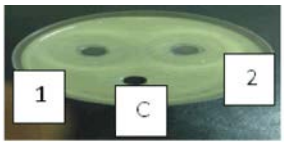

Fig. 9: Antibacterial activity of honey varieties from Libyan against *Klebsiella pneumonia* bacteria at 50% concentration 1= *Ziziphus lotus,* 2= *Arbutus pavarii,* C=DMSO

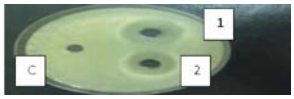

Fig. 10: Antibacterial activity of honey varieties from Libyan against *Klebsiella pneumonia* bacteria at 25% concentration 1= *Ziziphus lotus,* 2= *Arbutus pavarii,* C=DMSO

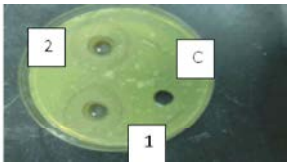

Fig. 11: Antibacterial activity of honey varieties from Libyan against *Pseudomonas aeruginosa* bacteria at 90% concentration. 1= *Ziziphus lotus,* 2= *Arbutus pavarii,* C= DMSO

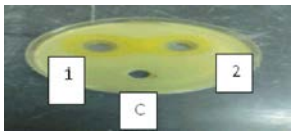

Fig. 12: Antibacterial activity of honey varieties from Libyan against *Pseudomonas aeruginosa* bacteria at 80% concentration 1= *Ziziphus lotus,* 2= *Arbutus pavarii,* C=DMSO

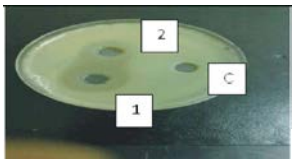

Fig. 13: Antibacterial activity of honey varieties from Libyan against *Pseudomonas aeruginosa* bacteria at 75% concentration 1= *Ziziphus lotus,* 2= *Arbutus pavarii,* C=DMSO

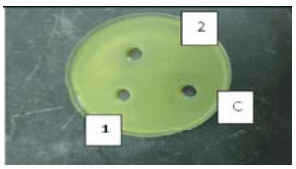

Fig. 14: Antibacterial activity of honey varieties from Libyan against *Pseudomonas aeruginosa* bacteria at 50% concentration 1= *Ziziphus lotus,* 2= *Arbutus pavarii,* C=DMSO

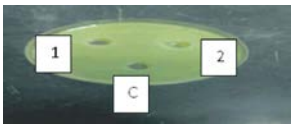

Fig. 15: Antibacterial activity of honey varieties from Libyan against *Pseudomonas aeruginosa* bacteria at 25% concentration 1= *Ziziphus lotus,* 2= *Arbutus pavarii,* C=DMSO

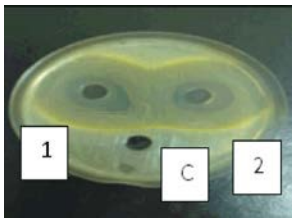

Fig. 16: Antibacterial activity of honey varieties from Libyan against *Staphylococcus aureus* bacteria at 90% concentration. 1= *Ziziphus lotus,* 2= *Arbutus pavarii,* C= DMSO

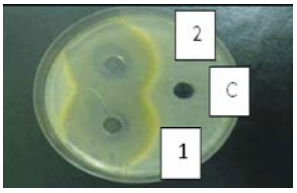

Fig. 17: Antibacterial activity of honey varieties from Libyan against *Staphylococcus aureus* bacteria at 80% concentration. 1= *Ziziphus lotus,* 2= *Arbutus pavarii,* C= DMSO

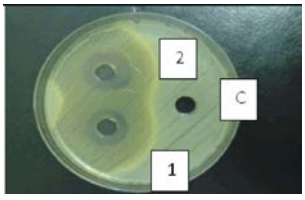

Fig. 18: Antibacterial activity of honey varieties from Libyan against *Staphylococcus aureus* bacteria at 75% concentration. 1= *Ziziphus lotus,* 2= *Arbutus pavarii,* C= DMSO

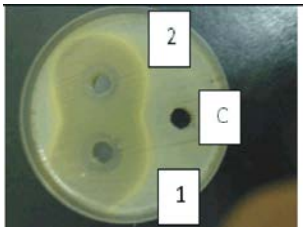

Fig. 19: Antibacterial activity of honey varieties from Libyan against *Staphylococcus aureus* bacteria at 50% concentration. 1= *Ziziphus lotus,* 2= *Arbutus pavarii,* C= DMSO

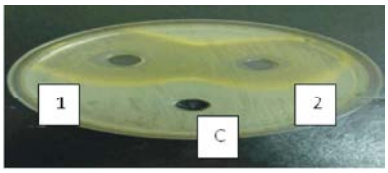

Fig. 20: Antibacterial activity of honey varieties from Libyan against *Staphylococcus aureus* bacteria at 25% concentration. 1= *Ziziphus lotus,* 2= *Arbutus pavarii,* C= DMSO

been used to evaluate antioxidants for their radical quenching capacity and to better understand their antioxidant mechanism of water extract of honey varieties was evaluated for radical scavenging activity against DPPH•. The decrease in absorbance of DPPH• radical is caused by antioxidant through the reaction between antioxidant molecule and radical results in the scavenging of the radical by hydrogen donation. The high amount of the phenolic compounds and reducing power having the highest percent DPPH• scavenging activity was shown by water extract of honey varieties [17]. Total phenolic content was high of *Ziziphus lotus* honey(0.585 - 2.879 µg/ml) Followed by *Arbutus paravii* honey (0.522-2.421 µg/ml and values of Pyrogallol content are(0.481 – 1.462 µg/ml) (Table1). In this study the high levels of Reducing power assay content was recorded in *Ziziphus lotus* sample (3.103µg/ml) followed by the *Arbutus paravii* (2.987 µg/ml) respectively, (Table 2). Results obtained on (DPPH) were higher values (90.9-97.3mg/ml) were recorded in *Ziziphus lotus* and lower values were recorded in *Arbutus paravii* (81.5-93.8mg/ml) While quercetin values (91.1-92.3 mg/ml), Table (3).

Antibacterial Activity Assay: The results of the well diffusion test revealed that the all type of honye shows a significant activity on bacteria tested with varying degrees of inhibition of growth, depending on the bacterial strains (Table4), antibiotic activity of different type of bacteria as compare to the standard (Table 5). These results agree with that obtained by [18, 19].

In the present study, the results for the antibacterial screening have shown that the *Ziziphus lotus* at all concentration from "25 to 90%" has higher activities than the *Arbutus pavarii* (Table 4) (Figures 1-25). This result agrees with that obtained by [20], who reported that honey also contains several compounds which function as antioxidants compounds that may help delay the oxidative damage to cells or tissues in our bodies. Known antioxidant compounds in honey are chrysin, pinobanksin, vitamin C, catalase and pinocembrin.

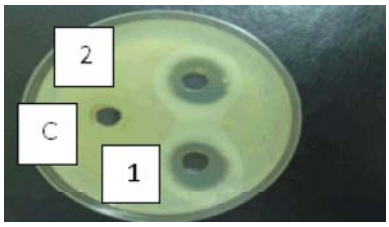

Fig. 21: Antibacterial activity of honey varieties from Libyan against A*cinetobacter sp*.bacteria at 90% concentration. 1= *Ziziphus lotus,* 2= *Arbutus pavarii,* C= DMSO

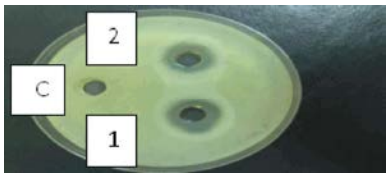

Fig. 22: Antibacterial activity of honey varieties from Libyan against A*cinetobacter sp*.bacteria at 80% concentration. 1= *Ziziphus lotus,* 2= *Arbutus pavarii,* C= DMSO

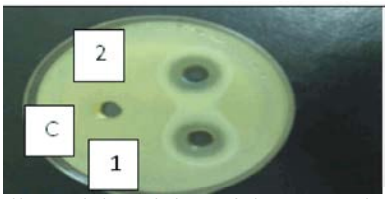

Fig. 23: Antibacterial activity of honey varieties from Libyan against A*cinetobacter sp*.bacteria at 75% concentration. 1= *Ziziphus lotus,* 2= *Arbutus pavarii,* C= DMSO

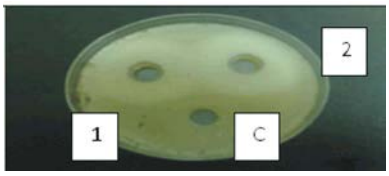

Fig. 24: Antibacterial activity of honey varieties from Libyan against A*cinetobacter sp*.bacteria at 50% concentration. 1= *Ziziphus lotus,* 2= *Arbutus pavarii,* C= DMSO

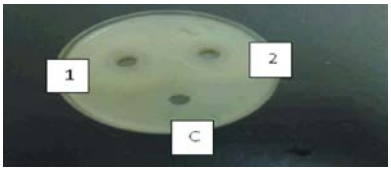

Fig. 25: Antibacterial activity of honey varieties from Libyan against A*cinetobacter sp*.bacteria at 25% concentration. 1= *Ziziphus lotus,* 2= *Arbutus pavarii,* C= DMSO

Also, [20] reported that honye extracts possesses antibacterial properties and could be used for the treatment of bacterial infections.

This study showed that *Ziziphus lotus* honey was more effective against *staphylococcus aurius* bacteria than *Ps. aeruginosa* bacteria in vitro with zones of inhibition ranging from (16 mm to 32 mm)and (2 mm to17 mm) respectively (Table 4) (Figures16 and 20) and (11-15) respectively. In contrast several studies that *Ps. aeruginosa* more resistant against (essential oils) because of the cell wall structure. Gram-negative bacteria have an outer lipopolysaccharide wall that can work as a barrier against toxic agents [21], which is similar to other reports describing the use of essential oils components [22].

Natural plant antioxidants include phenolic compounds may produce beneficial effects by scavenging free radicals [23]. Thus, phenolic compounds may help protect cells against the oxidative damage caused by free radicals, also reported that the extract plant rich in phenolic compounds leads to antibacterial activity.

CONCLUSION

It is observed that the *Ziziphus lotus* at all concentration from "100 to 500 µg/ml" has higher activities than the *Arbutus pavarii*. In general, it is found that the all Honey varieties contain phenolic compound which is responsible for the antioxidant properties. And also they give the higher reductive potential due to reducing capacity and DPPH free radical scavenging activity which serves as strong indicator of antioxidant activities.

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