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In vitro Antibacterial Activity of Honey against some pathogenic bacterial isolates from Sulaimani Teaching Hospital

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Abstract

Four different concentrations of original –natural- honey [0.5 v/v, 0.0.25 v/v, 0.125 v/v, and 0.0625 v/v] were tested for their inhibitory effects against [63] bacterial isolates including both Gram positive and Gram negative bacteria which were obtained from Sulaimani Teaching Hospital and re-identified in our laboratories. **Turbidometrical** method was depended for measuring the bacterial growth for studying the effects, through comparing the absorbency obtained from tested and control isolates. All concentrations showed their effects on the bacterial growth but in different degrees.

Depending on Duncan test, significant differences were observed between the growth rates of tested Gram positive bacteria and control isolates in regard to concentrations and bacterial isolate types [p= 0.000]. The effects of all four honey concentrations against Gram negative bacterial isolates were similar to that of Gram positive isolates, the growth rates were significantly differ regarding concentrations and bacterial isolate types[p= 0.000]. The obtained results from this study revealed that the honey have a great effects on the growth of both gram positive and gram negative bacteria of hospital origins and can be used in treating or preventing bacterial growth or as an anti-inflammatory agent.

Key Words: Antibacterial activity; Natural product, Honey, Gram positive and gram negative bacteria, Turbidometry

Introduction

Honey is among the most important agents which were used as a natural medical treatment for different types of diseases. It was used to treat infected wounds as long as 2000 years before bacteria were discovered to be the cause of infections [1].

The antibacterial property of honey has often concluded to be due to the osmotic effect of its high sugar content, as it has an osmolarity sufficient to inhibit microbial growth [2, 3, 4, 5], also it has been concluded that honey contains lysozyme, which is a well-known antibacterial agent [6]. The scientific analysis showed that honey contains seven tetracycline derivatives, fatty acids, lipids, amylases and ascorbic acid [7, 8, 9], while some believed that hydrogen peroxide is the main antibacterial agent present in honey [10, 11, 12]. Moreover other studies have shown that the non-peroxide activity is more important [13, 14], while other investigators showed that the low honey pH was besides the high osmolarity are responsible for the antibacterial activity [15]. Effects of honey against some fungi and bacteria have also been concluded when honey worked as an inhibitor against the growth of *Salmonella, E.coli, Aspergillus niger* and *Penicillium chrysogenium* [16]. More recently inhibitory effect of honey has been reported against a wide range of bacteria including aerobes and anaerobes, Gram-positives and Gram-negative ones [17].

Other investigators reported that honey inhibits the growth of microorganisms of the burns [18, 19], while some bacterial isolates including ***Pseudomonas aerugenosa, Clostridium***

oedematiens, and ***Streptococcus pyogenes*** showed to be resistant for honey when tested by other researchers [20].

In a comparative study on burn, it was noted that honey causes decline in the numbers of bacterial colonies [21], as well as the effects of honey were seen when diluted, especially against ***Staphylococcus aureus*** [22].

The aim of this study is to investigate the inhibitory effects of two different concentrations of honey on the growth rates of some clinical bacterial isolates including both Gram- positive and Gram- negative bacteria depending on the turbidometrical assay for bacterial growth, as well as if the honey can be used as an anti-inflammatory agent.

Materials and Methods

This work was achieved between Jan. 10 and Apr. 1 2004. Sixty three bacterial isolates were obtained from Sulaimani Teaching Hospital (tables 1&2) and re-identified in our laboratories biochemically. Gram positive isolates includes, 15 isolates of ***Staphylococcus aureus*** from burns; 7 ***S. epidermidis*** from wound , 4 from burn; and 3 isolates of ***α***-hemolytic ***Streptococci*** from burn also.

The Gram negative isolates include 5 ***Pseudomonas aeruginosa*** from burns and 12 from urine; 9 ***E.coli*** from urine and 4 from wounds as well as 4 ***Proteus mirabilis*** isolated from urine].

All isolates were tested for their susceptibility for four different concentrations of honey which were [0.5 v/v, 0.25 v/v, 0.125 v/v, and 0.0625 v/v].

Two fold dilution techniques was followed for preparing the first concentration of honey 0.5 v/v [1:1], so from the first concentration the second 0.25 v/v was prepared [also 1 ml from the first concentration to 1 ml sterile distilled water and so for the other two honey concentrations.

Each isolate was inoculated into five test tubes contained 2 ml of nutrient broth as basic components. The first tube was used as control [without adding honey], and for the second, third, fourth, and fifth tubes 0.5 ml of [0.5 v/v, 0.25 v/v, 0.125 v/v and 0.0625 v/v] of honey concentrations was added respectively. After overnight incubation at 37^0C the absorbency of all tubes were recorded at [540 nm], the blank was non inoculated nutrient broth.

For analyzing the results statistically, Duncan test was depended to decide if there were significant differences between the effects of concentrations and bacterial isolates from different sources.

Results

The Bacterial isolates and sources of isolations are illustrated in (table, 1), as well as (table, 2) clarify biochemical re-identification of all bacteria obtained from Sulaimani Teaching Hospital.

The turbidometrical method was followed to compare the growth rates of bacterial isolates expressed as absorbency at [540 nm], when four different concentrations of honey were added to the inoculated growth media.

The first concentration of honey (0.5 v/v) showed high antibacterial activity in compare to control isolates (Tables, 2, 3 and Fig. 3), the lowest growth rate [the lowest absorbency was 0.020] which was recorded with ***Proteus mirabilis*** from urine, while the highest growth rate [the highest absorbency was 0.218] which was for ***Pseudomonas aeruginosa*** from urine among the Gram negative isolates, (table, 4).

The effect of honey was different between the same bacterial isolates which were obtained from different clinical sources, (Fig.1, 2), while the effects declined when the honey diluted 16 folds, and some times undetectable or limited (Table, 3, 4 and Fig. 3).

Also the first concentration (0.5 v/v) showed higher antibacterial activity when tested against Gram-positive bacteria (Table, 3), especially against ***S. epidermidis*** from burn [the absorbency was 0.110], and the lowest activity [the highest absorbency was 0.231] was against ***S. aureus*** from burn.

Statistical analysis showed significant differences between concentrations and different bacterial isolates from various sources in regard to both Gram positive and Gram negative bacteria.

Table-1- Gram positive and Gram negative bacterial isolates from different sources

Bacterial isolates	Source of isolation	Number of isolates	Abbreviation
Staphylococcus aureus	Burn	15	SAB
Staphylococcus epidermidis	Burn	4	SEB
Staphylococcus epidermidis	Wound	7	SEW
α-hemolytic ***Streptococci***	Burn	3	α- SB
Pseudomonas aeruginosa	Burn	5	PAB
Pseudomonas aeruginosa	Urine	12	PAU
Proteus mirabilis	Urine	4	PMU
Escherichia coli	Urine	9	ECU
Escherichia coli	Wound	4	ECW
Total isolate numbers		**63**	

Table-2- Biochemical re-identification of bacterial isolates obtained from different sources in Sulaimani Teaching Hospital

Bacterial Isolates	100% positive biochemical activities
S. aureus	7-10% NaCl, Manitol fermentation, coagulase, catalase, and acetoin production, gram positive cocci
S. epidermidis	7% NaCl, no manitol fermentation, catalase production, non-coagulase production, Gram positive cocci
Streptococci	7% NaCl, no catalase production, alpha haemolytic activity on blood agar, Gram positive cocci.
P. aeruginosa	Growth on cetrimide agar, oxidase production, Gram negative coccobacilli, citrate utilization, motile, no H_2S production.
P. mirabilis	Gram negative, glucose fermentation, motile, no indole production, urease production, H_2S production and gas fro KIA.
E. coli	Fermentation of lactose, manitole, and glucose, no oxidase production, no H_2S production, indole production, gram negative.

Table-3- Effect of four concentrations of Honey on the growth rates of some Gram-positive bacteria.

Bacteria	Source	No. of isolates	Mean Absorbency [540 nm]				
			Control	0.5 v/v	0.25 v/v	0.125 v/v	0.0625 v/v
SAB	Burn	15	0.597	0.231	0.298	0.385	0.530
SEB	Burn	4	0.567	0.110	0.231	0.306	0.435
SEW	Wound	7	0.603	0.162	0.211	0.289	0.521
α-SB	Burn	3	0.482	0.203	0.310	0.375	0.401
Mean			**0.56225** ^{a*}	**0.1765** ^{b}	**0.2625**^{c}	**0.33875**^{cd}	**0.47175**^{e}

* Denotes significant differences.

Table-4- Effect of four concentrations of Honey on the growth rates of some Gram-Negative bacteria.

Bacteria	Source	No. of isolates	Mean Absorbency [540 nm]				
			Control	0.5 v/v	0.25 v/v	0.125 v/v	0.0625v/v
PAB	Burn	5	0.635	0.028	0.106	0.270	0.512
PAU	Urine	12	0.723	0.218	0.310	0.409	0.578
ECU	Urine	9	0.571	0.152	0.260	0.337	0.467
ECW	Wound	4	0.802	0.207	0.288	0.412	0.692
PMU	Urine	4	0.721	0.020	0.112	0.340	0.615
Mean			**0.690**[a*]	**0.125**	**0.215**	**0.353**	**0.572**

* Denotes significant differences.

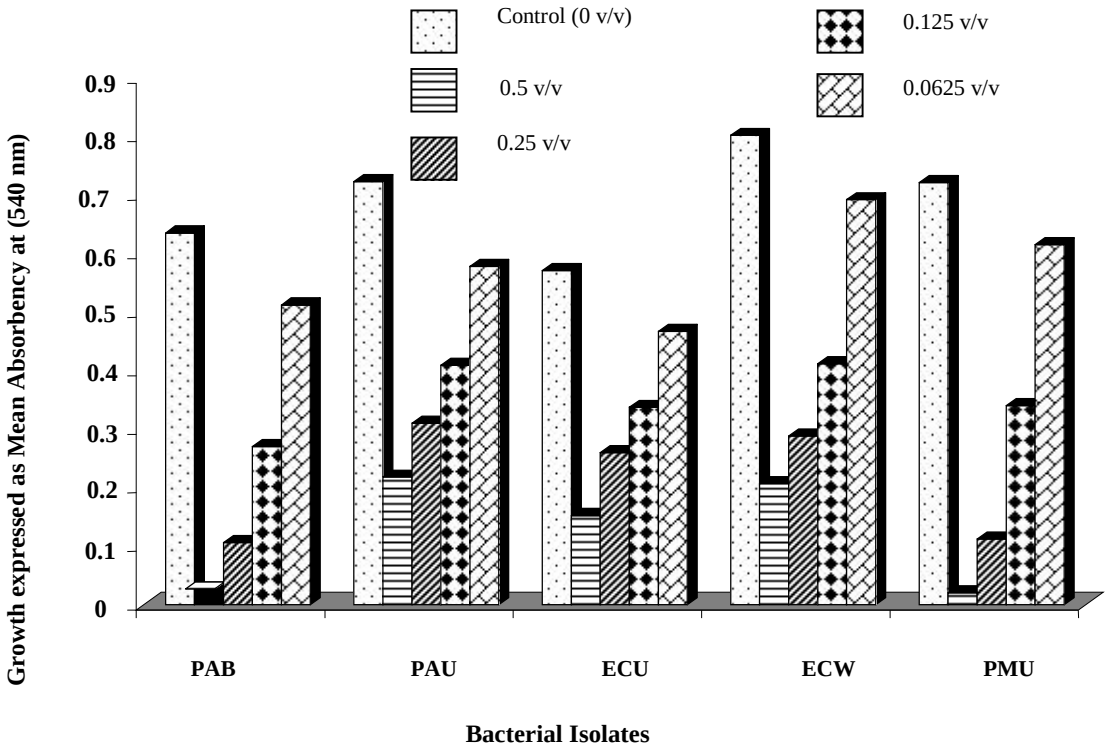

Figure- 1- Effects of different honey concentrations on different Gram negative bacterial isolates

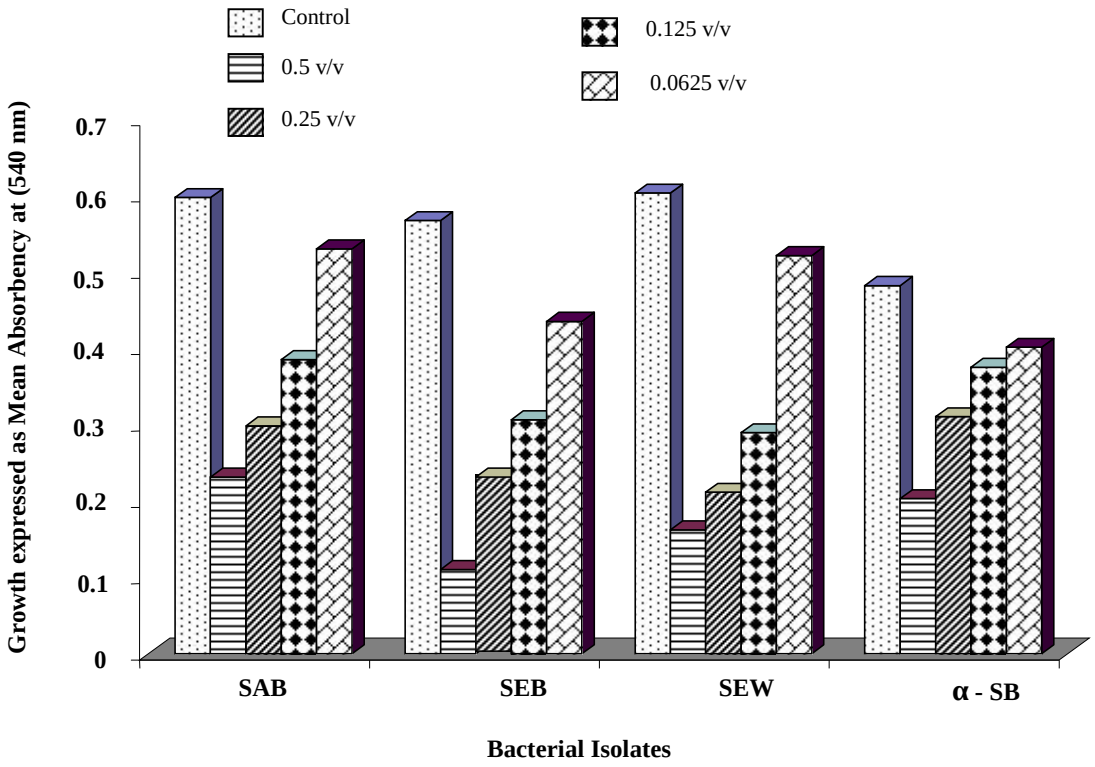

Figure-2- Effects of different honey concentrations on different Gram positive bacterial isolates

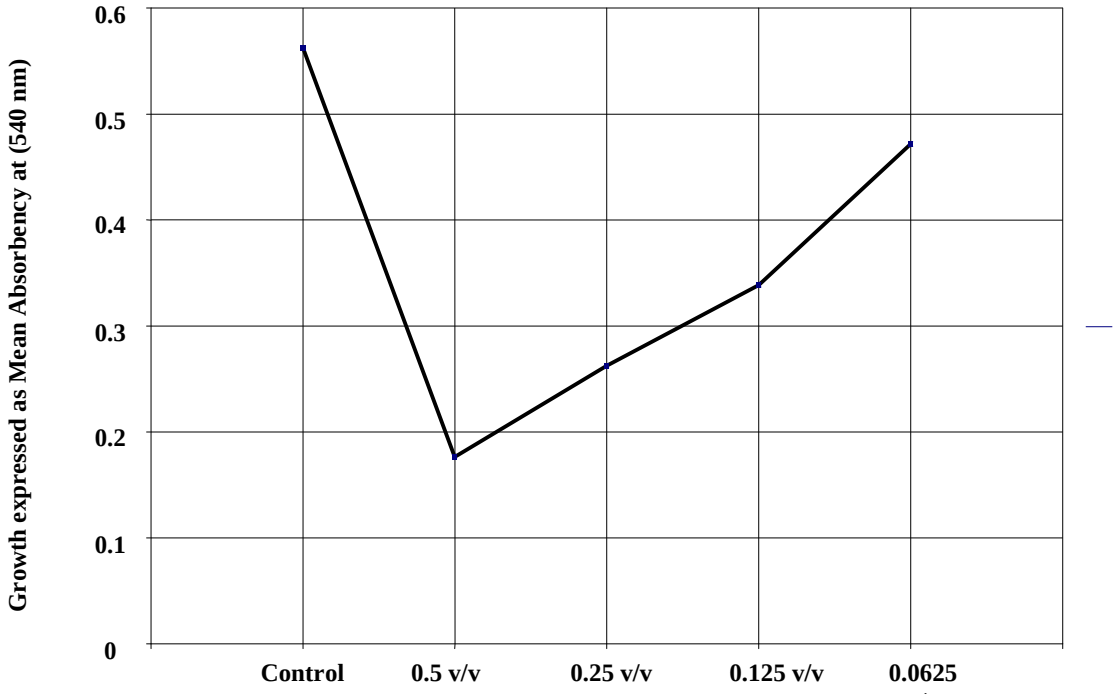

Figure -3- Comparison between the growth rates of controls and using different concentrations of honey on gram positive bacterial isolates

Discussion

Four different concentrations of honey were tested for their inhibitory effects on the growth of some clinical bacterial isolates of both Gram-positive and Gram-negative bacteria that are known with their ability to resist a wide range of antibacterial agents. The inhibitory effects of honey were shown in Table, 3 and 4.

The results revealed that there is a correlation between the concentration of tested honey and its effect on the bacterial growth expressed as absorbency (Fig. 1, 2 & 3). The higher the concentration, the higher the effects, this due to the higher contents of the antibacterial derivatives [7, 9] or the high osmolarity of the concentrated honey [2, 3, 5], although the enzymatic activity [6, 10, 12] may be responsible for the high antibacterial activity of the first concentration.

Results revealed that the effects of honey were higher against Gram- negative than that of Gram-positive bacteria [Tables, 3 and 4], this may due to the structure of the cell wall of the bacteria and correlated with the effects of the osmotic pressure of the honey [4, 5] which cause damage for Gram negative bacteria more than Gram positive, this observation was in agreement with results obtained by other researchers [16]. The inhibitory effects of honey due to its important chemical and physical properties [7, 23, 24, 25], including tetracycline derivatives [14], peroxidases, fatty acids, phenols, ascorbic acid, and amylases [20, 26]. Sometime the honey effects may due to non-peroxide activity, but may attribute to its acidity which may affect negatively against some bacteria [13, 14]; although some other researchers believed that the antibacterial effects might due to the hydrogen peroxide contents [10, 11, and 12]. Other results obtained by Quaradaghi and Kamaran when they concluded that honey was effective against wound infections due to *S. epidermidis* [30] supports the results of this study. Similar observations were recorded by other researchers who investigated the phenol content of honey which participate in its antibacterial activity [26, 27, and 28]. Also the results of this study were agreed with observations obtained by other researchers [22, 25, and 29]. Also the results in this work was agreed with results obtained by Subrahmanyam *et al.* when they reported that the honey acts as an antibacterial agent against different bacterial types [31], also the results of this work are similar to that obtained by French *et al.,* in 2005[32].

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