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*Article*

# Effectiveness of Royal Jell on Helicobacter Pylori Bacteria Isolated From Infected Patients

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**Abstract:** This project aimed to know the antibiotic effect of royal jelly produced by Apis mellifera bees against Helicobacter pylori bacteria isolated from infected people. Twenty samples that isolated from stomach infected patients by respiratory. Royal jelly showed an effective effect in inhibiting the bacteria. After knowing the minimum inhibitory concentration (MIC), the following concentrations were chosen (25, 50, 75)% with an average inhibition diameter of (20.00, 24.30, 30.60) respectively. The results showed that the inhibition effectiveness increases when royal jelly is used with the antibiotic clarithromycin at the same concentration, with an average inhibition diameter of (22.10, 28.60, 39.05). The antibiotic clarithromycin is currently used to treat stomach bacteria as part of the triple therapy used for stomach bacteria and ulcers. The effectiveness of royal jelly is due to its containing many active compounds, the most important of which is fatty acid, the most important of which is 10-HDA. (10-Hydroxy-trans-2-Decenoic Acid) which represents 80% of total fatty acids in royal jell and which was detected in this study using the HPLC apparatus High Performance Liquid Chromatography. Patients' irregularity in taking antibiotics leads to a decrease in the acidity of the stomach, which affects the effectiveness of the treatment. This in turn leads to making the bacteria more virulent and the possibility of bacterial mutations occurring at a high rate which made the medication ineffective Therefore, honey bee products are considered natural, do not cause genetic mutations or any bacterial resistance, and do not affect human health compere with antibiotic.

**Keywords:** Royal Jell, Helicobacter Pylori, Clarithromycin, 10HDA

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## 1. Introduction

Honey bee, known as the golden insect, is considered the most important insect from an economic aspect [1]. By biological management as pollinators in agriculture, it works to produce beneficial products for humans, whether they are therapeutic food with honey, royal jelly, beeswax, Propolis, bee venom and bee pollen, and all of these substances are great interest for humans, so the medication for bee products is a new science that called Apitherapia [2], [3].

Royal jelly is a white liquid tinged with yellow, thick in consistency, has a slightly acidic and slightly bitter taste, and represents the secretion of the hypopharyngeal glands of honey bee workers [4], [5]. Royal jelly is the only food given to all young larvae aged 1-3 days, while the queen has the specific food throughout her life. This is the reason for the long life of the queen bee, as the queen's life span ranges from 1-5 years and may live up

to 7 years, while the workers do not exceed 6 months and the males live up to nine months [6], [7].

The main components of royal jelly are proteins, the most important of which are Albumin, Globulin and Royalisin with a percentage of 12.50%, amino acids 11.5%, the most important of which are aspartic, lysine, leucine, glutamic, valine, proline, water at a rate of 65%, fatty acids, the most important of which is 10-HAD (10- Hydroxy- trans-2- decenoic acid), which accounts for 80% of the total acids, and it gave biological characteristics of royal jelly, carbohydrates by 11%, the most important of which are fructose, glucose, sucrose, minerals, the most important of which are (K, Mg, Na, Ca, Zn, Fe, Cu ,Mn) with a percentage of 2%, and little percentages of vitamins, the most important of which is Vt.C, folic acid, biotin, thiamine, riboflavin, niacin, pyridoxine and natural antibiotics such as flavonoids [8], [9].

In 1875, German scientists discovered spiral bacteria for the first time in the human stomach. After that, the scientist Walery published a study that included many types of spiral-shaped bacteria that he found in gastric washings [10], [11]. The work of the German and Polish scientists was not rediscovered till 1979 when Warren saw curved and spiral bacteria in the biopsy material from gastric mucosa. He and Marshall isolated and cultured the organism and named it Campylobacter pyloridis, They contended that most stomach ulcers and gastritis were caused by colonization with this bacterium. Following DNA sequences, the organism in 1990 was renamed H. pylori [12]. Helicobacter pylori is a gram negative (G-ve) bacterium, it infection occurs when the bacteria infect a human stomach.

## 2. Materials and Methods

### 2.1 Collect Royal Jelly

Royal jelly was also collected in a manner that orphan the cell from its queen, so the bees build royal houses on the homes of the larvae of young workers (1-3) days old. The quantities of royal jelly are collected from these houses by means of a vaccination needle after lifting the royal caterpillar from the royal house and to obtain royal jelly in large quantities, it is preferable to take it at the end of the fifth day from the homes of the queens, and put the royal jelly in an opaque bottle, then these bottles are placed in the refrigerator at a temperature of (5) C or in the freezer for long-term preservation [13].

### 2.2 High Performance Liquid Chromatography (HPLC)

A sample of the royal jelly was sent to the laboratories of Al-Mustansiriya University to conduct a chemical analysis of the sample, which showed the presence of the active compound at a percentage that is within the acceptable percentage for the typical sample [14].

The standard solutions were separated and the peak area of the standard model, the retention time and the height of the bands were identified. Then the retention time, area and height of the bands resulting from the injection of the samples were measured, and the concentration of the compounds in the model was calculated according to the following equation in figure 1 [15].

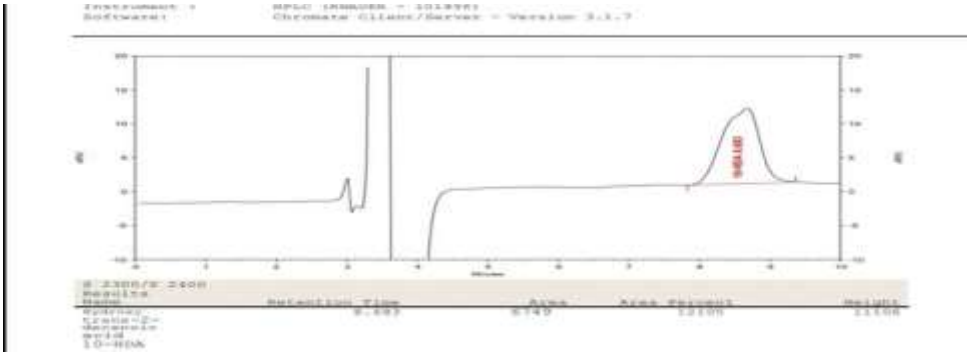

**Figure 1.** appearance 10-HDA by HPLC.

**2.3 Culture media**

Swabs were taken from patients infected with Helicobacter pylori by respiratory droplets in the laboratory of Dr. Manal Dheyaa Mohammed. The number of samples was 20 samples, cultured on blood agar for 24hr. at 37c . After the expiry of the period, the bacterial growth will be obvious, Then bacterial samples were streaked on blood agar .All the media were prepared according to manufacturer, sterilized by autoclave for 15 min at 121c [16].

**2.4 Minimum Inhibition Concentration (MIC)**

The minimum inhibitory concentration of RJ was found by preparing concentration (5, 10, 15, 20, 25, 30%) By adding (5, 10, 15, 20, 25, 30) ml-gm of the royal jell and complete the volume to 10 ml with distilled water, and it was mixed with Mueller Hinton agar medium by mixing 5 ml of (MIC) concentration + 20 ml medium Muller-Hinton, the plates were left for 5-10 minutes to cool, then plates were cultured with 0.1 ml of 24-hour-old bacterial suspension grown in the middle of the nutrient broth after equilibration with McFarland solution, and dishes were made at a rate of three replications for each concentration, with control dishes containing On the bacterial culture and the nutritional medium agar Muller-Hinton, to which no honey bee product was added, then left to dry at room temperature, then the dishes incubated at 37C° for 24 hours, and the presence of growth was observed or not, as the lowest concentration indicates no growth on it is the minimum inhibitory concentration [17].

**2.5 Antimicrobial susceptibility test**

Used method of the Agar-gap or well diffusion to test the inhibitory activity of the products on bacterial growth. It was done by taking 20 ml from agar medium into each dishes Petri, and after that, the dishes were incubated for 24 hours at a temperature of 37C° to sure the agar was not contaminated. Then inoculated the medium by using a sterile cotton swab. A swab was taken from the bacterial suspension and spread evenly on the Muller-Hinton Agar medium and then left for 15 minutes at room temperature for the purpose of absorbing the vaccine, then 3 holes that made in used dishes with a 6 mm diameter by perforator cork each hole representing a concentration, as 100µl of each of the three concentrations were added and placed in the hole designated for it, and at the same time 100 microliters of distilled water was added to one of the holes instead of the plant extract. The presence of bacterial growth around holes, and the dishes were incubated for 24h., after that, measured the diameters of the inhibition zones around the holes by using a ruler in millimeters, by taking the measurement of each fixation zone I and then subtracting the diameter of the hole Diameters of inhibition from the results obtained for the diameters of the inhibition areas, after that the average diameters of the inhibition areas were taken for three replicates [18].

**3. Result**

**Table 1.** Inhibition zone of R.J against H.pylori.

| RJ Concentration% | N | Mean | Std. Deviation | Minimum | Maximum |
|---|---|---|---|---|---|
| 25 | 20 | 20.00 A | 0.525 | 16 | 25 |
| 50 | 20 | 24.30 A | 0.733 | 22 | 26 |
| 100 | 20 | 30.60 C | 1.000 | 27 | 33 |
| ANOVA significant differences between mean zoon * | | | | | |

The treatment of patients with traditional antibiotics was becoming worldwide burden therefore multiple drug resistance bacterial infections are ineffective12, as we can see in table (1), the results of sensitivity Helicobacter pylori bacteria treated with royal jelly

showed that the diameter of the inhibition zone around the pits containing royal jell ranged between (16-33 mm) and as follows, as the diameter of the inhibition zone ranged between (27-33) mm when the concentration royal jell was 100%, while the area of inhibition ranged between (22-26) mm when the concentration of royal jelly was 50%, and at the concentration of 25% the diameter of the area of inhibition of bacteria around the pits ranged between (16-25 mm), as shown in Table (2) We notice an increase in the percentage of bacterial inhibition after adding the disc of antibiotic Clarithromycin at a concentration of 10 mg near the royal jelly in the same petri dish, and we notice an increase in inhibition as the percentage of royal jelly concentration increases, the pits containing royal jelly ranged between (19-45 mm) and as follows, as the diameter of the inhibition zone ranged between (32-45) mm when the concentration of royal jelly was 100%, while the area of inhibition ranged between (24-33) mm when the concentration of royal jelly was 50%, and at the concentration of 25% the diameter of the area of inhibition of bacteria around the pits ranged between (19-26 mm).

**Table 2. I**nhibition zone of RJ and clarthamycin against H.pylori.

| RJ Concentration% | N | Mean | Std. Deviation | Minimum | Maximum |
|---|---|---|---|---|---|
| 25 | 20 | 22.10 a | 0.787 | 19 | 26 |
| 50 | 20 | 28.60 b | 0.990 | 24 | 33 |
| 100 | 20 | 39.05 c | 1.400 | 32 | 45 |

ANOVA significant differences between mean zoon *

The results of this study agreed with the study of Fontana [19], which showed that royal jelly has a significant effect on some human pathogenic bacteria, as it has antibacterial properties against many gram-positive and gram-negative bacteria. This strong inhibition of bacteria is attributed to the royal jelly's containing of the soluble fatty acid 10-HDA (10-hydroxy decenoic acid), which was detected in this study by HPLC and its presence was 3.1%, and it is considered an excellent percentage as its concentration in royal jelly in figure 2. It does not exceed 5% of the other components of R.J., which is attributed to it giving biological characteristics of royal jelly and it has a significant role in influencing the synthesis of cell membrane proteins and cell plasmid proteins, and royal jelly contains many carbohydrates, the most important of which are Fructose, glucose and sucrose, which were also detected in this study, are attributed to the occurrence of osmosis, cell shrinkage and death.

**Figure 2.** Royal jelly contamination.

## 4. Discussion

Royal jelly also contains the proteins albumin, globulin and royalisin, and it contains many amino acids, including lysine and leucine, which in turn have an effective role ininhibiting microbial [20]. These results are consistent with what was stated by [21],[22] who suggested combining the triple treatment system (lansoprazole, Tinidazole, Clarithromycin) with milk, propolis, royal jelly, broccoli sprouts, honey, ginger, or broccoli or mango.

## 5. Conclusion

Royal jelly had an effective effect against H. pylori bacteria that isolated from patients respiratory that infected by it, and the effectiveness increases with increasing concentration. When royal jelly used with clarthamycin antibiotic that commonly in treatment Helicobacter pylori, that lead to increase inhibition bacterial zone, so that's means using royal jelly with the antibiotic leads to a speedy healing from the bacteria stomach.

1. Using royal jelly against other types of bacteria.
2. Using other natural products and materials to treat stomach bacteria because they do not lead to an increase in the virulence factors of the bacteria if they are not used regularly as is the case with antibiotics.
3. Study of the effect of royal jelly on the genetic composition of the bacteria under study.

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