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# Evaluation The Inhibitory and Synergistic Action of Aqueous and Alcoholic Extracts of Bee Honey and Its Products on Some Bacterial Species Isolated from Otitis Media

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**Abstract**

The current study, honey bee products were used antibiotics the treatment of many diseases affect humans, including treatment against bacteria cause otitis media. The current study included use aqueous and alcoholic extracts honey bee products in treating bacteria isolated from otitis media. The results the study showed the effectiveness honey bee products against Gram-positive and Gram-negative bacteria. The current study included (124) swabs from patients with otitis media visiting Hawija General Hospital using swabs medium then cultured on media (blood agar, MacConkey agar), (96) positive samples (77.42%) and (28) negative samples (22.58%) were obtained. The study included three bacterial species. *Proteus mirabilis Staphylococcus aureus ,Psedomonas aeruginosa*, the results showed *P. mirabilis* is the most sensitive types of bacteria the aqueous extract royal jelly with average diameter of inhibition (23.78 mm) concentration 100 mg/ml, followed by the alcoholic extract of propolis with average diameter the zone inhibition (19.46 mm) concentration 100 mg/ml against *S. aureus, P. aeruginosa* bacteria, the aqueous extract honey showed inhibitory effect (17.84 mm) concentration 100 mg/ml and lowest average inhibitory effect alcoholic propolis (8.84 mm) concentration 75 mg/ml against *P. aeruginosa*. While aqueous extract wax did not show any inhibitory effect for all concentrations used against types bacteria under study.

## Introduction

Microorganisms are now characterized by their therapeutic resistance to antimicrobial resistance, which makes traditional antibiotics fewer in number and less effective [1]. As for the treatment method using the same antibiotics repeatedly for the same bacterial type, it led to the bacteria acquiring resistance as a result of changes in their cell walls, the secretion of hydrolyses, or changing the locations of receptors, i.e. the emergence of new strains [2]. As well as for genetic reasons, it becomes unaffected by these types of antibiotics, thus reducing the effectiveness of chemotherapy drugs for treatment [3]. Given the ability of bacteria to generate internal resistance through their possession of many resistance genes [4]. Some bacterial strains have acquired the ability to resist most antibiotics [5].

Which has motivated researchers in recent years to increase their interest in seeking alternative solutions to traditional antibiotics [6]. To discover new factors to overcome microbial resistance through the use of alternatives such as natural products and herbs as raw materials or as sources of active ingredients that are included in drug formulation [7]. Including honey bee products with a natural composition that have the ability to heal and enhance the patient's immunity at the same time [8]. In addition to the vital role that *Apis mellifera Linnaeus* plays as pollinators in agriculture, they work to produce honey bee products that are beneficial to humans, whether food or medicinal. These products include honey, royal jelly, bee venom, propolis, grain pollen, and bee's wax. All of these materials are of great benefit to humans. These ancient natural materials date back to the prehistoric era that humans used in food and medicine to treat many diseases [9]. Due to the success of these natural compounds in using antibiotics as therapeutic agents, it has taken a wide space in medical science. This has led to the spread of treatment. Honey bee products are widely available in pharmacies [10].

Modern medicine has proven the importance of honey bee products for human health [11], and it has been used in treating bones [12]. It has also been scientifically proven that honey bee products contain antioxidants, anti-bacterial, viral, and fungal infections, a natural treatment for cancer, and improve immune status [13]. Propolis is known for its antiviral, anti-inflammatory, antibacterial, anesthetic, antioxidant, anticancer, and antifungal properties [14]. Honey is important to humans as an antioxidant that makes it effective in protecting the skin from free radical damage. Anti-aging by regulating skin enzymes, used as an ointment to dress burns and wounds, protects the heart and blood vessels, and anti-bacterial by controlling wound inflammation [15]. This may be due to its complex composition rich in antioxidants. Oxidation because it contains carbohydrates, minerals, proteins, fatty acids, and other biologically active substances, including flavonoids, polyphenolic hydrocarbons, and indoles [16]. Royal jelly is also an important food source for humans because it contains proteins, and because it contains peptide compounds and biologically active fats, it is therefore used in pharmacies as an antibacterial [17].

Among the important diseases that are widespread and that affect large numbers of people of both sexes around the world and cause them health problems is otitis media, which is linked to bacterial or viral pathogens [18]. Otitis media is the most common among groups. At a young age, this is due to their incomplete immune system and weak physical structure [19]. Otitis media occurs in children and adults, but its incidence in children is higher than in adults [20]. Infection occurs through the entry of pathogens, whether bacterial, viral, or fungal directly to the ear or after injuring the respiratory system through the nose or throat through the Eustachian canal, causing pain and inflammation in the area of infection and its transmission to the ear space [21]. The most common bacterial species causing otitis media are *Escherichia coli, P. aeruginosa, Klebsiella pneumoniae, Staph. Spp.,* and *P. mirabilias* [22].

The aim of the study is to evaluate the inhibitory effect of honey bee products against bacterial species isolated from patients with otitis media.

**Working Methods**

**Collection of honey bee models:**

Samples of honey bees were collected directly from beehives from farms in Kirkuk Governorate/Hawija District. After catching, they were placed in a glass bottle with a capacity of (0.5) Liters containing 0.25 Liters of medical alcohol to kill the insects, and a large amount of glycerine was added to maintain the flexibility and vitality of the insect. Then, they were placed in a plastic box containing a piece of cork inside that is tightly fixed. The insect was fixed on the cork using pins stuck in the middle of the insect's chest. Information is fixed on the box, which is the name of the student, university, college, department, type of study, time of collection, and it was transferred to the research centre and museum. Natural History/University of Baghdad for the purpose of classification.

**Collection of bee products (honey, royal jelly, wax, propolis, bee venom):**

Honey bee products were collected from honey bee hives directly from farms in the city of Hawija, which depend on apricot, apple and grape trees for their food. The models were stored in sterile, tightly sealed bottles of 200 ml, and then special information was fixed on each bottle, including the type of model and date of collection [23].

**Preparing extracts and solutions of honey bee products:**

**Preparation of aqueous extracts:**

Aqueous extracts were prepared by adding sterile distilled water. Four ascending concentrations of bee products (honey, venom, propolis, wax, and royal jelly) were prepared. The study included concentrations (25%, 50%, 75%, 100%) (V/V) [24].The products were prepared each according to their own method. Aqueous solutions of royal jelly, honey, and wax were obtained by diluting each of them with distilled water. The aqueous extract of the wax was prepared by taking 40 grams of the dry matter of bee wax and placing it in a pre-sterilized glass beaker contains 200 ml of distilled water, where the ratio is (1 millilitre to 5 weight), and the mixture was mixed well at a temperature of 40 °C for two hours. then it was placed in a centrifuge for 15 minutes at a speed of 4000 rpm, and then placed in an electric oven at a temperature of 35°C for 4 hours to obtain the concentrated extract, which. The standard concentration was considered the stock solution, from which the required dilutions under study were prepared, and it was stored in dark, tightly sealed bottles at a temperature of (5°C) until use [25]. The hot aqueous extract of propolis was prepared by weighing 40 grams of propolis powder and adding 160 millilitres of distilled water, the ratio being (1 millilitre to 5 weight), and the mixture was mixed well for half an hour to mix the propolis with the water. a thermal magnetic mixer to mix the propolis with water well for half an hour at a temperature of 55 degrees Celsius, and then after.

The resulting solution was filtered using a piece of gauze to get rid of the insoluble components, and the solution was placed in bottles and kept at room temperature for 48 hours for the purpose of soaking, shaking for a period of time every half hour. Then it was transferred to the centrifuge for 15 minutes at a speed of 4000 revolutions per minute, after which the solution was placed. the solution was transferred to electrical oven at a temperature of 35°C for 4 hours obtain the concentrated extract, which was considered the standard concentration of the stock standard solution, from which the rest of the concentrations (25-50-75-100 mg/ml) were prepared under study. These solutions and extracts were sterilized using filters with a diameter of 0.2 micrometres and stored in sterile, opaque bottles and sealed in the refrigerator until use [25].

**Preparing the alcoholic extract of propolis:** The alcoholic extract of propolis was prepared by adding 10 grams of propolis powder to 100 ml of absolute alcohol (99%) at a ratio of 1:10 weight: volume, in a glass beaker and left for five days at laboratory temperature with daily shaking for 15 minutes using a magnetic stirrer, and then filtered. The resulting solution was used with a piece of gauze to get rid of the insoluble components. Then the process of alcoholic evaporation of the solution was carried out by placing it in the oven and setting the temperature at 45°C until it turned into a dry state (solid). The product was left to cool, and then it was placed in the refrigerator at (5°C) to solidify. It is crushed with a blender and 1 gm of propolis is taken from it with 9 ml of distilled water. The latter is considered the standard concentration of the stock standard solution from which it is diluted. The concentrations (25-50-75-100 mg/ml) are being studied and this extract is sterilized using filters with a diameter of 0.2 micrometres. It was stored in sterile, dark-coloured, tightly sealed bottles in the refrigerator until use [26].

**Sample Collection**

96 positive samples and 28 negative samples were isolated from a total of 124 samples isolated from patients with otitis media visiting the ear and throat department at Hawija General Hospital/Kirkuk Governorate. Using cotton swabs with carrier medium, then cultured on media (blood agar) and (MacConkey agar).

**Testing the inhibitory effectiveness of honey and other bee products:**

It was confirmed that the product was sterile and free of microbial contamination by conducting tests on honey bee products against microorganisms. The results showed that there was no bacterial growth on the Nutrient agar medium after 48 hours of incubation at a temperature of 37°C [27]. The Agar-well diffusion method was used to test the inhibitory effectiveness of the products on bacterial growth, The medium was inoculated using a sterile cotton swab. A swab of the bacterial suspension was taken and spread evenly on the Muller-Hinton Agar medium. It was then left for 15-20 minutes at room temperature for the purpose of absorbing the inoculum. After that, 4 holes were made in each dish using a cork perforate with a diameter of 6. Each hole mm represents a specific concentration, as 75 microliters of each concentration was added using micropipettes. From the four concentrations, the plates were incubated for 24 hours [28]. After the end of the incubation period, the plates were incubated for 24 hours. Measuring the diameters of inhibition around the Wells holes using a ruler in millimetres, by taking the measurement of each inhibition zone and then subtracting the diameter of the hole (diameters of inhibition) from the results obtained for the diameters of the inhibition zones. After that, the average diameters of the inhibition zones were taken for three replicates [29].

**Testing the synergistic effectiveness of honey bee product extracts on bacterial growth*:***

The synergistic effect of honey bee products was tested in a ratio of (1:1) (volume: volume). The etch diffusion method was used on Muller Hinton agar medium. Then the plates were incubated at a temperature of (37°C) for 24 hours, and the diameters of inhibition were measured in millimetres to demonstrate the synergistic effectiveness, replicates for each sample [30].

**Determine the minimum inhibitory concentration value for honey bee products:**

The MIC for honey bee products was found according to the method of [31]. By preparing solutions or aqueous extracts of honey bee products at concentrations of (5, 10, 15, 20, 25,

30%) by adding (5, 10, 15, 20, 25, 30) ml of products in succession and complete the volume to 100 ml, i.e. (95, 90, 85, 80, 75, 70) ml of sterile distilled water and mix them well, then Mueller-Hinton agar medium is prepared. These concentrations of products are mixed with the medium was mixed by mixing (5 ml of MIC + 20 ml of Mueller-Hinton medium). The plates were left for 5-10 minutes to cool. After that, the plates were planted with 0.1 ml (µ100) of the 24-hour-old bacterial suspension grown in the broth medium. The feeder was balanced with McFarland's solution, and the plates were made at a rate of three replicates for each concentration, with control plates made containing the bacterial culture and the Mueller-Hinton agar nutrient medium, to which no honey bee products were added. Then they were left to dry at room temperature. After that. The cultured petri dishes were then transferred bacteria were transferred and incubated at a temperature of 37 °C for 24 hours, and then the presence of bacterial growth was observed or not, as the lowest concentration at which growth did not appear indicates the minimum inhibitory concentration [32].

**Statistical Analysis**

The results obtained during the study were collected into a computer database and processed using the program (Statistical Package for Social Sciences (SPSS)) to estimate the means and standard deviation (St. Deviation). The differences between the means were compared based on the Duncan test the probability level ($P < 0.05$) [33].

**Results and Discussion:- Category**

The classification results for the specimens sent to the Natural History Research Center and Museum/Department of Insects and Invertebrates/University of Baghdad showed that the specimens sent were diagnosed and classified as follows: Apis mellifera Linnaeus, 1758 (Hymenoptera, Apidae).

**The inhibitory results of honey bee product extracts on bacterial growth are under study**

The results showed an inhibitory activity against bacterial isolates of extracts of both types of honey bee products (alcoholic and aqueous) and at the different concentrations under study (25-50-75-100) mg/ml, as follows:

***P. aeruginosa:*** The results shown in Table 1 the inhibitory effect of extracts of honey bee products on P. aeruginosa bacteria isolated from otitis media at a concentration of 100 mg/ml. This is the honey product that actually showed an inhibitory effect against the isolated bacteria with no significant differences ($P<0.05$) with a diameter of 17.84 mm, then followed by the effect of honey at a concentration of 75 mg/ml, where the diameter of inhibition reached a diameter of 12.35 mm. It is consistent with a study conducted by [34] to evaluate the antibacterial effect of honey on Gram-negative and positive bacteria, which indicates that high concentrations that are more than (80 mg/ml) are more effective than low concentrations, While the results of this study did not agree with the results of the study conducted by Al-Jubouri [35]. Which indicated that high concentrations of honey (100 mg/ml) did not record any effect. The antibacterial activity of honey is attributed to many properties, the most important of which is that it contains a ripening enzyme such as $H_2O_2$.

The inhibitory effect of the aqueous extract of propolis comes with an inhibitory diameter of (10.88 ml). The results of this study agreed with [36] while the results differed with [37]

and the inhibitory effect of the alcoholic extract of propolis comes with an inhibitory diameter of (10.77 ml), both at the concentration (100 mg/ml), and royal jelly came after that with its inhibitory effect on the bacteria isolated with a diameter of 9.25 ml. The results of the study agreed with ]25[ who indicated that royal jelly has an effective effect against some bacterial species that infect humans, as it has antibacterial properties against Gram-positive and Gram-negative bacteria. This is due to royal jelly containing soluble fatty acids. There was no significant effect of beeswax on the isolated bacteria for all concentrations, and the results of this study were consistent with the study [38] which indicates that the effect of wax on the bacteria *P. aeruginosa* was very low regarding Gram-positive and Gram-negative bacterial isolates.

**Table 1:** Inhibitory effectiveness of aqueous and alcoholic honey bee product extracts against *P. aeruginosa* bacteria isolated from otitis media, measured in millimetres (mm).

| The focus mg/ml | The diameter of the inhibition zone is measured in millimetres for the bacteria *P. aeruginosa* | | | | |
|---|---|---|---|---|---|
| | Aqueous extract | | | | Alcoholic extract |
| | Wax mean±std | Honey mean±std | Royal jelly mean±std | Propolis mean±sd | Propolis mean±std |
| 25% | f0.00±0.00 | f0.00±0.00 | f0.00±0.00 | f0.00±0.00 | f0.00±0.00 |
| 50% | f0.00±0.00 | f0.00±0.00 | f0.00±0.00 | f0.00±0.00 | f0.00±0.0 |
| 75% | f0.00±0.00 | b12.5±0.43 | f0.00±0.00 | d9.8±0.31 | e8.84±0.32 |
| 100% | f0.00±0.00 | a17.84±0.65 | a17.4±0.65 | c10.88±0.52 | c10.77±0.44 |

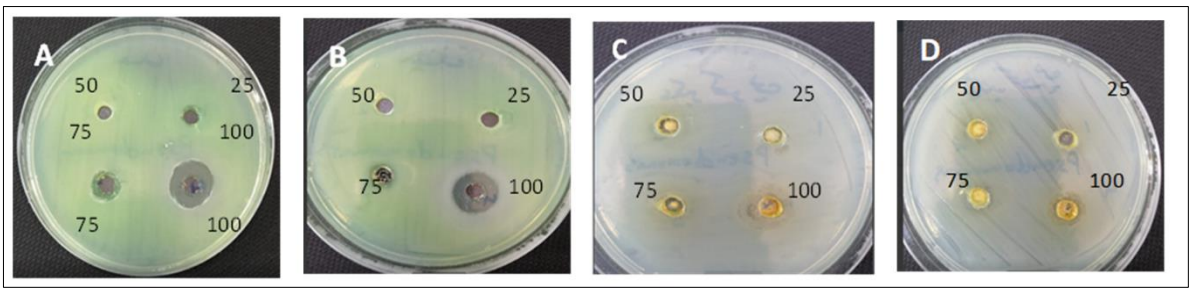


**Fig. 1** *Effect of honey bee product extracts against P. aeruginosa bacteria.*
*A Effect of aqueous extract of honey*
*B Effect of aqueous extract of royal jelly*
*C Effect of alcoholic extract of propolis*
*D Effect of aqueous extract of propolis*

***Staphylococcus aureus:*** Table 2 shows the inhibitory effect of honey bee product extracts on bacteria *S. aureus* as the alcoholic extract of propolis was most effective with an inhibitory diameter of (19.46 mm) at a concentration of (100 mg/ml), as the effect of this extract was directly proportional to the concentration used, and with an inhibitory diameter of (17.73 mm) at a concentration of 75 mg/ml) against the isolated bacteria. The results of this study agreed with [39], which obtained diameters ranging between (14-22 mm) when the concentration was (100 mg/ml). This study also agreed with [40], who showed that the

alcoholic extract of propolis has an inhibitory effect on bacteria. The inhibitory action of the aqueous extract of honey comes in second place with a diameter of 12.47 mm, with significant differences ($P < 0.05$). This study is consistent with what was found [34] when evaluating the activity of honey against Gram-negative and Gram-positive bacteria, that the honey's activity was more than (80 mg/ml). It also agrees with the results of [39] and found that the diameters of inhibition ranged between (10-20 mm) at the concentration (100 mg/ml). However, the current study did not agree with the study of [35], which showed that honey at a concentration (100 mg/ml) did not record any effect on many bacterial species, and that diluted honey has a higher effect than concentrated honey.

This activity of honey against bacteria is due to It contains many factors and characteristics, including osmotic pressure and its high acidity resulting from the enzymes secreted by worker bees on the nectar when they transform it into mature honey, such as the enzyme Glucosoxidase, and the presence of other substances, the most important of which is ascorbic acid. As for the highest effect of royal jelly, it had an inhibitory diameter of (10.83 mm) at a concentration of (100 mg/ml) and an inhibitory diameter of (9.16 mm) at a concentration of (75 mg/ml), while it had no effect at concentrations (25-50 mg/ml). The results of the current study agreed with [39] and [25], which showed an effect on Gram-negative and positive bacteria. This effect is attributed to royal jelly because it contains soluble fatty acids [41]. The inhibitory action was for the aqueous extract of the wax with a diameter of 10.32 mm. While the aqueous extract of propolis had the least inhibitory effect at the concentration (75 mg/ml) with an inhibitory diameter (7.10 mm), while the aqueous extract of propolis did not have any inhibitory effect at the concentration (25 mg/ml). This study agreed with the study of [37], which showed that the aqueous extract of propolis has little effect on bacteria. As for the aqueous extract of the wax, it did not have any inhibitory effect at the concentrations used (25-50-75-100 mg/ml). These results agreed with the results of [38], as they confirmed that beeswax had no significant effect on microorganisms.

**Table 2:** Inhibitory effectiveness of aqueous and alcoholic honey bee product extracts against *S. aureus* bacteria isolated from otitis media, measured in millimetres (mm).

| The focus mg/ml | The diameter of the zone of inhibition is measured in millimetres for bacteria *Staph. aureus* | | | | |
|---|---|---|---|---|---|
| | Aqueous extract | | | | Alcoholic extract |
| | Wax mean±std | Honey mean±std | Royal jelly mean±std | Propolis mean±std | Propolis mean±std |
| 25% | j0.00±0.00 | h8.53±0.33 | j0.00±0.00 | j0.00±0.00 | e11.16±0.54 |
| 50% | j0.00±0.00 | g9.46±0.41 | j0.00±0.00 | i7.10±0.30 | c16.17±0.66 |
| 75% | j0.00±0.00 | e11.49±0.53 | g9.16±0.37 | i7.10±0.31 | b17.73±0.32 |
| 100% | f10.32±0.40 | d12.47±0.52 | f10.83±0.41 | g9.10.±0.39 | a19.46±0.63 |

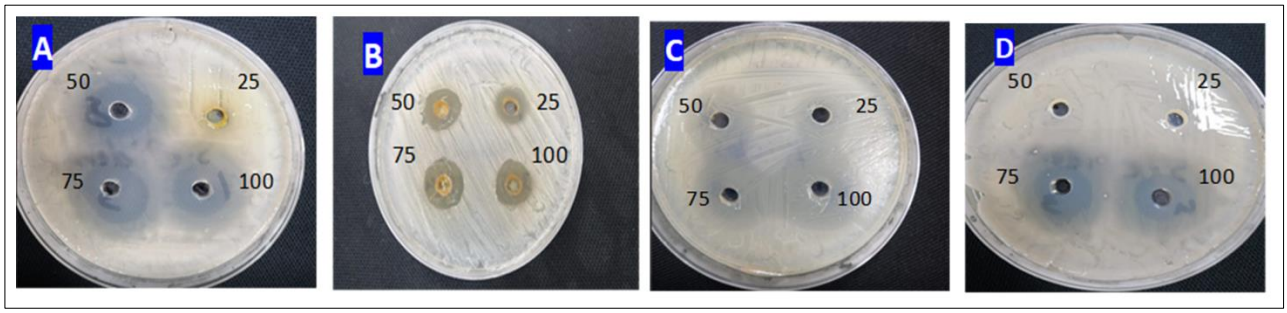


**Fig. 2** *Effect of honey bee product extracts against bacteria Staph. aureus.*

*A Effect of aqueous extract of propolis*

*B Effect of alcoholic extract of propolis*

*C Effect of aqueous extract of honey*

*D Effect of aqueous extract of royal jelly*

***Proteus mirabilis:*** The results shown in Table 3 that the most effective extract of honey bee products against the isolated bacteria at a concentration of 100 mg/ml is the aqueous extract of royal jelly, which actually showed an inhibitory effect with significant differences ($P<0.05$) with inhibitory diameter rates directly proportional to the concentrations (100-75-50-25 mg/ml) with inhibitory diameters respectively (23.78-21.27-19.46-17.61 mm). The results of the current study showed that royal jelly extract has a high effect on bacteria isolated from otitis media. This is due to royal jelly containing the soluble fatty acid (10-Hydroxytrans decenoic acid), which has effective biological properties and affects the synthesis of cell membrane proteins and plasmid proteins. The cell, as well as containing carbohydrates that have an effective effect on bacteria through the occurrence of osmosis, cell shrinkage and death. In addition, royal jelly contains amino acids, including leucine, which has an effective role in inhibiting microbes [41]. These results are consistent with the findings of [41]. Next comes the aqueous extract of honey with an inhibitory diameter of 16.43 mm and the effect of the alcoholic extract of propolis comes with an inhibitory effect a diameter of 16.42 mm at concentration of 100 mg/ml. Then came the effect of the alcoholic extract of propolis when the concentration was (75 mg/ml) with an inhibitory diameter (14.50 mm) and with an inhibitory diameter of (14.13 mm) when the concentration was (50 mg/ml). The effect of the alcoholic extract of propolis on bacteria is by preventing the process of cell division and reproduction, inhibiting protein synthesis inside the cell, as well as on the permeability of cytoplasmic membranes, and inhibiting enzymatic activity and bacterial movement [42].

Then comes the effect of the aqueous honey extract with an inhibitory diameter (12.78 mm) when the concentration was (75 mg/ml), and the effect of the alcoholic extract of propolis with an inhibitory diameter of (12.41 mm) came in last place when the concentration was (25 mg/ml). The inhibitory effect of honey against bacteria was due to its possession of properties such as high osmotic pressure, as honey has the ability to osmosis resulting from the high sugar in the honey, so water is absorbed from the bacterial cells, which leads to dehydration, contraction of the bacteria and their death [43]. The results of the current study agreed with the study [44], while the aqueous extracts of propolis and wax did not show any inhibitory effect at all concentrations, and the aqueous extract of honey did not show any inhibitory effect at a concentration of 25 mg/ml.

**Table 3:** The inhibitory activity of aqueous and alcoholic honey bee product extracts against *P. mirabilis* bacteria isolated from otitis media, measured in millimeters (mm).

| The focus mg/ml | The diameter of the inhibition zone is measured in millimeters for the bacterium *P. mirabilis* | | | | |
|---|---|---|---|---|---|
| | Aqueous extract | | | | Alcoholic extract |
| | Wax mean±std | Honey mean±std | Royal jelly mean±std | Propolis mean±std | Propolis mean±std |
| 25% | i0.00±0.00 | i0.00±0.00 | d17.61±0.67 | i0.00±0.00 | g12.41±0.52 |
| 50% | i0.00±0.00 | h9.52±0.37 | c19.46±0.72 | i0.00±0.00 | f14.13±0.61 |
| 75% | i0.00±0.00 | g12.78±0.53 | b21.27±0.83 | i0.00±0.00 | f14.50±0.63 |
| 100% | i0.00±0.00 | e16.43±0.59 | a23.78±0.88 | i0.00±0.00 | e16.42±0.56 |

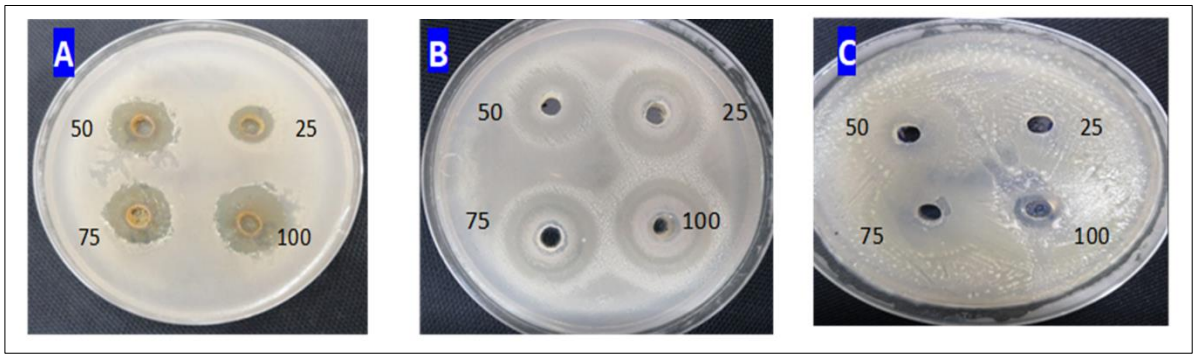


**Fig. 3** *Effect of honey bee product extracts against bacteria P. mirabilis.*
*A Effect of alcoholic extract of propolis*
*B Effect of aqueous extract of royal jelly*
*C Effect of aqueous extract of honey*

**The synergistic inhibitory results of honey bee product extracts on bacterial growth are under study:**

The results of the current study showed an inhibitory activity of extracts of honey bee products when mixing both extracts with each other against the bacteria under study isolated from otitis media. The highest inhibitory results against *P. mirabilis* bacteria were obtained for the synergistic extract (honey and royal jelly) with an inhibitory diameter of 21.76 mm, followed by the synergistic extract (propolis and royal jelly) with an inhibitory diameter of 21.57 mm. The results of the two synergistic extracts (propolis and honey) and (propolis and wax) had inhibitory diameters. 20.87 and 20.44 mm, respectively, and the extract (wax and honey) has a diameter of 10.23 mm. The synergistic extract (wax and royal jelly) has the least inhibitory effect, with an inhibitory diameter of 8.17 mm against bacteria *P. mirabilis*. Then came the inhibitory effect against bacteria *S. aureus* for synergistic extract (honey and Royal jelly) with a diameter of 14.34 mm. Then the inhibitory action of the synergistic extracts (propolis and royal jelly) with an inhibitory diameter of 10.56 mm and (propolis and honey) with a diameter of 10.44 mm against bacteria increases *S. aureus*, and the synergistic extract (honey and royal jelly) with a diameter of 10.49 mm against bacteria *P. aeruginosa*. And the synergistic extract (wax and honey) with a diameter of 9.87 mm against bacteria *P. aeruginosa.*

The synergistic action of the extracts of (royal jelly and propolis) and (royal jelly and honey) is due to the properties of each product, as the effect is due to the royal jelly because it contains the soluble fatty acid (10-Hydroxytrans decenoic acid), which in turn affects the synthesis of cell membrane proteins and the cell plasmid protein. Royal jelly also contains carbohydrates that affect bacteria through the occurrence of osmosis, cell shrinkage and death [40]. The effect of alcoholic propolis extract comes from preventing the process of cell division, preventing protein synthesis, inhibiting the enzymatic activity of the bacteria, and affecting the permeability of the cytoplasmic membranes and thus the death of the bacteria [42] as for the inhibitory effectiveness of honey due to its possession of properties, including high osmotic pressure, which leads to the dehydration of bacteria by absorbing their water, as well as its containment of benzoic acid, which inhibits bacteria [43] as shown in Table 4.

**Table 4:** Synergistic inhibitory activity of aqueous and alcoholic extracts of honey bee products against the bacteria *P. aeruginosa, S. aureus, P. mirabilis* isolated from otitis media, measured in millimeters (mm).

| Bacterial treatment | Mean | Std |
|---|---|---|
| *P. aeruginosa* | | |
| Propolis + wax | g0.00 | 0.00 |
| Propolis + honey | g0.00 | 0.00 |
| Propolis + royal jelly | g0.00 | 0.00 |
| Wax + honey | e9.87 | 0.34 |
| Wax + royal jelly | g0.00 | 0.00 |
| Honey + royal jelly | d10.49 | 0.47 |
| *S. aureus* | | |
| Propolis + wax | g0.00 | 0.00 |
| Propolis + honey | d10.44 | 0.43 |
| Propolis + royal jelly | d10.56 | 0.39 |
| Wax + honey | g0.00 | 0.00 |
| Wax + royal jelly | g0.00 | 0.00 |
| Honey + royal jelly | c14.34 | 0.77 |
| *P. mirabilis* | | |
| Propolis + wax | b20.44 | 0.77 |
| Propolis + honey | b20.87 | 0.82 |
| Propolis + royal jelly | a21.57 | 0.81 |
| Wax + honey | d10.23 | 0.32 |
| Wax + royal jelly | f8.17 | 0.51 |
| Honey + royal jelly | a21.76 | 0.83 |

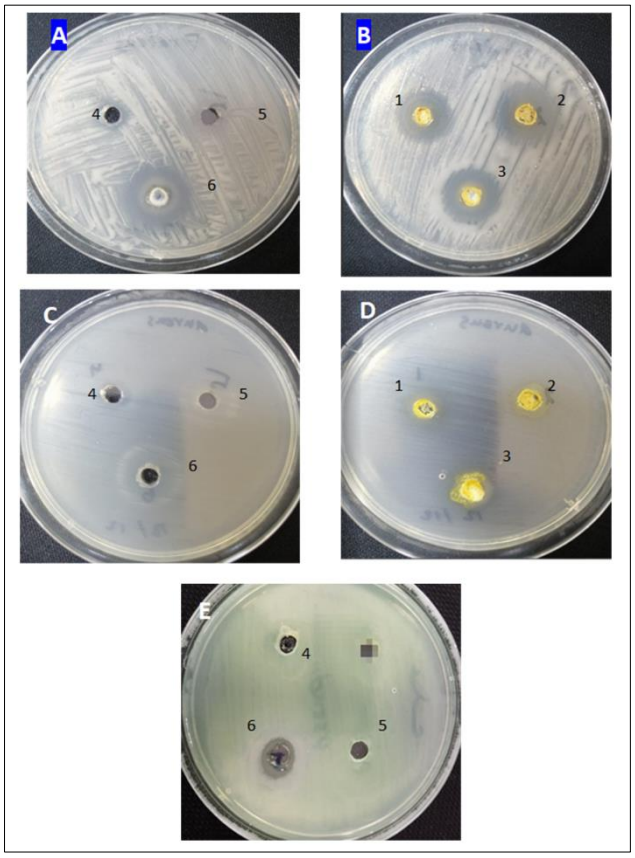


**Fig. 4** The inhibitory effect of synergistic extracts of honey bee products against the bacteria under study.

*A4 Effect of (wax + honey) extracts against bacteria P. mirabilis*
*A5 Effect of (wax + royal jelly) extracts against bacteria. P. mirabilis*
*A6 Effect of extracts (honey + royal jelly) against bacteria P. mirabilis*
*B1 Effect of (propolis + wax) extracts against bacteria P. mirabilis*
*B2 Effect of (propolis + honey) extracts against bacteria P. mirabilis*
*B3 Effect of extracts (propolis + royal jelly) against bacteria P. mirabilis*
*C2 Effect of (propolis + honey) extracts against bacteria S. aureus*
*C6 Effect of (honey + royal jelly) extracts against bacteria S. aureus*
*D3 Effect of (propolis + royal jelly) extracts against bacteria S. aureus*
*E4 Effect of (wax + honey) extracts against bacteria P. aeruginosa*
*E6 Effect of (honey + royal jelly) extracts against bacteria P. aeruginosa*

**The inhibitory results of the minimum values of honey bee product extracts on the growth of bacteria are being studied:**

The MIC results of the study showed to find the limit value for inhibition by extracts of honey bee products at concentrations (5%-10%-15%-20%-25%-30%). *P. aeruginosa* bacteria have been shown to be resistant to all honey bee products and to all concentrations. The high resistance is attributed to its resistance to weak disinfectants as well as to many commonly used antibiotics. These characteristics are considered important factors for its environmental success, which also helps explain its ubiquitous spread of the organism and its emergence as a pathogen [45]. where the growth of *P. aeruginosa* bacteria appeared on the culture medium. The results of the current study on *S. aureus* bacteria also showed, the minimum level of inhibition was at a concentration of 30% for the alcoholic propolis extract, while it showed resistance to the rest of the honey bee products and to all concentrations used in the current

study. The results of the study on the bacteria *P. mirabilis* also showed that the minimum inhibition value was at a concentration of 30% for the aqueous extract of royal jelly, while it showed resistance to the rest of the honey bee product extracts and to all concentrations under study. The resistance of the Gram-negative bacteria *P. aeruginosa* and *P. mirabilis* is attributed to the production of the beta-lactamase enzyme, which depends on plasmids, and it also has the advantage of retaining this enzyme within the plasma membrane [46].

## Conclusions

1. Royal jelly ranked first among other honey bee products in terms of its inhibitory effect on bacteria isolated from otitis media.
2. The alcoholic extract of propolis showed high effectiveness at a concentration of 100 mg/ml, and this effect decreased at higher concentrations, while the aqueous extract of propolis was little effective against bacterial isolates.
3. Concentrated raw honey gave a high effectiveness, and this effectiveness decreased as the concentration decreased.
4. The aqueous extract of the wax did not show any inhibitory effect against bacterial isolates from otitis media.
5. The synergistic extracts (honey and royal jelly) and (propolis and royal jelly) had the highest effect on bacteria, followed by the synergistic extract (propolis and honey) that had the least effect, while the bacteria, Psedomonas aeruginosa Staph. aureus was highly resistant to the synergistic extracts (propolis and wax) and (honey and wax).
6. Regarding finding the minimum value, it was found that Psedomonas aeruginosa bacteria were highly resistant to all the applied concentrations.

## References


1. Naknaen, A., Samernate, T., Wannasrichan, W., Surachat, K., Nonejuie, P., & Chaikeeratisak, V. (2023). Combination of genetically diverse *Pseudomonas* phages enhances the cocktail efficiency against bacteria. *Scientific Reports, 13*(8921), 13. doi:10.1038/s41598-023-36034-2
2. Wahyuni, T. D., Hatta, M., Bukhari, A., Santoso, A., & Massi, M. N. (2021). Increasing natural resistance associated macrophage protein 1 serum level after Miana treatment in BALB/c induced klebsiella pneumoniae experimental research. *Annals of Medicine and Surgery, 65*, 102262. doi:10.1016/j.amsu.2021.102262
3. Tacão, M., Moura, A., Correia, A., & Henriques, I. (2014). Co-resistance to different classes of antibiotics among ESBL-producers from aquatic systems. *Water Research, 48*, 100-107. doi:10.1016/j.watres.2013.09.021
4. Chen, Z., Min, H., Hu, D., Wang, H., Zhao, Y., Cui, Y., . . . Luo, K. (2020). Performance of a novel multiple draft tubes airlift loop membrane bioreactor to treat ampicillin pharmaceutical wastewater under different temperatures. *Chemical Engineering Journal, 380*, 122521. doi:10.1016/j.cej.2019.122521
5. Tenover, F. C. (2006). Mechanisms of antimicrobial resistance in bacteria. *The American journal of medicine, 119*(6), S3-S10. doi:10.1016/j.amjmed.2006.03.011
6. Tian, Z., Feng, Q., Sun, H., Liao, Y., Du, L., Yang, R., . . . Xia, Q. (2018). Isolation and purification of active antimicrobial peptides from *Hermetia illucens* L., and its effects on CNE2 cells. *bioRxiv*, 353367. doi:10.1101/353367

7. Breijyeh, Z., Jubeh, B., & Karaman, R. (2020). Resistance of gram-negative bacteria to current antibacterial agents and approaches to resolve it. *Molecules, 25*(6), 1340. doi:10.3390/molecules25061340
8. Gorlenko, C. L., Kiselev, H. Y., Budanova, E. V., Zamyatnin Jr, A. A., & Ikryannikova, L. N. (2020). Plant secondary metabolites in the battle of drugs and drug-resistant bacteria: new heroes or worse clones of antibiotics? *Antibiotics, 9*(4), 170. doi:10.3390/antibiotics9040170
9. Zainol, M. I., Mohd Yusoff, K., & Mohd Yusof, M. Y. (2013). Antibacterial activity of selected Malaysian honey. *BMC Complementary and Alternative Medicine, 13*(129), 1-10. doi:10.1186/1472-6882-13-129
10. Al-Zubaidi, A. N. (1998). Feeding Honey Bees with Alternatives and Supplements to Honey and Pollen and Their Effect on Brood Production and Honey and Pollen Collection. Ph.D. Thesis. University of Baghdad.
11. Šuran, J., Cepanec, I., Mašek, T., Radić, B., Radić, S., Tlak Gajger, I., & Vlainić, J. (2021). Propolis extract and its bioactive compounds—From traditional to modern extraction technologies. *Molecules, 26*(10), 2930. doi:10.3390/molecules26102930
12. Martinotti, S., & Ranzato, E. (2018). Honey, wound repair and regenerative medicine. *Journal of Functional Biomaterials, 9*(2), 34. doi:10.3390/jfb9020034
13. Ahmed, S., Sulaiman, S. A., Baig, A. A., Ibrahim, M., Liaqat, S., Fatima, S., . . . Othman, N. H. (2018). Honey as a potential natural antioxidant medicine: an insight into its molecular mechanisms of action. *Oxidative Medicine and Cellular Longevity, 2018*, 19. doi:10.1155/2018/8367846
14. Kurek-Górecka, A., Rzepecka-Stojko, A., Górecki, M., Stojko, J., Sosada, M., & Świerczek-Zięba, G. (2014). Structure and antioxidant activity of polyphenols derived from propolis. *Molecules, 19*(1), 78-101. doi:10.3390/molecules19010078
15. Biluca, F. C., da Silva, B., Caon, T., Mohr, E. T. B., Vieira, G. N., Gonzaga, L. V., . . . Dalmarco, E. M. (2020). Investigation of phenolic compounds, antioxidant and anti-inflammatory activities in stingless bee honey (Meliponinae). *Food Research International, 129*, 108756. doi:10.1016/j.foodres.2019.108756
16. Forbes-Hernández, T. Y., Giampieri, F., Gasparrini, M., Mazzoni, L., Quiles, J. L., Alvarez-Suarez, J. M., & Battino, M. (2014). The effects of bioactive compounds from plant foods on mitochondrial function: A focus on apoptotic mechanisms. *Food and Chemical Toxicology, 68*, 154-182. doi:10.1016/j.fct.2014.03.017
17. Alu'datt, M. H., Rababah, T., Sakandar, H. A., Imran, M., Mustafa, N., Alhamad, M. N., . . . Al-Tawaha, A. R. (2018). Fermented food-derived bioactive compounds with anticarcinogenic properties: Fermented royal jelly as a novel source for compounds with health benefits. In *Anticancer plants: properties and application* (pp. 141-165). Springer, Singapore.
18. Schilder, A. G., Chonmaitree, T., Cripps, A. W., Rosenfeld, R. M., Casselbrant, M. L., Haggard, M. P., & Venekamp, R. P. (2016). Otitis media. *Nature Reviews Disease Primers*, *2*(16063), 1-18. doi:10.1038/nrdp.2016.63
19. DeAntonio, R., Yarzabal, J.-P., Cruz, J. P., Schmidt, J. E., & Kleijnen, J. (2016). Epidemiology of otitis media in children from developing countries: A systematic review. *International Journal of Pediatric Otorhinolaryngology, 85*, 65-74. doi:10.1016/j.ijporl.2016.03.032
20. Rijk, M. H., Hullegie, S., Schilder, A. G., Kortekaas, M. F., Damoiseaux, R. A., Verheij, T. J., & Venekamp, R. P. (2021). Incidence and management of acute otitis media in adults: a

primary care-based cohort study. *Family Practice, 38*(4), 448-453. doi:10.1093/fampra/cmaa150
21. Swain, S. K., Behera, I. C., & Sahu, M. C. (2019). Role of Betadine irrigation in chronic suppurative otitis media: Our experiences in a tertiary care teaching hospital of East India. *International Journal of Health & Allied Sciences, 8*(1), 29-32. doi:10.4103/ijhas.IJHAS_13_18
22. Shaheen, S., Ramzan, S., Khan, F., & Ahmad, M. (2019). Adulteration in Herbal Drugs: A Burning Issue, Springer Cham, 1st ed.
23. Abbas, H. M., Hasan, M. A., & Ali, S. D. (2019). Effect of bee venom on MRSA isolated from patient's wounds at Tikrit teaching hospital. *Indian Journal of Public Health Research & Development, 10*(10), 956. doi:10.5958/0976-5506.2019.02945.0
24. Al-Daoudi, H. F. A., Omar, A. K. F., & Al Bayati, A. J. A. R. (2018). A Comparative Study Between the Effect of Some Plant Extracts and Some Antibiotics on Staphylococcus Species Isolated from The Nostril Area Among Male and Female Students at Tikrit University. MSc Thesis. Tikrit University.
25. Al-Obaidi, O. H. S., & Al-Hiti, W. F. H. (2014). Aqueous and Alcoholic Extracts of Some Effective Compounds from Beeswax and Studying their Effectiveness against Bacteria and Cancerous Tumors. Ph.D. Thesis. Anbar University.
26. Huo, H. Z., Wang, B., Liang, Y. K., Bao, Y. Y., & Gu, Y. (2011). Hepatoprotective and antioxidant effects of licorice extract against CCl4-induced oxidative damage in rats. *International Journal of Molecular Sciences, 12*(10), 6529-6543. doi:10.3390/ijms12106529
27. Mahdi, F. M. (2020). Evaluation of the Molecular Effect of Alcoholic Extract of Pomegranate and Fenugreek Peels and Some Antibiotics on Escherichia Coli Bacteria Isolated from People with Urinary Tract Infections. MSc Thesis. Tikrit University.
28. Ivančajić, S., Mileusnić, I., & Cenić-Milošević, D. (2010). In vitro antibacterial activity of propolis extracts on 12 different bacteria in conditions of 3 various pH values. *Archives of Biological Sciences, 62*(4), 915-934. doi:10.2298/ABS1004915I
29. Shinkafi, S., & Dauda, H. (2013). Antibacterial activity of Allium cepa (onion) on some pathogenic bacteria associated with ocular infections. *Scholars Journal of Applied Medical Sciences 1*(3), 147-151.
30. Al-Ghazali, L. H. T. (2005). Study of the Inhibitory Action of Some Plant Extracts against Some Types of Bacteria and Dermatophytes. MSc Thesis. Karbala University.
31. Weinstein, M. P., & Lewis, J. S. (2020). The clinical and laboratory standards institute subcommittee on antimicrobial susceptibility testing: background, organization, functions, and processes. *Journal of Clinical Microbiology, 58*(10), 1128. doi:10.1128/jcm.01864-19
32. Al-Rawi, K. M. (2000). Introduction to Statistics. 2nd ed.
33. Hussain, M. B., Hannan, A., Akhtar, N., Fayyaz, G. Q., Imran, M., Saleem, S., & Qureshi, I. A. (2015). Evaluation of the antibacterial activity of selected Pakistani honeys against multi-drug resistant *Salmonella typhi*. *BMC Complementary and Alternative Medicine, 15*(32), 1-9. doi:10.1186/s12906-015-0549-z
34. Al-Jubouri, W. A. K. (2019). Study of the Effectiveness of Honey Bee Products (Apis Mellifera) (Hymenoptera Apidae) in Salah Al-Din Governorate on Some Microorganisms. MSc Thesis. Tikrit University.

35. Ugur, A., & Arslan, T. (2004). An in vitro study on antimicrobial activity of propolis from Mugla province of Turkey. *Journal of Medicinal Food, 7*(1), 90-94. doi:10.1089/109662004322984761
36. Abu Shaala, F. A. B., Al-Asawi, M. Q. M., & Abu Shuwaiqer, P. (2019). Studying the effect of Libyan propolis extracts on some bacterial species responsible for human brood care and contaminating food. *Journal of Basic and Life Sciences*.
37. Fontana, R., Mendes, M. A., De Souza, B. M., Konno, K., César, L. l. M. M., Malaspina, O., & Palma, M. S. (2004). Jelleines: a family of antimicrobial peptides from the Royal Jelly of honeybees (Apis mellifera). *Peptides, 25*(6), 919-928. doi:10.1016/j.peptides.2004.03.016
38. Muhammad, M. D. (2022). Evaluation of the Effectiveness of Apis Mellifera Honey Bee Products against Methicillin-Resistant Staphylococcus Aureus (MRSA) Bacteria Isolated from Burn Injuries. PhD Thesis. Tikrit University.
39. Allawi, M. Y. A. (2008). Inhibitory Effect of Local Propolis Extracts on Some Fungi Isolated from Different Food Sources. MSc Thesis. University of Mosul.
40. Nugent, R., Back, E., & Beith, A. (2010). The race against drug resistance. chrome-extension://efaidnbmnnnibpcajpcglclefindmkaj/https://www.cgdev.org/sites/default/files/1424207_file_CGD_DRWG_FINAL.pdf
41. Loundon, N., Roger, G., Thien, H. V., Bégué, P., & Garabédian, E. N. (1999). Evolution of the bacteriologic features of persistent acute otitis media compared with acute otitis media: a 15-year study. *Archives of Otolaryngology–Head & Neck Surgery, 125*(10), 1134-1140. doi:10.1001/archotol.125.10.1134
42. Siqueira, A. B. S., Rodriguez, L. R. N. D. A., Santos, R. K. B., Marinho, R. R. B., Abreu, S., Peixoto, R. F., & Gurgel, B. C. d. V. (2015). Antifungal activity of propolis against *Candidaspecies* isolated from cases of chronic periodontitis. *Brazilian Oral Research, 29*, 1-6. doi:10.1590/1807-3107BOR-2015.vol29.0083
43. Dinkov, D., Stratev, D., Balkanska, R., & Sergelidis, D. (2016). Antibacterial activity of royal jelly and rape honey against methicillin-resistant Staphylococcus aureus strains. *Journal of Food and Health Science, 2*, 67-73. doi:10.3153/JFHS16007
44. Mandal, M. D., & Mandal, S. (2011). Honey: its medicinal property and antibacterial activity. *Asian Pacific Journal of Tropical Biomedicine, 1*(2), 154-160. doi:https://doi.org/10.1016/S2221-1691(11)60016-6
45. Wu, W., Jin, Y., Bai, F., & Jin, S. (2015). Pseudomonas aeruginosa. In *Molecular medical microbiology* (pp. 753-767). Elsevier.
46. Cesur, M. F., Siraj, B., Uddin, R., Durmuş, S., & Çakır, T. (2020). Network-based metabolism-centered screening of potential drug targets in Klebsiella pneumoniae at genome scale. *Frontiers in Cellular and Infection Microbiology, 9*, 447. doi:10.3389/fcimb.2019.00447

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# تقييم التأثير التثبيطي والتآزري للمنتجات المائية والكحولية لعسل النحل ومنتجاته على بعض الأنواع الالبكتيريا المعزولة من التهاب الأذن الوسطى

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**معلومات البحث:**
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**الكلمات المفتاحية:**
*المستخلصات الكحولية، المستخلصات المائية، لبكتيريا، منتجات عسل النحل و التهاب الاذن الوسطى*

**معلومات المؤلف**
الايميل
الموبايل:

**الخلاصة:**
أستخدم في الدراسة الحالية منتجات نحل العسل كمضادات حيوية في علاج الكثير من الأمراض التي تصيب الأنسان من ضمنها علاج ضد البكتريا التي تسبب التهاب الأذن الوسطى، حيث شملت الدراسة الحالية أستخدام مستخلصات المائية والكحولية لمنتجات نحل العسل بالتراكيز (100،75،50،25 مليغرام/مليلتر) في علاج البكتريا المعزولة من التهاب الأذن الوسطى، أظهرت نتائج الدراسة كفائة فعالية منتجات نحل العسل ضد البكتريا الموجبة والسالبة لصبغة كَرام، شملت الدراسة الحالية (124) مسحة من المرضى المصابين بالتهاب الأذن الوسطى المراجعين لمستشفى الحويجة العام بواسطة مسحات قطنية مع وسط ناقل ثم زرعت على الأوساط (آگار الدم) و(آگار الماكونكي)، تم الحصول على (96) عينة موجبة النمو بنسبة (77.42%) و(28) عينة سالبة النموبنسبة (22.58%)، شملت الدراسة ثلاثة أنواع بكتيرية *Proteus mirabilis*، *Staphylococcus aureus*، *Psedomonas aeruginosa*، أظهرت النتائج أن بكتريا *P. mirabilis* من أكثر أنواع البكتريا حساسية للمستخلص المائي للغذاء الملكي بمتوسط قطر تثبيطي (23.78 ملم) عند التركيز 100ملغم/مل، يليه المستخلص الكحولي للبروبوليس بمتوسط قطر منطقة تثبيط (19.46 ملم) عند التركيز 100 ملغم /مل ضد *S. aureus*. أما بكتريا *P. aeruginosa* فقد أظهر المستخلص المائي للعسل تأثير تثبيطي (17.84 ملم) عند التركيز 100 ملغم /مل وأدنى متوسط قطر تثبيطي للعكبر الكحولي (8.84 ملم) عند التركيز 75 ملغم/مل ضد *P. aeruginosa*. بينما المستخلص المائي للشمع لم يظهر أي فعل تثبيطي ولجميع التراكيز المعمولة ضد أنواع البكتريا قيد الدراسة.