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Research Article

Inhibiting the growth of two types of bacteria using honeybee extract (*Apis mellifera* or: Hymenoptera) of Samarra city-Iraq

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Abstract

An inhibitory action test was conducted in which honeybee body lotion was selected as an inhibitor with extracts of the following insects, social insects. Test experiments on Gram-negative bacteria, *Escherichia coli* and *Pseudomonas auroginosa*. The solvents are polar ethanol and hexane. The tests were carried out by both methods of discs and drilling. cationic antibiotics (AMP) Ampicillin and (STRPTO) streptomycin. As for the results that showed less sensitivity to the two types of *Ps. Aruginosa*. In all extracts, however, the inhibition values appeared to double compared to antibiotics in the event of their positive effect, which were occupied by antibiotics CN and TE by an inhibitor of 18.6 and 14.3 mm against *E. coli*. The rest of the treatments for the three antibiotics were negative against the two types of bacteria above. As for insecticidal lotion at concentrations. 3.5, 1.0, 2.5 and 5.0 mg/ml, inhibited the growth of all types of bacteria with a range of 5.0-3.0 mm.

Keywords Cationic antibiotics, Gram-negative bacteria, Honeybee, Inhibitors

Introduction

Insects and the materials extracted from them are used as medical sources by people and cultures. In addition to their medicinal uses, they have a magical role in treating many diseases. Scientists have proven the presence of medicinal properties in insects' bodies, including anti-bacterial, allergic, immune, and diuretic (Meyer-Rochow, 2017). The use of insect bodies and their products for medicine has been common since a very ancient history as a therapeutic source in the healing of many ailments suffered by man. The knowledge of man about poisonous insects and nerve stimulants goes back to the beginning of his knowledge of food plants that he was guided to by mistake and right without real knowledge of the nature of their components, and this prompted researchers to return to nature again and pay attention to medicinal insects, as each plant or herb can be an important natural source for

the compounds that are used in the preparation of various medicinal drugs by containing many active substances that interact with each other in a balanced manner and to which the medicinal effect is attributed. Physiology for the treatment of pathological conditions. Bees are considered one of the best medicinal insects since a long time ago and have been used in treating mouth ulcers, head pain, burns, coughs, chest injuries, colds, and insect stings. The resistance of pathogens (bacteria, fungi, viruses, and protozoa) to drugs has necessitated the need to research and find effective antibiotics for these microorganisms (Aslam et al., 2018).

Bacteria adapt by acquiring resistance against antibiotics and sterilizers, so it is necessary to use new antibiotics and strategies with these techniques (Lopez Romo and Quirós, 2019). as traditional antibiotics are no longer able to meet the current therapeutic needs, but research and development of new antibiotics to suit the rise Infection rates are due to

microorganisms, which prompts scientists to pay more and more attention to research and development of new anti-bacterial drugs (Wicha et al., 2021). Insects play an effective role in transmitting pathogenic bacteria and fungi in home and hospital environments without being affected by them (Jordan & Tomberlin, 2017). Even though insects account for about 90% of the animal kingdom, they have different compounds that have many benefits for health (Kumar & Kumar, 2019). Insects - at all stages of their lives - are rich in protein, fat, and many other nutrients (Roos, 2018). These insects may develop several ways to protect themselves against pathogenic microorganisms, which made scientists and researchers find new sources of antimicrobials through bio-mimicry of insects in protecting themselves from these microorganisms. It helps them survive insects (Kachhawa, D. 2017).

There is a long history of using insect extracts in folk medicine, which has motivated scientists to develop effective (viable) medical treatments for various diseases, such as viral infections and problems associated with the emergence of antibiotic resistance (Roope et al., 2019). The fact that insects are resistant to microorganisms has been known for a long time, and a well-studied part of the mechanism of their resistance is their production of antimicrobial peptides (Sierra et al., 2017). Antibiotics extracted from living organisms are called biological antibiotics. Due to the high resistance of pathogenic bacteria to conventional antibiotics, scientists and researchers are currently seeking to find new natural sources of antibiotics. Several studies have been conducted to test the ability of insect extracts against pathogenic bacteria, especially anti-microbial peptides. Diagnosing that insects have had effective immune defenses in the past years through their production of compounds against various pathogenic microorganisms. The beginning of the initial extraction and purification of antimicrobial protein (AMP) from the hemolymph of the giant silk moth *Hyalophora cecropia* by the Boman research group, coincided with the observation of the effect of this antibacterial protein since 1980 (Hultmark et al., 1980).

Material and Methods

Biochemical Test

Catalase Test

This test is carried out by transferring part of the young colonies on Nutrient agar medium to a clean glass slide using a sterile loop and adding a drop of hydrogen peroxide H_2O_2 to the bacterial culture. The appearance of gas bubbles within a few seconds of addition indicates a positive test (Prescott, 2002).

Oxidase test

This test is carried out by transferring part of the young colony from the nutrient agar by a sterile wooden stick to a filter paper saturated with oxidase reagent (Tetramethyl *p*-phenyl

diamine dihydrochloride at a concentration of 1% 1, the appearance of violet color indicates the positiveness of the test (Jerde et al., 2011).

Indol test

The test is carried out by inoculating tubes containing peptone water with the bacteria to be tested and incubated for 24-48 hours at a temperature of 37 °C, then 0.5 ml of Kovacs reagent is added to the inoculated tubes. Pink or dark red after adding the reagent is an indication of a positive test (Jerde et al., 2011).

Maintenances of bacteria isolates

Bacterial isolates are preserved in two ways: * Short-term preservation: the bacterial isolates are preserved on a nutrient medium (slant) in a refrigerator at a temperature of 4 °C, taking into account that the isolates are renewed periodically every month by reactivating them in the heart and brain infusion medium and re-developing them. On a tilted planting, medium to ensure its survival throughout the study period. Long-term preservation: the diagnosed bacterial isolates are kept in a sterilized heart-brain infusion medium, sterilized by sterilizer, distributed in tubes, at an amount of 5 ml per tube (to preserve their genetic characteristics and ensure that their characteristics do not change), add 1 ml of glycerol 20% to the tubes and shake the tube well to the top. and the bottom and memorized it at -20 °C.

Antibiotics Sensitivity test

Sensitivity to antibiotics is tested on the isolates under study by the disc diffusion method and according to the modified Baure-Kiby method described by the World Health Organization (Gnecchi et al., 2006). A suspension of bacterial isolates is prepared, a single colony of the isolates under study is transferred to tubes containing (Nutrient broth) and the tubes are incubated at a temperature of 35°C ° for 18-24 hours, and we compare the growth in the tubes with the tube containing MacFarland solution, which is equal to Approximately (1.5*10⁸) cells/ml, and using a sterile cotton swab, the bacteria were spread on the Muller-Hinton Agar medium and left for 15 minutes to dry the dishes, the antibiotic tablets are distributed by sterile forceps on the surface of the medium, and the dishes were incubated for 24 At 37 °C, the results are read by measuring the diameter of the inhibition zone using a standard ruler and comparing it with the standard tables (Humphries et al., 2019), in order to know the resistant, sensitive and moderately sensitive bacteria to antibiotics.

Insect materials

Insects are collected from their natural environment, such as samples of bees prepared from apiaries, and for the purpose of drying, it is according to the seasons. In the summer, they are dried in the shade until they are fragile and ready to be crushed. But when the laboratory temperature drops, they are

dried in the oven at a temperature of 35 degrees Celsius, kept in containers Sealed away from moisture.

Preparation of Insect extracts Preparation of extract from insect powder

The first step is to extract the powder of each of the insects under study by crushing 15 g of each sample from each sample, and the crushing is either by an electric bee grinder.

The extraction is done using organic solvents of different polarity to allow the extraction of various active substances against bacterial growth, namely: ethanol alcohol, and hexane, and then the solvent is mixed with powdered insect body paste, left for three days under laboratory conditions with continuous stirring with a magnetic stirrer device. Magnetic stirrer. Large impurities are removed with gauze and then filtered twice with No. 1 filter paper under low-pressure condition. To get rid of the solvents, the filtrate is poured into glass dishes with a diameter of 15 cm and left in front of an electric fan in summer or an air conditioner in winter. The dry extract, whose weight varies according to the type of insect and the solvent used in the extraction, is collected and the name of each extract is put on it and kept at a temperature of 4 °C until the start of experiments.

Preparation of concentrates

For the purpose of preparing a stock solution for any of the extracts, a concentration of 400 mg/ml is prepared by dissolving one gram of the extract in 2.0 ml of Dimethyl sulfoxide (DMSO) or dissolving 400 mg of the extract in 4 ml of DMSO to obtain a concentration of 100 mg/ml. For the purpose of using the desired concentration for experiments and to prepare the appropriate dilution, the equation is used:

$$N_1 \times V_1 = N_2 \times V_2$$

N1 - Concentration of the original solution

N2_ Required concentration

V1_ volume of the original solution

V2_ Desired volume of concentrate preparation

Bacteria and media used

Experiments to test the efficacy of insect extracts against bacteria use two types of pathogenic bacteria, Gram-negative types, *Escherichia coli* and *Pseudomonas auroginosa*. In order to obtain pure culture media from the laboratory of bacteria and microorganisms.

Culture medium

The medium used in cultivating the above bacteria to maintain their presence and to conduct sensitivity

experiments on them is Nutrient Agar and to prepare the culture medium and follow the following steps:

1. Weigh 14.0 g of dry paper prepared previously.
2. Transfer the medium to a conical flask, and add 500 ml of distilled water to it.
3. Shake to obtain a homogeneous mixture, and to obtain a clear solution, place the flask on a magnetic stirrer for this purpose.
4. The culture medium was sterilized by placing the flask in the Autoclave.
5. Lift the beaker from the sterilizer and leave it exposed to the air to reduce its temperature, and be prepared for the next step.
6. Pour the medium by distributing it on Petri dishes 9.0 cm diameter petri dishes and under aseptic conditions in the Hood.
7. It is left in the sterilization position until the medium hardens and its temperature is reduced to get rid of condensation of water vapor on the inner surface of the dish cover.
8. Sterilized dishes should be kept upside down at 4.0°C.

Bacterial sensitivity test to insect extracts

The discs

The test disc is 0.5 cm in diameter and 0.1 mm thick. The discs are prepared. The discs are placed in a sealed glass vial, sterilized by a sterilizer, and kept in the refrigerator until use.

The pits

The sensitivity test is carried out by drilling, by preparing a hole in the culture medium with a 6 mm diameter cork piercing tube.

Bacterial culture

The four types of bacteria are cultured in previously prepared Petri dishes and kept in the refrigerator at a temperature of 4 °C by swab method, and left to grow in the Program control system type incubator (Jlab tech LCL-050EL) for 24 hours, to be prepared for conducting allergy experiments.

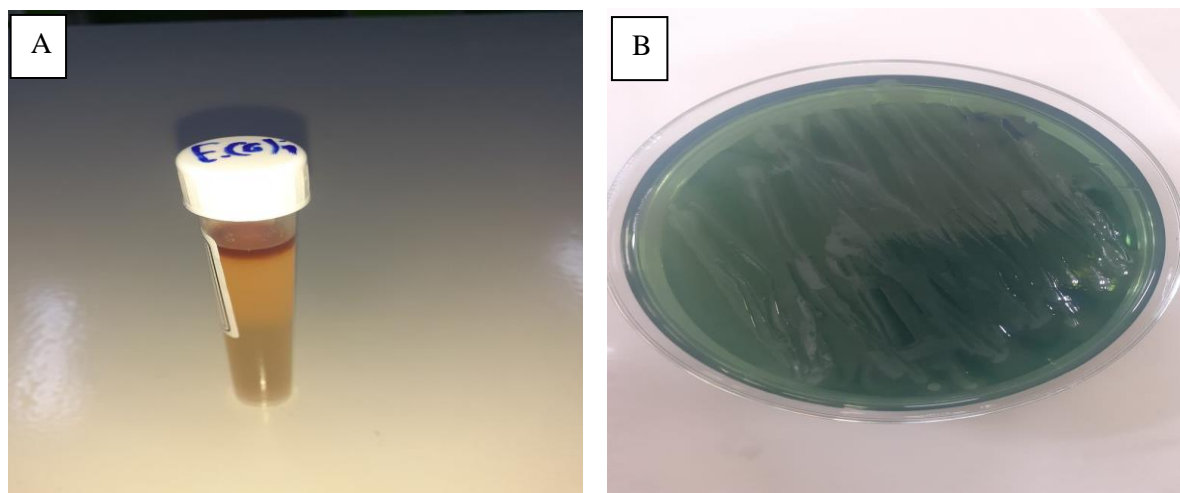


Fig. 1. Biochemical Characteristics of *E.coli* (A) and *Ps. Aeruginosa* (B)

Table 1: Inhibitory action of *Apis mellifera* bee body extract against negative bacteria (*E.coli*. and *Ps. Aeruginosa*) at concentration (5.0 mg/mL). It was prepared with ethanol and hexane solvents, and by tablet test

	T0	T1	T2	T3	T4	T5	T6	T7	T8	T9	T10
		<i>E.coli</i>	<i>PS</i>	<i>E.coli</i>	<i>PS</i>	<i>E.coli</i> Streptomycin	<i>PS</i> Streptomycin	Tetra	Tetra	Amp	Amp
1-	0	3.75 b	1.0 d	2.5 c	5 a	1.0 d	1.0 d	0	0	0	1.0 d

Addition of insect extracts to cultured dishes with bacteria

If the sensitivity test is in tablets, then the tablets are placed in the specified concentration for half an hour, then lifted and left for a little while to dry so that they spread steadily and uniformly on the (bacterial surface) of the dish. If the test is by drilling, it is placed in each hole (100 micro-ml) with a microliter syringe. The identification marks shall be placed on the back (the second side) of the dish in the case of testing with discs, and if the test is carried out by drilling, the marks shall be fixed on its cover.

Result & Discussion

Bacterial growth inhibition test during disc diffusion

The effect of contrasting solvents on the effectiveness of the insect extract (effectiveness in the whole body of the insect) Different polarity organic solvents were used to precipitate the components.

Table (1) shows the use of each of the hexane solvents in treating the body powder of (honey bee) *Apis mellifera*, and testing the effect of the extract at concentration, (5 mg/ml) on the growth of Gram-negative bacteria, *Pseudomonas aeruginosa*, *Escherichia coli*. There was a difference in the effect of both hexane and ethanol (3.5, 5.0 mM) in inhibiting the growth of *Ps. aeruginosa*, *E.coli* .As for the variation in the growth inhibition effectiveness of the bee extract for each of the two solvents, Table (1) shows the difference in the effect of the extract with hexane (3.5, 5.0 ml) in inhibiting the growth of bees. *E. coli*, *Ps. aeruginosa* and 0.5, 1.0, and 11. millimeters in types. Tetracycline, Ampicillin, and Streptomycin.

Conflict of Interest

The author hereby declares no conflict of interest.

Consent for publication

The author declares that the work has consent for publication

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