



Effect of Crude Extracts of Natural Compounds from local Iraqi insects of Worker bees and Ladybirds as Antimicrobial Activity on Pathogens

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Abstract

Scholars encourage to research and explore new resources of antibiotics; insects were openly used throughout history for medical curative, so it is considered as a new source of antibiotic.

It was observed that two insects of ladybirds and honeybee workers are able to inhibit various species of bacterial growth by using agar diffusion test on Gram-negative bacteria like *Pseudomonas*, *Proteus*, and *Escherichia coli* and Gram-positive bacteria like *Staphylococcus aureus*, all extracts inhibited the tested bacterial species except *Proteus* and *Pseudomonas* which have ability to grow in low concentration of honeybee workers extract, while ladybirds extract gave better result than honeybee workers extract.

It is concluded that these extracts have antibacterial activity and can provide a new source of antibiotic.

INTRODUCTION

Recent years, the antibiotic resistance has become a danger problem to human health, and if everyone does not act against this threat, the world is on the verge of a post-antibiotic. This phenomenon is not a regional and affects the poor countries beside the rich countries. It can happen in all countries of the world (WHO). Consequently, scholars' research sparked to explore new resources. Insects have been widely used throughout history for medical treatment, and they are a common part of the component of Traditional Chinese Medicine [1].

Insects are potentially a vast new area of medicinal research, because insect have different compounds offered many benefits for health. To date, insects have been relatively neglected as sources of modern drugs, [2]. Insects represent 80% of all fauna and are the most widespread group within the animal kingdom. Insects and insect-derived products have been widely used in folk healing in many parts of the world since ancient time. Several live organisms are used as treatments: leeches used to cure everything from headaches to hemorrhoids and osteoarthritis and the helminthes *Trichuris suis* and *Necator americanus* for inflammatory bowel disease, lastly, it has been reported that the insects possess antimicrobial activities, which promising preliminarily treatments have been studied experimentally by [3,4]

Unlike conventional antibiotics, insect peptides are known to have successfully eliminated many types of bacterial pathogens from the insect body for millions of years. A few peptides have already entered clinical trials for the treatment of impetigo, diabetic foot ulcers and gastric helicobacter infections [5]. The harlequin ladybird beetle *Harmonia axyridis* is a large coccinellid beetle. The colour of this beetle ranges from yellow-orange to black, and the number of spots between none and twenty two. It is native to eastern Asia [6]. The harlequin ladybird beetle has emerged as a model species in invasion biology because of its strong resistance against pathogens [7,8]

There are many uses for bee products such as the use Propolis, pollen and honey as anti-bacterial [9,10,11] but

there is no research to indicate the use of insect extracted from bee workers as Antibiotic.

This study is trying to describe the use of compounds derived from natural sources with a focus on finding and discovery of new active substances in these natural products as a crude extract and evaluate antimicrobial potential of these extract against microorganisms species that causing diseases.

MATERIAL AND METHODS

Insect collection sites

Insects were collected in sufficient numbers from different areas of Baghdad, Ladybirds between March and April while worker bee during spring season.

Preparation of insects extracts

Insect's materials were washed thoroughly in clean sterile water and dried for 24 hours at 37°C. The whole bodies of the beetle and worker bee were used for extraction of antimicrobial compounds.

Eight grams of respective dry insects material was blended into powder and soaked in 10 ml of acidified methanol (methanol/ water/ acetic acid; 90/9/1: v/v/v) according [12] for 48hrs in sterile glass container and shaken vigorously to allow for proper extraction. The slurry was filtered using a sterile muslin cloth after which the extract obtained, the homogenate was centrifuged at 5000 rpm for 5 min, and the supernatant filtered with Millipore filter and subjected to evaporation by air dried to remove acidified methanol and stored at 4°C until required according to the method of [13], the output of this method is 1 g was dissolved in sterile distilled water. From the stock of 10 mg/ml extract, made one dilution of 5 mg/ml.

Antibacterial assay

The human bacterial pathogens such as *Staphylococcus aureus*, *Escherichia coli*, *Proteus* and *Pseudomonas aeruginosa* were selected for antibacterial screening and their susceptibility patterns to mixed two antibiotics of Gentamycin - Penicillin suspension of 25000 µg/µl to each antibiotic was determined. Bioassay was carried out by Agar well diffusion method. Tenmicro liter of the bacterial

broth culture was transferred to the sterile agar plates and spread the culture uniformly with the help of a sterilized loop.

A well of 6mm diameter was punched off into agar medium with a sterile cork borer and filled with 100µl of the tow extracts concentrations and suspension of antibiotics were dropped into each well by using micropipette in an aseptic condition and labeled, then they were kept in a refrigerator to allow the extracts to diffuse into the agar for 30minutes and further incubated in an incubator at 37⁰C for 24hrs. The antibacterial activity was evaluated by measuring the zone of inhibition. The experiment was done in triplicate and the mean diameter of radius of clear inhibition of zones (mm) was calculated. The work has been conducted in the Microbiology Lab of the Biology Department of Science College/ Mustansiriyah University.

RESULT AND DISCUSSION

Insects have a various compounds whether structure of insects or its products and they are benefit for health and it became potentially a wide new field of medicinal research, up to now. This topic have been neglected as sources of modern drugs, and because of the unexpected resistance to the antibiotic, scientists research on alternatives natural biological material to be used in treating diseases causing by bacteria resistant to antibiotics was the idea of our research is to find a crude extract using the whole bodies of insects and used easy way to extracted and we have chosen honey worker bee for easy to get it and it is an acceptable insect by people because of it has many benefits unlike the rest of most insects which are disgusted by people in

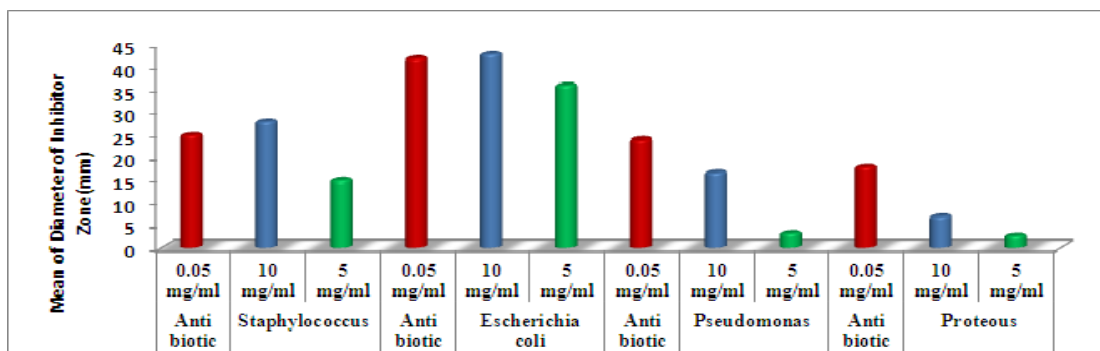
general, and therefore we looked for another insect that would be somewhat acceptable, so we chose the Ladybird as well as containing pigments that may have antibacterial activity, so the results of the use of an insect extract Ladybird against pathogenic bacteria better than the use of insects honey bee workers extract as we note below.

Ladybird species and honey worker bees were collected from sources available in Baghdad such as gardens, apiaries, and farms.

Antimicrobial activity of two insects extracts from honey worker bee and Ladybird were studied against *S. aureus*, *E. coli*, *Pseudomonas* and *Proteus*. Results are shown in Table 1 and clarified in Figure 1 and 2 indicated that extracts had an obvious inhibitor zone against four species of tested bacteria.

ANOVA test showed that there was significant difference in the sensitivity of all bacteria towards work bee extracts in comparison to mixed antibiotics solution, while Ladybird extract showed that there was non-significant difference in the sensitivity of all bacteria except *Proteus* especially in 10 mg/ml concentration towards work bee extracts in comparative with mixed antibiotics solution Table (1)

The diameter of the inhibition zone for all treated bacteria was directly proportional to the concentration as we see in Table (1). the 10mg/ml is more effective than lower concentration. The mean of inhibition zones presented in Table (1) showed that the Ladybird extract was the most effective extract on all bacteria species and the worker bees the least effective.



Figure(1) :Antibacterial activity ofLadybirdsextract against different species of bacteria

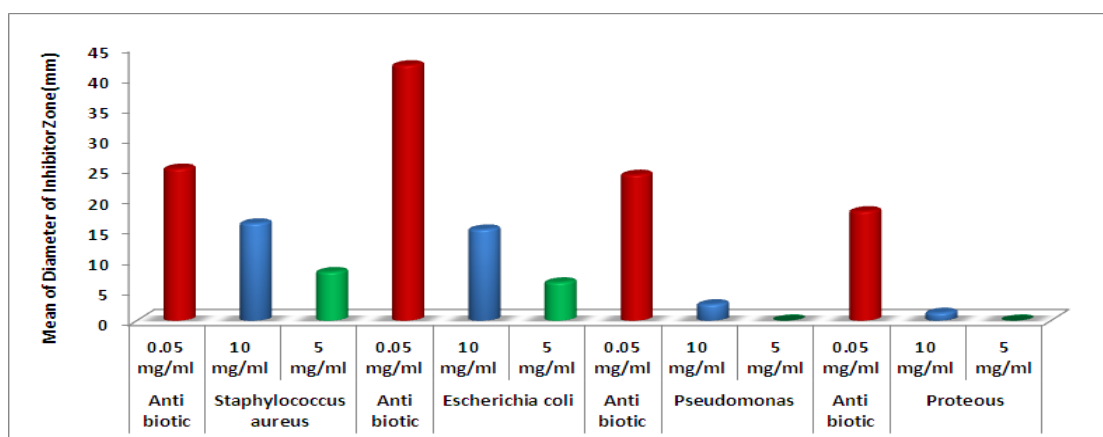


Figure (2): Antibacterial activity ofhoney bees workersextract against different species of bacteria

Table (1): The effect of insects extracts on inhibition zone (mean±SD) in comparative with mixed antibiotic and two concentrations

	Statistical relations		<i>Staphylococcus</i>	<i>Escherichia coli</i>	<i>Pseudomonas</i>	<i>Proteus</i>
Antibiotics		0.05mg/ml	25±1.7	42±13.1	24±12	18±3
Worker bee	Between 10 mg/ml con & antibiotics	10mg/ml	16±3.5	15±5.2	2.7±2.3	1.3±2.3
		P value	0.016*	0.029*	0.039*	0.002**
	Between 5 mg/ml con & antibiotics	5 mg/ml	8±3.5	6.3±2.5	0.000	0.000
		P value	0.002**	0.01**	0.026*	0.000**
	Between two concentrations	P value	0.047*	0.06 ^{NS}	0.116 ^{NS}	0.37 ^{NS}
Ladybird	Between 10 mg/ml con & antibiotics	10mg/ml	28±9.2	43±9.6	15.7±8.02	7±1.73
		P value	0.61 ^{NS}	0.92 ^{NS}	0.37 ^{NS}	0.005**
	Between 5 mg/ml con & antibiotics	5 mg/ml	15±3	36±6	3.3±3.1	3.33±3.1
		P value	0.007**	0.5 ^{NS}	0.045*	0.004**
	Between two concentrations	P value	0.08 ^{NS}	0.35 ^{NS}	0.07 ^{NS}	0.06 ^{NS}
Between worker bee & Ladybird (10mg/ml)		P value	0.1 ^{NS}	0.01**	0.05*	0.02*

Escherichia coli was more affected or sensitivity to the treatment by Ladybird extract followed by *Staphylococcus*, *Pseudomonas*, and *Proteus* respectively.

Insects are considered as food sources because of their richness in essential substances such as proteins, carbohydrates, and fats [14].

Natural antibiotics gained from microorganisms are called biological antibiotics origin, now the scientific researchers are looking to find new natural sources of antibiotic due to a highly resistant pathogenic bacteria to classic antibiotics, although insects were vastly applied during history to treat different diseases on nearly everywhere but a few researches on medical entomological have been reviewed since the advent of antibiotics but now there are many researchers try to use different insects and their parts [15,13,16,17], there are number of studies that have tested ability of the insect extracts against pathogenic bacteria, especially antimicrobial peptides' (AMPs) extracted from various insects maggots [16] dung beetles [13] Red Palm Weevil [17] pupae of the giant silk moths [18] etc.

During the research phase, we tried to obtain other studies that used honey bee workers and Ladybird, but we did not get only one research dealing with honey bee workers [19] and another talking about Ladybird [7] which is pointed that Ladybird had used as antibacterial as *Mycobacterium tuberculosis* and antimalarial that caused by (*P. falciparum*). So Röhrich and his colleagues recommended for further studies that provide a base for the development of new anti-malarial drugs.

[19] indicate that antibacterial peptide families are promising material due to the bacteria until now do not develop any resistance and it is mechanism of action remains unknown but it has been reported that disturb cell membrane.

CONCLUSION

We concluded that insects' extracts can use an antibacterial material, which means that insects as new sources of antibiotics after different species of bacteria showed strong resistance to classic antibiotics, so it needs further studies to understand the mechanism and functions of these

extracts including the effect of each compound of crude extract.

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