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The Antibacterial Activity of *Crocothemis servilia* Extract Against *Staphylococcus aureus and Escherichia coli*

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ABSTRACT

An effective test of the insect body extract of *Crocothemis servilia* was performed Extraction was done using organic solvents such as hexane and ethanol alcohol to inhibit the growth of *Staphylococcus aureus* and *Escherichia coli*. While the antibiotics used in this study are Ciprofloxacin (CIP) and Ceftazidime (CAZ). Use the pits method for *Crocothemis servilia* extract and the disks method for antibiotics The results showed a higher effect of the extract with hexane solvent against *S. aureus* Compared to its inhibition of *E. coli* growth. As for the effectiveness of the extract with an ethanol alcohol solvent Higher inhibitory activity against *E. coli* than against *S. aureus*. However, the extract has good inhibitory efficacy compared to the standard comparator antibiotics The hexane extract against *S. aureus* has a diameter of inhibition (16.67) mm. For the ethanol alcohol extract, the diameter of inhibition is (19.67) mm against *E. coli*.

INTRODUCTION

Insects have a wide use in folk medicine (traditional) in different parts of the world. In Chinese medicine, insects have been used to heal many diseases for more than 3,000 years. Insects have been used in folk medicine because they possess an innate immune response that is highly effective and able to survive in highly polluted environments despite the absence of an adaptive immune system (Buonocore *et al*., 2021). Insect resistance to microorganisms has been extensively studied, with the production of anti-pathogenic peptides identified as the main mechanism of resistance (Hernando *et al*., 2020).

It has considered some of the most important recent advances in identifying and bioengineering natural products from insects with use or potential use in medicine, as well as in using insects as models to study basic mammalian processes such as immune responses to pathogens. Cherniak (2010) and Dossey (2010) present recent reviews detailing the global use of medicinal insects and exploring their potential in drug discovery.

Antimicrobial resistance is an important and critical issue in our societies. The widespread, indiscriminate and irregular use of antibiotics has led to the Development and spread of antibiotic resistance has been a long-standing problem due to lack of discovery (Chen *et al*., 2019).

Over the past few years, new antibiotics have been introduced, but their costly production and associated side effects have exacerbated the risks to public health, because it limits the effectiveness of treatment, so patients remain carriers of infection for a longer period, which increases the risk of transferring antibiotic-resistant pathogens to healthy people (World Health Organization, 2016). Staphylococcal resistance to most antibiotics, especially those of the beta-lactam group, arises from their ability to produce the enzyme beta-lactamase (Chen *et al*., 2020), and this led to the interest of researchers in natural materials because of their great effectiveness and their healing ability in controlling microbial resistance, as it is considered natural treatment and does not cause genetic mutations that lead to the emergence of resistance genes and immune booster at the same time (Gorlenko *et al*., 2020)

MATERIALS AND METHODS

Specimens Collection:

The samples were transferred to the laboratory by means of a swab, and they were planted directly on the culture media of the primary isolation, which are mannitol medium and Maconki agar medium, and the dishes were incubated in the incubator at (37) °C for 24 hours.

Phenotypical Diagnosis:

The growing bacterial colonies were identified on the culture media of the primary culture, primarily on the basis of the culture characteristics in terms of shape, size, color, texture, odor, and fermentation of lactose sugar in the medium of the Maconki agar, then the isolates were subjected to microscopic examination by taking a swab, fixing it with heat, staining it with a gram stain, and then Examination under the oil lens of a light microscope.

Biochemical Tests:

Many biochemical tests were carried out to diagnose the bacteria isolated. The tests included catalase, oxidase test and IMViC (Indole, MR-VP cimmon citrate) tests, urease and coagulase test. (Kasprowicz *et al*., 2018; Brown and Smith, 2014; Tille, 2017; Forbes *et al.*, 2007).

Insect Materials:

Insects were collected from where they are located in their natural habitats, as the samples of the tick were collected by hand-made traps in their places of existence. In the summer, they were dried in the shade until they were ready to be crushed, while in the winter and lower temperatures inside the laboratory, the insects were dried in the oven at a temperature of 35 degrees Celsius, and then kept in closed containers away from moisture.

Preparation of Extracts:

The first step was to extract *Crocothemis servilia* powder by crushing (50 g). Using a mortar due to the high percentage of fat in it, makes it doughy.

The extraction process was carried out by using organic solvents of different polarities to extract various active substances against the growth of bacteria, namely: ethanol and hexane, and then mixing the organic solvent with crushed or paste insect bodies. Leave for three days under laboratory conditions with continuous stirring by a magnetic stirrer device. Large impurities were removed with a piece of gauze cloth and filtered twice with filter paper No. 1 under conditions of low pressure, then the insecticide was poured into glass dishes with diameters (15 cm) and then it was left in front of the electric fan in summer or the air conditioner in winter to get rid of the solvents. The dry extract was collected, whose weight varies according to the type and the solvent used in the extraction. The name of each extract was placed on it and kept at a temperature of 4 degrees Celsius until the start of the experiments.

Antibiotics Sensitivity Test:

The disc diffusion method was used to evaluate antibiotics susceptibility test as mentioned by (Bauer et al., 1996) against *S. aureus* and *E. coli* as specified by the Clinical and Laboratory Standards Institute (CLSI, 2019). For each antimicrobial disc, the

diameter of the inhibition zone (mm) was determined.

Antibacterial Activity of *Crocothemis servilia* Extract:

The antibacterial activities of insect extract were evaluated against *S.aureus* and *E.coli* using the Well Diffusion method. 0.2 ml of fresh cultures of each organism was inoculated into 5 ml of sterile nutrient broth (Himedia/ India) and incubated for 3–5 h to standardize the culture to McFarland standard (1.5×10^8 CFU/ml). 0.1 ml of each culture of microorganism was spread on Mueller Hinton Agar (Himedia/ India). Wells were made using gel puncture (6 mm) according to (Egorove, 1985), and then 0.1 mL of a concentration of (100) mg/ml of insect extract was added to the well. The petri plates were incubated at 37 ºC for 24 hours in the incubator during which activity was evidenced by the presence of a zone of inhibition (mm) surrounding the well.

RESULTS AND DISCUSSION

Table (1) shows the effect of *C. servilia* extracts on *S. aureus* and *E. coli*. The hexane extract showed a significant effect on *S. aureus* with an inhibition zone (of 16.67) mm while the ethanol extract had a value of (11.67) mm. The results shown in table (1) that the ethanol extract was more effective than the hexane extract, as the inhibition value of the ethanol extract towards *E. coli* was (19.67) mm, *S. aureus* was (14.67) mm as shown in Figure 1. The antibacterial effect of *C. servilia* extracts was more effective than Ceftazidime antibiotics, While the isolates showed resistance to the extract compared to Ciprofloxacin. The results of this study were not consistent with the results of (Dhanalakshmi and Reniprabha, 2019) who indicated that *Diplacodes trivialis* inhibited *E.coli* (16.6)mm, CIP had a diameter of inhibition (13) mm and CAZ (17) mm. The antimicrobial peptides in insects may be a good alternative to antibiotic-resistant bacteria. Since these insects are normally present in all habitats, they are exposed to many invasive and harmful microbes in nature. In the past century, studies concluded that the insect immune system is able to cope with invading microbes (Boman *et al*., 1991 and Hoffman *et al*., 1993). The differentiation in the inhibitory activity of the extract and the preparation with more than one solvent is due to the difference in the degree of polarity and its specialization in dissolving lipid and glycosidic compounds, proteins, polypeptides and peptides.

Table 1. Antibacterial activity of *Crocothemis servilia* body extract against pathogenic bacteria**.**

Solution / Bacteria	Hexane	Ethanol	CIP	CAZ
S. aureus	16.67 a	11.67 b	20	9
E.coli	14.67 b	19.67 a	30	17

* Horizontally similar lowercase letters mean that there are no significant differences between them

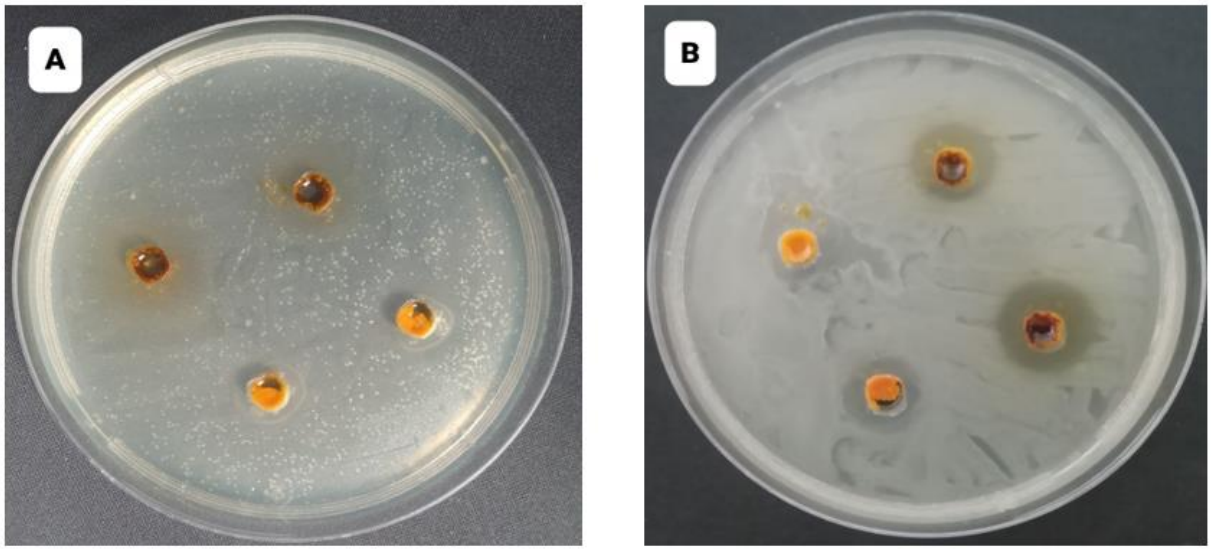

Fig .1 inhibition of extracts *Crocothemis servilia* to growth *S. aureus (*A*)* and *E.coli* (B)

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