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Antibacterial potentials of extracts from *Gryllotalpa gryllotalpa*, *Pentodon algerinum* grubs, and *Gypsonoma euphraticana* larva frass

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ABSTRACT

Objectives: The overuse and abuse of antibiotics have accelerated antibiotic resistance, and to solve this problem, it has been found that many insect species have potential antimicrobial properties against a wide range of resistant pathogens. Our study tests the antibacterial activity of microbial defensive compounds included in body extract of insects inhabiting contaminated environments and frass of phytophagous insects.

Materials and Methods: Through sequential extraction method by acidic methanol, chloroform, and hexane solvents, insect body extract of *Gryllotalpa gryllotalpa*, grubs of *Pentodon algerinum* besides feces of *Gypsonoma euphraticana* larvae were tested against Gram-positives *Bacillus cereus, Bacillus coagulans, Staphylococcus aureus,* and *Salmonella typhi, Escherichia coli,* and *Klebsiella pneumoniae*. The antibiotics ceftriaxone (CRO) and ampicillin (AM) were used as standard drugs. The antibacterial growth inhibition was estimated by well diffusion methods.

Results: High significant antibacterial activity against the tested bacteria by acidic methanol then chloroform extracts, while hexane extract of all the three insect species only produced significant growth inhibition of *S. aureus*. In addition, growth inhibition 20.0 mm or more was induced by: MeOH extracts of *G. gryllotalpa* and *P. algerinum* for *S. typhi* and *E. coli*, besides chloroform *G. gryllotalpa* extract for *S. typhi*. The tested bacteria *S. aureus*, *S. typhi*, and *K. pneumoniae* were AM-resistant, while *E. coli* was both AM and CRO-resistant.

Conclusion: Acidic meOH and chloroform body extract of *G. gryllotalpa* and *P. algerinum* and larvae *G. euphraticana* feces extract possess bioactive compounds with promising antibacterial properties, for overcoming antibiotic resistance.

Keywords: Insect extracts, antibacterial, antibiotic resistance, *Gryllotalpa gryllotalpa*, *Gypsonoma euphraticana*, *Pentodon algerinum*

INTRODUCTION

The beneficial uses of most insects relate to honey and edible insects as food, silk for clothing, pollinator insects for plant pollination, and few traditional medicinal applications, but little is known about developing potential drugs from insect bodies depending on their innate immunity properties as reservoirs of antimicrobial agents. The overuse of antibiotics since the last decade of the 20th century has led to the emergence of antibiotic resistance.[1] Moreover, many pathogenic bacteria acquire resistance to more than one antibiotic, which so referred to

as multidrug resistance, some of them are even resistant to any known antibiotics and so named pan-drug resistance.[2,3] Now, drug resistance is one of the 10 problems that threaten the world,[4,5] with annual proportional increasing resistance of the fatal pathogenic species to present antibiotics.[6] Today, drug resistance encourages searching for new alternative resources. One of these resources deals with the insect world, which aims to separate active antibacterial ingredients as templates for a new generation of the drug industry. Most studies in this field were first emphasized as survey study on the insect body extracts,[7-9] or bacterial inhibition by parts of the insect.[10-13] In more advanced studies, peptides with low molecular weights had been identified, and their growth inhibition activity was tested against a wide spectrum of Gram-negative and Gram-positive pathogenic bacteria. Therefore, many active metabolic compounds were separated and identified, with promising bacteria growth inhibition.[14,15] Moreover, many of the present drug-resistant bacteria are sensitive to insect antimicrobial peptides,[16-19] or epicuticular content lipids of the exoskeleton,[20-22] with promising results. Despite the huge diversity of the insect taxa, there has been slow progress in insect therapeutics, for instance, melittin from bees and alloferon from blow flies.[23,24]

In the light of the adaptation hypothesis, insects in polluted habitats have evolved high antimicrobial defense mechanisms. On this scope, the insect body extracts of the imago mole cricket, *Gryllotalpa gryllotalpa*, scarab beetle *Pentodon algerinum* grubs, and feces the leaf silk-webbing *Gypsonoma euphraticana* inhabited the host plant *Populus euphratica* were tested on the growth inhibition *in vitro* the pathogenic bacteria; *Bacillus cereus, Bacillus coagulans, Staphylococcus aureus, Salmonella typhi, Escherichia coli,* and *Klebsiella pneumoniae*.

MATERIALS AND METHODS

Insects

The tested insects were reared from their native environment in Mosul province/Iraq (36° 22′35 43° 08′32″ E). Mole cricket *G. gryllotalpa* was collected manually from the house garden infested with the pest around a light source in the rainy season. Specimens of the scarab grubs, *P. algerinum* (about 30 mm long) were picked up from the earthen cells in a depth of about 30 centimeters the last spring. Feces were removed from the *Populus Euphratica* leaves housing the *G. euphraticana*.

Bacteria isolates

The human pathogenic bacteria had been used as references for evaluating *in vitro* antibacterial activity of the insect extracts. The Gram-positives are *B. cereus, B. coagulans,* and *S. aureus,* while *S. typhi, E. coli,* and *Klebsiella pneumoniae* are Gram-negatives. Bacteria isolates were identified and brought from the Microbiology laboratory/Department of Biology/College of Education for Pure Sciences/Mosul University/Iraq.

Culture media

The culture growth media, Muller–Hinton agar from NEOGEN Culture Media (foodstafety.neogen.com) had been purchased.

Extraction solvents

The insect body extracts were prepared using the following polar solvents with descending polarity indices values; water (10.2), dimethyl sulfoxide DMSO (7.2), acetic acid (6.0), methanol (5.1), chloroform (4.1), and hexane (0.1).

Bacteria isolation

Each of the bacteria species was inoculated on a new nutrient agar plate by loop full bacteria and then incubated for 24 h. at 37°C to obtain an active cultivar. The prepared plates were used either for experimental testing or kept at 4°C as stock inoculums for subsequent experiments.

Insect crude extracts

The mole crickets and scarab grubs were killed by lowering their temperatures in the refrigerator, then in the oven dried at 35°C. 100 g of dried insects and 25 g of larval feces were grounded by an electric mill, sequential separation of active constituents through a 3-stage solvent elution method which modified after.[7,25] The first step includes extraction by acidic methanol (90% meOH + 9% H_2O + 1% CH_3COOH) solvent, then the filtrate dried, and the precipitate secondly eluted by chloroform, and within the last (third) stage of the elution by hexane solvent. The three obtained dried extracts for each insect material were preserved at 4°C. For experimentation, the dried extract dissolved in DMSO, and the applied concentration for all the experimental treatments was 250 mg/mL.

Antibacterial susceptibility assay

Antibacterial activity was evaluated by the well diffusion method. The inhibition zones were recorded in millimeters (mm) using a ruler. Briefly, Muller–Hinton agar (MHA) plates were inoculated with the activated model bacteria isolates under aseptic conditions, and the wells (diameter = 8 mm) were filled by the test samples and incubated at 37 °C for 24 h. Together, discs of standard drugs Ceftriaxone (CRO) and Ampicillin (AM) were fixed in MHA plates. The diameter of the clear growth to inhibition zones was measured. Inhibition rank was categorized according to Mohtar *et al.*[26] as follows:

≥8 mm (good), 6–7 mm (moderate), 4–5 mm (weak), and 2–3 mm (very weak).

Data analysis

All treatments were repeated in three replicates. The data was tabulated as means ± standard deviation. Mean differentiations at $P \leq 0.5$ were conducted; using a one-way Analysis of Variance Duncan's multiple range test.[27]

RESULTS

Antibacterial effect of the insect extracts

The present study deals with the antibacterial ability of the dry body ingredients of insects inhabiting polluted environments by means of growth inhibition zones of pathogenic bacteria. The antibacterial activity of body extracts of *G. gryllotalpa*, grubs of *P. algerinum,* and grounded feces of the leaves webbing moth, *G. euphraticana*, is shown in Tables 1-3. These extracts were prepared by sequential elution by gradual polarity indices of the applied solvents. Th e determined growth inhibition zone depended on the source of the extract and bacterium species.

For *G. gryllotalpa* extract, Table 1 exhibits growth inhibition of all the testing Gram-positive bacteria (*B. cereus, B. coagulans, and S. aureus*) by the three applied polar solvents ranging from 21.5 mm (for *B. cereus*) to 12.0 mm (for chloroform extract). While, only acidic methanol and chloroform inhibited the growth of the treated Gram-negative bacteria; *S. typhi, E. coli,* and *K. pneumoniae,* with higher clear zones of 25.3 mm for *K. pneumoniae* at chloroform extract and lower growth inhibition zone 18.0 mm for *S. typhi* and *E. coli* at acidic methanol and chloroform extracts, respectively.

The fecal extract of moth larvae, *G. euphraticana* inhibiting all Gram-positive bacteria except hexane extract for *B. coagulans*, is evoked in Table 2. On the other hand, only *K. pneumoniae* from Gram-positive bacteria were inhibited by hexane extract with 9.8 mm.

The grub beetle, *P. algerinum* extract with all three polar solvents, inhibited growth of the Gram-positives which ranged between 17.7 mm for *S. aureus* by hexane and 10.2 mm for *B. coagulans* with chloroform extract [Table 3].

Table 1: Antimicrobial activity of body extracts of mole cricket, *Gryllotalpa gryllotalpa* against pathogenic bacteria by inhibition of clear zone parameter.

Bacteria species	Sequential solvents used in extraction		
	Acidic meOH	Chloroform	Hexane
Bacillus cereus	19.0±0.0^{b}	21.5±0.5^{a}	16.0±0.0^{c}
Bacillus coagulans	13.5±0.5^{a}	12.0±1.0^{b}	11.5±0.5^{b}
Staphylococcus aureus	15.5±0.5^{b}	16.8±0.8^{ab}	18.0±1.0^{a}
Salmonella typhi	18.0±1.0^{b}	21.7±0.8^{a}	0.0±0.0^{c}
Escherichia coli	20.5±0.5^{a}	18.0±1.0^{c}	0.0±0.0^{c}
Klebsiella pneumoniae	19.0±1.0^{b}	25.3±1.0^{a}	0.0±0.0^{c}

Horizontal means±standard deviations with different letters are significantly different at $P \leq 0.05$ (Duncan's test)

Growth inhibit ability at each solvent extract bacteria

Each of the Tables 1-3 revealed how long the growth inhibition zones obtained by the extracts of *G. gryllotalpa*, *G. euphraticana*, larva feces and grubs *P. algerinum*, which were separately prepared by the following solvents; acidic methanol (mixed solvents), chloroform, and hexane.

For acidic methanol extract: The clear zones between 18.0 and 20.5 mm are shown in Table 4 demonstrated by the action of *G. gryllotalpa* against *S. aureus*, *B. cereus*, *K. pneumoniae, and E. coli*. Besides, feces extract gave a diameter clear zone ranging from 14.5 to 16.2 mm for the bacteria *S. typhi, S. aureus, K. pneumoniae,* and *B. cereus,* respectively. The extract of the grub beetle *P. algerinum*

Table 2: Growth inhibition zones (mm) of marker bacteria caused by fecal extract of moth larvae *Gypsonoma euphraticana*.

Bacteria species	Sequential solvents used in extraction		
	Acidic meOH	Chloroform	Hexane
Bacillus cereus	16.2±0.3^{a}	13.7±0.3^{b}	10.7±0.6^{c}
Bacillus coagulans	10.0±0.0^{b}	12.2±2.5^{a}	0.0±0.0^{c}
Staphylococcus aureus	15.7±0.6^{b}	15.20.3^{c}	17.0±0.0^{a}
Salmonella typhi	14.5±0.5^{a}	10.5±0.5^{b}	0.0±0.0^{c}
Escherichia coli	11.7±0.6^{a}	10.2±0.3^{b}	0.0±0.0^{c}
Klebsiella pneumoniae	15.5±0.5^{a}	10.8±0.3^{b}	9.8±0.3^{c}

Horizontal means±standard deviations with different letters are significantly different at $P \leq 0.05$ (Duncan's test)

Table 3: Antimicrobial activity of body extracts of white grub larvae, *Pentodon algerinum* extract represented by growth clear zones.

Bacteria species	Sequential solvents used in extraction		
	Acidic meOH	Chloroform	Hexane
Bacillus cereus	15.2±0.8^{a}	10.8±0.3^{b}	14.3±0.9^{a}
Bacillus coagulans	13.7±0.6^{a}	10.2±0.3^{b}	10.8±0.3^{b}
Staphylococcus aureus	11.7±0.6^{c}	14.0±1.0^{b}	17.7±0.6^{a}
Salmonella typhi	21.8±0.8^{a}	10.0±1.0^{c}	13.7±0.6^{b}
Escherichia coli	20.0±0.0^{a}	7.0±0.0^{b}	0.0±0.0^{c}

Horizontal means±standard deviations with different letters are significantly different at $P \leq 0.05$ (Duncan's test)

inhibited the growth of *S. aureus, K. pneumoniae, B. coagulans*, and *B. cereus*, while 20.0 and 21.8 mm for *E. coli* and *S. typhi*, respectively.

The diameters of growth inhibition zones of the cultured plates treated with extracts of the second phase chloroform are shown in Table 5. For mole *G. gryllotalpa* extract, the growth inhibition zone is mostly between 12.0 and 18.0 mm, except for *B. cereus* and *K. pneumoniae* 21.5 and 25.3 mm, respectively. However, *P. algerinum* grub extract was less effective with a range of 7.0–14 mm for all the experimental bacteria.

The antibacterial sensitivity variation between the marker bacteria treatment with the third (last) elution phase by hexane is illustrated in Table 6. Except for the bacteria, *B. cereus*, *B. coagulans*, and *S. aureus* were inhibited by extract *G. gryllotalpa* 16.0, 15.0, and 18.0 mm, respectively. Only, the bacteria *B. cereus* and *S. aureus* were affected by moth *G. euphraticana* larval frass with zones of inhibition 10.7 and 17.0 mm. It was found that *E. coli* resistant to grub *P. algerinum* hexane extract, and growth inhibition zones were determined (8.7, 13.8) for Gram-negative *K. pneumoniae*, and *S. typhi*, and 10.8, 14.3, and 17.7 mm for *B. coagulans*, *B. cereus*, and *S. aureus*, respectively.

Inhibition comparison between standard drugs and insect extracts

CRO caused antibacterial action (24.3, 26.0 mm) at treatment of the bacteria *S. typhi* and *K. pneumoniae*, and 11.0, 15.3, and 17.2 mm for *B. cereus, B. coagulans,* and *S. aureus,* respectively, but *E. coli* was not affected. The zones of inhibition by amoxicillin were restricted (14.7, 22.3 mm) with only *B. cereus* and *B. coagulans,* whereas the latters (*S. aureus*, *S. typhi*, and *K. pneumoniae*) were completely not responsive to the applied standard drugs [Table 4].

After testing with acidic meOH [Table 4] (with perpendicular columns); *B. cereus* was more inhibited (19.0, 16 2, and 15.0 mm) at *G. gryllotalpa*, *G. euphraticana,* and *P. algerinum* and then amoxicillin standard drugs (11.0 and 14.6 mm), respectively. The tested standard drugs were more effective than all the tested extracts. For *S. aureus*, their growth was inhibited with 17.2 mm by CRO (standard drug), whereas for *G. gryllotalpa*, *G. euphraticana,* and *P. algerinum* ranged between 16.5 and 11.6 mm, respectively. *S. typhi* was inhibited by CRO (24.3 mm) and the extracts were between 21.8 and 14.5 mm. Sensitivity of *E. coli* to the extracts was about 20.0 mm for *G. gryllotalpa* and *P. algerinum* and resistant to the standard drugs. *K. pneumoniae* was only inhibited by

Table 4: Antibacterial inhibition by acidic meOH body extracts *G. gryllotalpa* and fecal extract of the moth *G. euphraticana* and Scarab grub *P. algerinum* against the marker bacteria.

Insect extract	The growth inhibition zone (mm) of the bacteria					
	B. cereus	***B. coagulans***	***S. aureus***	***S. typhi***	***E. coli***	***K. pneumoniae***
G. gryllotalpa	19.0 ± 0.0^{bA}	13.5 ± 0.5^{dC}	15.5 ± 0.5^{cB}	18.0 ± 1.0^{bC}	20.5 ± 0.5^{aA}	19.0 ± 1.0^{bB}
G. euphraticana	16.2 ± 0.3^{aB}	10.0 ± 0.0^{dD}	15.7 ± 0.6^{aB}	14.5 ± 0.5^{bD}	11.7 ± 0.6^{cB}	15.5 ± 0.5^{aC}
P. algerinum	14.7 ± 1.5^{cB}	13.7 ± 0.6^{cdC}	11.7 ± 0.6^{eC}	21.8 ± 0.8^{aB}	20.0 ± 0.0^{bA}	12.7 ± 0.6^{deD}
CRO (ve+)	110.0 ± 0.5^{cC}	15.3 ± 1.5^{bB}	17.2 ± 0.8^{bA}	24.3 ± 1.2^{aA}	0.0 ± 0.0^{dC}	26.0 ± 0.0^{aA}
AM (ve+)	14.7 ± 0.6^{bB}	22.3 ± 0.6^{aA}	0.0 ± 0.0^{cD}	0.0 ± 0.0^{cE}	0.0 ± 0.0^{eC}	0.0 ± 0.0^{cE}

Horizontal means±standard deviations with different (small) letters are significantly different at $P\leq0.05$ (Duncan's test). Means that vertical different (capital) letters are significantly different at $P\leq0.05$ (Duncan's test). *G. gryllotalpa: Gryllotalpa gryllotalpa, P. algerinum: Pentodon algerinum, G. euphraticana: Gypsonoma euphraticana, B. cereus: Bacillus cereus, B. coagulans: Bacillus coagulans, S. aureus: Staphylococcus aureus, S. typhi*: *Salmonella typhi*, *E. coli: Escherichia coli, K. pneumonia: Klebsiella pneumoniae.* CRO: Ceftriaxone, AM: Ampicillin

Table 5: Antibacterial activity of Chloroform body extracts *G. gryllotalpa* and fecal extract of the moth *G. euphraticana* and Scarab grub *P. algerinu*m against the marker bacteria.

Insect extract	Growth inhibition zone (mm) of the bacteria					
	B. cereus	***B. coagulase***	***S. aureus***	***S. typhi***	***E. coli***	***K. pneumoniae***
G. gryllotalpa	21.5 ± 0.5^{bA}	12.0 ± 0.0^{dC}	16.8 ± 0.8^{cA}	21.7 ± 0.6^{bD}	18.0 ± 1.0^{cA}	25.3 ± 1.0^{aB}
G. euphraticana	13.7 ± 0.5^{bC}	12.2 ± 2.5^{cD}	15.2 ± 0.3^{aB}	10.5 ± 0.5^{cdC}	10.2 ± 0.3^{dB}	10.8 ± 0.3^{cdB}
P. algerinum	10.8 ± 0.3^{cD}	10.2 ± 0.3^{cdD}	14.0 ± 0.0^{aC}	10.0 ± 1.0^{dC}	7.0 ± 0.0^{eC}	12.0 ± 0.0^{bB}
CRO (ve+)	11.0 ± 0.5^{cD}	15.3 ± 1.5^{bB}	17.2 ± 0.8^{bA}	24.3 ± 1.2^{aA}	0.0 ± 0.0^{dD}	260 ± 2.0^{aA}
AM (ve+)	14.7 ± 0.6^{bB}	22.3 ± 0.6^{aA}	0.0 ± 0.0^{cD}	0.0 ± 0.0^{cB}	0.0 ± 0.0^{cD}	0.0 ± 0.0^{eC}

Horizontal means±standard deviations with different (small) letters are significantly different at $P\leq0.05$ (Duncan's test). Means with vertical different (capital) letters are significantly different at $P\leq0.05$ (Duncan's test). *G. gryllotalpa: Gryllotalpa gryllotalpa, P. algerinum: Pentodon algerinum, G. euphraticana: Gypsonoma euphraticana, B. cereus: Bacillus cereus, B. coagulans: Bacillus coagulans, S. aureus: Staphylococcus aureus, S. typhi*: *Salmonella typhi*, *E. coli: Escherichia coli, K. pneumonia: Klebsiella pneumoniae.* CRO: Ceftriaxone, AM: Ampicillin

CRO (26.0 mm) and less with a range of 19.0–12.6 mm for the applied extracts.

Growth inhibition by chloroform extracts; zone diameters of *B. ceseus* with *G. gryllotalpa* and *G. euphraticana* extracts 21.5 and 13.7 mm, and less than (11.0, 14.7) for CRO and AM (+ve). In the case of *B. coaculans*, growth inhibition was 22.3 and 15.3 mm for the antibiotics(+ve) AM and CRO, and between 10.2 and 11.0 mm for the tested extracts. It was found only CRO inhibits the growth of *S. aureus* with near results for *G. gryllotalpa* and *G. euphraticana* extracts. The *S. typhi* is resistant to AM but sensitive (24.3 mm) to CRO 21.3 for *G. gryllotalpa* and 10.5 mm for both *G. euphraticana,* and *P. algerinum* extracts. *E. coli* and *K. pneumoniae* were resistant to the tested standard drugs except the second ones 26.0 mm with CRO, whereas growth inhibition by *G. gryllotalpa*, *G. euphraticana, and P. algerinum* (18.0, 10.0, and 7.0 mm) and (25.3, 10.8, and 12.0 mm) for *E. coli* and *K. pneumoniae,* respectively.

In comparison antibacterial treatment with hexane insect extracts with (standard drugs) CRO and AX: *B. cereus* inhibition (16.0 mm) with *G. gryllotalpa* more than that of the other two extracts, besides the antibiotics (11.0, 14.7 mm) CRO and AM. But (+ve) CRO and AM were more effective than tested insect extracts for *B. coaculans*. CRO had nearly the same antibacterial activity (17.2) with the applied *G. gryllotalpa*, *G. euphraticana, and P. algerinum* extracts against *S. aureus*. Only CRO had growth inhibition (24.3, 26.0 mm) to *S. typhi* and *K. pneumoniae*. However, *E. coli* is resistant to all antibiotics and insect extracts [Table 6].

DISCUSSION

Insects like other invertebrates have only innate immune system, therefore, have highly developed immune systems. Theoretically, because of their feeding habit and habitat like some other studied insects.[7,14,28,29] subterranean insects such as *G. gryllotalpa, P. algerinum* larvae, and webbing *G. euphraticana* larvae are in direct exposure to the pathogenic microbial agents. According to this hypothesis, our study gives promising results of potentially significant antibacterial properties. Due to overuse and abuse present antibiotics were led to overcoming annual antibiotic resistance to pathogenic and opportunistic bacteria. Insect body extracts and purified constituents from insect body parts were proven as one of the future antibiotics, and they took continuous interest by many alternative natural product researchers.[17,30,31] In the present study, the measured growth inhibition zone of any tested marked bacteria was related to the tested bacterium, source of the insect body extract, and polarity of the solvent used in extraction. Therefore, according to Mohtar *et al.*[26] susceptibility rank of the antibacterial agents, acidic meOH *G. gryllotalpa* extract had more significant activity (19.0 mm) for both *B. coagulan*s and *K. pneumoniae* and 20.5 mm for *E. coli* [Table 1], while 5 of the 6 marked bacteria treated by *G. euphraticana* and *P. algerinum* were more significantly caused growth inhibition in relation to chloroform and hexane extracts, which ranged between good to moderate inhibition [Tables 1-3]. It was found qualitative and quantitative inhibition by chloroform after acidic methanol extracts through the sequential method so that only *G. gryllotalpa* extract caused growth inhibition between 21.5 and 25.3 mm for *B. cereus*, *S. typhi* and *K. pneumoniae*, and *G. euphraticana* and *P. algerinum* extracts were less than 15.2 mm for all the tested bacteria. On the other hand, the largest growth inhibition by hexane extract was 18.0 mm at *S. aureus* by *G. gryllotalpa* extract.

It is illustrated in Tables 1-3 that *G. gryllotalpa* extracted by all the three sequential polar solvents had more significant growth inhibition *B. cereus* than the standard drugs. It was found nearly the same effect of *G. gryllotalpa* extracted by all the solvents and CRO on *S. aureus* which is completely resistant to AM. Besides, equal moderate effect of all the applied extracts with hexane and CRO, and complete resistance to AM. *S. typhi* was inhibited by all the extracts, but less significant than CRO and resistant (0.0 mm) to AM. All the extracts had growth inhibition to E. coli, while

Table 6: Antibacterial inhibition by Hexane extracts *G. gryllotalpa* and fecal extract of the moth *G. euphraticana* and Scarab grub *P. algerinum* against the pathogenic bacteria.

Insect extract	Growth inhibition zone (mm) of the bacteria					
	B. cereus	***B. coagulans***	***S. aureus***	***S. typhi***	***E. coli***	***K. pneumoniae***
G. gryllotalpa	16.0 ± 0.0^{bA}	11.5 ± 0.5^{C}	18.0 ± 1.0^{aA}	0.0 ± 0.0^{cC}	0.0 ± 0.0^{dA}	0.0 ± 0.0^{dC}
G. euphraticana	10.7 ± 0.6^{bC}	0.0 ± 0.0^{cD}	17.0 ± 0.0^{aA}	0.0 ± 0.0^{cC}	0.0 ± 0.0^{cA}	0.0 ± 0.0^{cC}
P. algerinum	14.3 ± 1.0^{bB}	10.8 ± 0.3^{cC}	17.7 ± 0.6^{aA}	13.8 ± 0.6^{bB}	0.0 ± 0.0^{eA}	8.7 ± 0.6^{dB}
CRO (ve+)	11.0 ± 0.5^{cC}	15.7 ± 0.8^{bB}	17.2 ± 0.8^{bA}	24.3 ± 1.2^{aA}	0.0 ± 0.0^{dA}	26.0 ± 2.0^{aA}
AM (ve+)	14.7 ± 0.6^{bB}	22.3 ± 0.6^{aA}	0.0 ± 0.0^{cB}	0.0 ± 0.0^{cC}	0.0 ± 0.0^{cA}	0.0 ± 0.0^{cC}

Horizontal means±standard deviation with different (small) letters is significantly different at $P\leq0.05$ (Duncan's test). Means with vertical different (capital) letters are significantly different at $P\leq0.05$ (Duncan's test). *G. gryllotalpa: Gryllotalpa gryllotalpa*, *P. algerinum*: *Pentodon algerinum*, *G. euphraticana: Gypsonoma euphraticana, B. cereus: Bacillus cereus, B. coagulans: Bacillus coagulans, S. aureus: Staphylococcus aureus, S. typhi*: *Salmonella typhi*, *E. coli: Escherichia coli, K. pneumonia: Klebsiella pneumoniae.* CRO: Ceftriaxone, AM: Ampicillin

at the same time had not responded to CRO and AM. *K. pneumoniae* was resistant to AM but sensitive (26.0 mm) to CRO which was better than the extracts, so all the extracts had significant inhibition in relation to AM.

CONCLUSION

In the present study, the good antibacterial activity of whole body extract of the insects that inhabiting polluted niches with pathogenic bacteria and other microbes, so the insect body reflex was represented by the production of antibiotic constituents. Therefore, a wide spectrum of Gram-positive and Gram-negative bacteria exhibited good sensitivity to body extract from subterranean *G. gryllotalpa* and grubs of *P. algerinum* and frass pellets of the confined living *G. euphraticana* larvae. Most of the extracts, especially acidic methanol have better activity than the (CRO and AM antibiotics) standard drugs.

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Ethical approval

Institutional Review Board approval is not required.

Declaration of patient consent

Patient's consent is not required as there are no patients in this study.

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Conflicts of interest

There are no conflicts of interest.

Use of artificial intelligence (AI)-assisted technology for manuscript preparation

The authors confirm that there was no use of artificial intelligence (AI)-assisted technology for assisting in the writing or editing of the manuscript and no images were manipulated using AI.

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