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Antibacterial potentials body extracts of *Gryllotalpa gryllotalpa*, grubs *Pentodon algerinum* and *Gypsonoma euphraticana* larva feces

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Abstract
The overuse and abuse of antibiotics have been accelerated antibiotic resistance, to solve this problem, it was found many insect species have potential antimicrobial properties against wide range of resistant pathogens. Through sequential extraction method by acidic methanol, chloroform and hexane solvents, insect body extract of *Gryllotalpa gryllotalpa* and grubs of *Pentodon algerinum* besides feces of *Gypsonoma euphraticana* larvae were tested against Gram positives *Bacillus cereus*, *Bacillus coagulans* and *Staphylococcus aureus* and Gram negatives *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 method. High significant antibacterial activity against the tested bacteria by acidic methanol then chloroform extracts, while hexane extract of all the three insect species only caused significant growth inhibition of *Staph. aureus*. Also, it was found growth inhibition 20.0 mm or more were induced by: Acidic 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 *Staph. aureus*, *S. typhi*, and *K. pneumoniae* were resisted AM standard drug, while *E. coli* resistant to both AM and CRO antibiotics. Acidic meOH and chloroform body extract of *G. gryllotalpa* and *P algerinum* have bioactive compounds with promising antibacterial properties, for confrontation overcoming antibiotic resistance.

Keywords: Insect body extract, antibacterial, *Gryllotalpa gryllotalpa*, *Gypsonoma euphraticana*, *Pentodon algerinum*

Introduction
The most insect benefit usages are relating with honey and edible insects as food, silk for clothing and pollinator insects for plant pollination, and few traditional medicinal applications, but little known about developing potential drugs from insect bodies depending on their innate immunity properties as reservoir of antimicrobial agents. The abuse of the available antibiotics at the last decade of 20th century and decades of 21st century were emerged of antibiotic resistance [1]. Moreover, many pathogenic bacteria acquired resistance for more one antibiotic and so referred as multidrug resistance, some of them even resistant to any known antibiotics and so named pan - drug resistant [2, 3]. Now, drug resistance is one of the ten problems threaten the world [4, 5], with annual proportional increasing resistant 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 insects world which aimed to separate active antibacterial ingredients as templates for new generation of drug industry. Most studies in this field were firstly emphasized as a survey studies on the insect body extracts [7-9], or bacterial inhibition by parts of the insect [10-13]. In more advanced studies, peptides with the low molecular weights had been identified and their growth inhibition activity were tested against 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 (AMPs) [16-19], or epicuticular content lipids of the exoskeleton [20-22], with promise results. But, in spite of the huge diversity of the insect taxa, only very low progress in the insect therapeutics, for instance melittin from bees and alloferon from blow flies [23, 24].

In the light of the adaptation hypothesis, alive insects in a polluted habitats have evolved high antimicrobial defense ability. 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

Materials

Insects

The tested insects were reared from their native environment in Mosul province/ Iraq (36 ° 22′35 43 ° 08′32 ″ E). Mole cricket *Gryllotalpa gryllotalpa* was collected by hand from the house garden infested with the pest around a light source in rainy season. Specimens of the scarab grubs, *Pentodon algerinum* (with length about 30 millimeters) were picked up from the earthen cells in depth about 30 centimeters at the last spring. Feces were removed from the *Populus Euphretica* leaves housing the *Gypsonoma 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 *Bacillus cereus*, *Bacillus coagulans* and *Staphylococcus aureus*, while *Salmonella typhi*, *Escherichia coli* and *Klebsiella pneumoiae* are Gram negatives. Bacteria isolates were identified and brought from 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 by using the following polar solvents with descending polarity indices values; water (10.2), dimethyl sulphoxide DMSO (7.2), acetic acid (6.0), methanol (5.1), chloroform (4.1) and hexane (0.1).

Bacteria isolation

Each of the bacteria species were inoculated on new nutrient agar plate by loop full bacteria, then incubated for 24 hrs. at 37 ºC to obtain an active cultivars. The prepared plates were used either for experimental testing nor kept at 4 ºC as stock inoculums for subsequent experiments.

Insect crude extracts

The mole crickets and scarab grubs were died by lowering their temperatures in refrigerator, then in oven dried at 35 ºC. 100 grams of dried insects and 25 grams of larval feces were grounded by electric mill, sequential separation of active constituents through three - stage solvent elution method which modified after [25, 26]. 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 obtaining dried extracts for each insect material were preserved in 4 ºC. For experimentation, the dried extract dissolved in DMSO, and the applied concentration for all the experimental treatments were 250 mg/ml.

Antibacterial susceptibility assay

Antibacterial activity were evaluated by well diffusion method. The inhibition zones were recorded in millimeter (mm) using a ruler. Briefly, Muller - Hinton agar (MHA) plates were inoculated with the activated model bacteria isolates under aseptic condition, the wells (diameter = 8 mm) were filled by the test samples, and incubated at 37 ºC for 24 hours. 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 Mohtar [27] as fellows; ≥ 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 were tabulated as means ± standard deviation. Mean differentiations at $p \leq 0.5$ were conducted by one way ANOVA Duncan's multiple range test [28].

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 growth inhibition zones of pathogenic bacteria. Tables 1, 2 and 3 had been showed the antibacterial activity of body extracts of *Grylloptalpa gryllotalpa*, grubs of *Pentodon algerinum* and grounded feces of the leaves webbing moth, *Gypsonoma euphraticana*. These extracts were prepared by sequential elution by gradual polarity indices of the applied solvents. The determined growth inhibition zone were depended on the source of the extract and bacterium species.

For *G. gryllotalpa* extract, table1exhibits growth inhibition of all the testing Gram positive bacteria (*B. cereus*, *B. coagulans and Staph. aureus*) by the three applied polar solvents, which ranged between 21.5 mm for *B. cereus* to 12.0 mm for chloroform extract. While, only acidic methanol and chloroform inhibited growth of the treated Gram negative bacteria; *S. typhi*, *E. coli* and *K. pneumoiae*, with higher clear zones 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.

Table 1: Antimicrobial activity of body extracts of mole cricket, *Gryllotalpa gryllotalpa* against pathogenic bacteria by inhibition 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 ±0.5b
Staphylococcus aureus	15.5 ± 0.5b	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 ± SDs with different letters are significantly different at $p \leq 0.05$ (Duncan, s test).

(Table 2) evokes the fecal extract of moth larvae, *Gypsonoma euphraticana* inhibiting all Gram positive bacteria except hexane extract for *B. coagulans*. On the other hand, only *K. pneumoiae* from Gram positive bacteria inhibited by hexane extract with 9.8 mm.

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
Staphyllococcus aureus	15.7±0.6 b	15.20.3 c	17.0± 0.0a
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 ± SDs with different letters are significantly different at $p \leq 0.05$ (Duncan, s test).

The grub beetle, *Pentodon algerinum* extract with all the three polar solvents were inhibited growth of the Gram positives which ranged between 17.7 mm for *Staph. aureus* by hexane and 10.2 mm for *B. coagulans* with chloroform extract (Table 3).

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
Klebsiella pneumoniae	12.7±0.6 a	12.0±0.0 a	8.7±0.6 b

- Horizontal means ± SDs with different letters are significantly different of $p \leq 0.05$ (Duncan, s test).

Growth Inhibitability at each solvent extract Bacteria

Each of the tables 1, 2 and 3 had revealed to how long the growth inhibition zones obtained by the extracts of *G. gryllotalpa*, *G. euphraticana*, larva feces and grubs *Pentodon algerinum*, which separately prepared by the following solvents; acidic methanol (Mixed solvents), chloroform and hexane.

For acidic methanol extract: Table 4 shows the clear zones between 18.0 to 20.5 mm were demonstrated by action of *G. gryllotalpa* against *Staph. aureus*, *B. cereus*, *K. pneumoniae and E. coli*. Besides, ////feces extract gave diameter clear zone ranged from 14.5 to 16.2 mm for the bacteria *S. typhi*, *Staph. aureus*, *K. pneumoniae* and *b. cereus* respectively. The extract of the grub beetle *Pentodon algerinum* inhibited growth of *Staph. aureus*, *K. pneumoniae*, *B. coagulans* and *B. cereus*, while 20.0 and 21.8 mm for *E. coli* and *S. typhi* respectively.

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

Insect extract	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 cC	0.0±0.0 cE

- Horizontal means ± SDs with different (small)l letters are significant different at $p \leq 0.05$ (Duncan's test)
- Means with vertical different (capital) letters are significantly different at $p \leq 0.05$ (Duncan's test)

(Table 5) shows diameters of growth inhibition zones of the cultured plates treated with extracts of the second phase chloroform. For mole *G. gryllotalpa* extract, growth inhibition zone mostly between 12.0 and 18.0 mm, except for *B. cereus* and *K. pneumoniae* 21.5 and 25.3 mm respectively. However, *Pentodon algerinum* grub extract less effective with range 7.0 to 14 mm for all the experimental bacteria.

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

Insect extract	Growth inhibition zone (mm) of the bacteria					
	B. cereus	***B. coagulanse***	***S. aureus***	***S. typhi***	***E. coli***	***K. pneumonae***
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 cCD	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 cC

- Horizontal means ± SDs with different (small)l letters are significant different at $p \leq 0.05$ (Duncan's test).
- Means with vertical different (capital) letters are significantly different at $p \leq 0.05$ (Duncan's test).

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

Table 6: Antibacterial inhibition by Hexane extracts *G. gryllotalpa* and fecal extract of the moth *G. euphraticana* and Scarab grub *Pentodon 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 cC	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 ± SDs with different (small)l letters are significant different at $p \leq 0.05$ (Duncan's test).
- Means with vertical different (capital) letters are significantly different at $p \leq 0.05$ (Duncan's test).

Inhibition comparison between standard drugs and insect extracts

The antibiotics; Ceftriaxone (CRO) was caused antibacterial action (24.3, 26.0 mm) at treatment the bacteria *S. typhi* and *K. pneumoniae*, and 11.0, 15.3 and 17.2 mm for *B. cereus*, *B. coagulans* and *Staph. aureus* respectively, but *E. coli* had not affected. The zones of inhibition by Amoxicillin (AM) was restricted (14.7, 22.3 mm) with only *B. cereus* and *B. coagulans*, whereas the latters (*Staph. aureus*, *S. typhi* and *K. pneumoniae*) are completely not responded to that applied standard drugs (Table 4).

After testing with acidic meOH (table 4 with perpendicular columns); *B. cereus* was more inhibited (19.0, 16 2, 15.0 mm) at *G. gryllotalpa*, *G. euphraticana* and *P. algerinum* and then AM standard drugs (11.0, 14.6 mm) respectively. The tested standard drugs were more effective than all the tested extracts. For *Staph. aureus*, their growth inhibited with17.2 mm by (CRO) as standard drugs, whereas for *G. gryllotalpa*, *G. euphraticana* and *P. algerinum* ranged between 16.5 – 11.6 mm respectively. *S. typhi* was inhibited by CRO (24.3 mm) and the extracts between 21.8 – 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* only inhibited by CRO (26.0 mm) and less with range 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 case *B. coaculans*, growth inhibition 22.3 and 15.3 mm for the antibiotics (+ve) AM and CRO, and between 10.2 – 11.0 mm for the tested extracts. It was found only CRO inhibits growth *Staph. aureus* with near results for *G. gryllotalpa* and *G. euphraticana* extracts. The *S. typhi* resistant to AM but sensitive (24.3 mm) to CRO and 21.3 for *G. gryllotalpa* and 10.5 mm for both *G. euphraticana* and *P. algerinum* extracts. *E. coli* and *K. pneumoniae* were resistant for the tested standard drugs except the second ones26.0 mm with CRO, whereas growth inhibition by *G. gryllotalpa*, *G. euphraticana and P. algerinum* (18.0, 10.0, 7.0 mm) and (25.3, 10.8, 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 same antibacterial activity (17.2) with the applied *G. gryllotalpa*, *G. euphraticana and P. algerinum* extracts against *Staph. aureus*. Only CRO had growth inhibition (24.3, 26.0 mm) to *S. typhi* and *K. pneumoniae*. However, *E. coli* resistant to all antibiotics and insect extracts. (Table 6).

Discussion

Insects like other invertebrates have only innate immunity system, therefore have highly developed immunity system. Theoretically, because of their feeding habit and habitat alike some other studied insects [25-31]. Subterranean insects as *Gryllotalpa gryllotalpa* and *Pentodon algerinum* larvae and sheltered living leave - webbing *Gypsonoma euphraticana* larvae are in direct exposure with the pathogenic microbial agents. According to this hypothesis, the present study gives encouragement results through 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 proved as one of future antibiotics and they took continuous interest by many alternative natural product researches [32-34]. In the present study, the measured growth inhibition zone of any tested marked bacteria was related with the tested bacterium, source of the insect body extract and polarity of the solvent used in extraction. Therefore, according to Mohtar [28] susceptibility rank of the antibacterial agents, acidic meOH *Gryllotalpa gryllotalpa* extract had more significant activity (19.0 mm) for both *B. coagulans* and *K. pneumoniae* and 20.5 mm for *E. coli* (table 1), while five of the six marked bacteria treated by *Gypsonoma euphraticana* and *Pentodon algerinum* were more significantly caused growth inhibition in relation to chloroform and hexane extracts, which ranged between good to moderate inhibition (Tables 1, 2, 3). It was found qualitative and quantitative inhibition by chloroform after acidic methanol extracts through sequential method, so that, only *G. gryllotalpa* extract was caused growth inhibition between 21.5 to 25.3 mm for *B. cereus*, *S. typhi* and *K. pneumoniae*, and *Gypsonoma euphraticana* and

Pentodon 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 *Staph. aureus* by *G. gryllotalpa* extract.

Tables 1, 2 and 3 (Capital letters) are illustrate 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 same effect of *G. gryllotalpa* extracted by all the solvents and CRO on *Staph. aureus* which complete resistant to AM. Besides, equal moderate effect of all the applied extracts with hexane and CRO, and complete resistant to AM. *S. typhi* 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 in same time had not responded to CRO and AM. *K. pneumoniae* resistant to AM but sensitive (26.0 mm) to CRO which better than the extracts, so, all the extracts had significant inhibition in relation to AM.

Conclusion

In the present study, good antibacterial activity of whole body extract of the insects which inhabiting polluted niche with pathogenic bacteria and other microbes, so the insect body reflex was represented by production antibiotic constituents. Therefore, wide spectrum of Gram positive and Gram negative bacteria were exhibited good sensitivity by body extract of each the subterranean *Gryllotalpa gryllotalpa* and grubs of *Pentodon algerinum* and feces of the confined living *Gypsonoma euphraticana* larvae. Most the extracts especially acidic methanol have better activity than the (CRO and AM antibiotics) standard drugs.

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