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Antibacterial activity body extracts of developmental stages the blowfly, *Calliphora vomitoria* (Diptera: Calliphoridae)

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Abstract
The antibacterial effectiveness of the bluebottle fly, *Calliphora vomitoria* developmental body extracts filtrated by sequent polar solvents were examined. These investigations appeared the presence of bacterial inhibitors within larval, pupal and imaginal body extracts active against both Gram positive (*Staphylococcus aureus*) and Gram negative (*Klepsiella pneumoniae*) bacteria. These extracts were shown high stability in laboratory condition and, exhibiting high activity against clinical infectious bacteria. Immature extracts with especially pupal extract can be a source of antibiotic like compounds which may be used for pathogenic microbial control for the fight antibiotic resistance.

Keywords: *Calliphora vomitoria*, developmental stages, antibacterial, *Staphylococcus aureus*, *Klebsiella pneumonia*e

Introduction
The treatment by antibiotics is associated with Pencillin discovery by Alexander Felming in 1928 [1]. Antibiotics are used in antimicrobial disease treatment [2]. In precision, antibacterial drugs have been used over many decades, but the overuse and misuse of antibiotic drugs have accelerated antibacterial multidrug resistance [3]. However, evolution of new antibiotics has mainly focusing on new resources [4]. Therefore, antibacterial experiments have been conducted on insect extractives to find new antibiotics [5, 6]. Insects have known ability to resist infectious diseases [7]. Cuticle of insects is consist of three layers, which protect internal organs against pathogenic infection, toxins, desiccation and injuries [8, 9, 10]. Besides, hemolymph of both larvae and pupae of *Musca domestica* have lytic ability against Gram negative and positive bacteria [11]. Body extract of *Lucillia sericata*, *Vespa orientalis* and *Schestocerca gregaria* had found antibacterial and antifungal activity, and these activities were depended on the used solvents [12].
Calliphorid flies had taken more attractant attention by physicians and entomologists for the last century, the concept "surgery maggots" is well known in chronic infectious wounds and ulcers, so they can be candidate as producers of antimicrobial active compounds [13]. The isolated secretions from larvae of *L. serecata* have antibacterial properties to both Methicillin – resistant and Methicillin – sensitive *Staphylococcus aureus* and *Staph. pyogenes* with less effect on Gram – negative *Pseudomonus aeruginosa* [14]. On the other hand, antimicrobial resistance – bacterial pathogens have evoked one of the greatest challenge to the health care in the twenty - first century [15]. To be searching alternatives, searching focused on insects inhabiting niches contaminated with pathogenic agents, so extraction / secretion of *Calliphora vomitoria* were more inhibited than Ceftriaxone antibiotic the growth of sensitive and resistance Piperacillin *Staph. arereus, Escherichia coli*, P. aeruginosa and *Klebsiella pneumoniae* [16]. Cuticular and internal fatty acids of *C. vomitoria* had exhibited antimicrobial activity [17]. For extraction antibacterial active compounds, whole body dried or fresh extracted by many solvents such as; petroleum ether, hexane, acetone, ethyl acetate and acidic methanol had been used [18, 19].

Materials and methods
Insect culturing
The full grown larvae of *Callphora vomitoria* were reared in the beginning of the wandering stage, which borrowing the substrate soil for pupation**.** Proper wooden cage (2 × 1 × 1m) for education the carrion blow fly, *C. vomitoria* on fish remaining which bringing from fish market/ Mosul city/ Mosul / Iraq (36 ° 22′35 N 43°08′32″E). The insectary condition had been modulated for education as whether in February month the seasonal occurrence of the wild blow fly, *C. vomitoria*; 25± 3 C, 60± 5 R/H and 11: 13 h (L: D) photoperiod.

Bacteria and culture media
The positive – Gram Staphylococcus aureus and Gram – negative *Klebsilla pneumonia* were chosen as model bacteria for testing the in vitro antibacterial activity of immature stage extracts of *C. vomitoria*. The isolated stocks of the applied bacteria were supplied by Dr. Alaa Taha Younis / Laboratory of Microbiology /

Postgraduate Study Unit / Biology Department /Mosul University Iraq. For in vitro bacterial inoculation; Muller – Hinton agar media were purchased from NEOGEN Culture Media (foodstafety.neogen.com**)**.

Preparation immature extracts

Separately, 150 grm. from each fresh full grown larvae and four – day old pupae were sufficiently homogenized by porcelain jar. After homogenizing, consequent mixing and stirring overnight separately with each of the following solvents: hexane, chloroform, acetone, and methanol, the superannuated extracts of the larvae and pupae with each solvent had been dried and preserved at 4 C for future experimental treatments.

Bioassay of antibacterial activity

The antibacterial activity was assessed by standard agar diffusion disc - test (growth inhibition zone) after Kirby–Bauer testing method [20]. In addition to extracts of larvae pupae and adults of blue bottle fly (BBF), Ceftriaxone (CRO) discs and Dimethyl sulfoxide (DMSO) were used as positive and negative controls respectively. Before use, the extracts were dissolved in DMSO solvent according to the fractionated concentration. Discs with 6.0 mm diameter of Watmann filter paper No. 1 were temperature sterilized and dipped in the prepared extract concentrations for 10 min., then air dried for 30 min., the discs were fixed on the inoculated agar plates with three replicates, these Petri plates were incubated at 37 C for 24 hrs.. The inhibition zone was represented by the diameter of the inhibited growth edge minus the paper disc diameter (6.0 mm) and measured by digital caliper [21]. The data were analyzed by ANOVA, Duncan Multiple Range Test at P 0.05 [22]. Evaluation of the Inhibition zones were ranked according to the extract antibacterial activity based on Mohtar [23] chart; ≥ 8 mm good, 6 – 7 mm moderate, 4 – 5 mm weak and 2 – 3 mm very weak.

Results

Table 1 evokes the inhibition zones of Gram – positive, *Staphylococcus aureus* as response to experimental treatments by extracts of larva, pupa and adult *Calliphora vomitoria* at 10 and 50 mg/ ml concentrations of the sequential solutions beginning by hexane solvent and obeyed by chloroform, acetone and ethanol solvents respectively. The extracts were prepared by each solvent appeared variation in growth zones which depending on BBF stage. However, pupal extract more effective than larval and imago extracts at all the solvents.

For hexane, diameter of inhibited growth zones the bacterium *Staphylococcus aureus* at 10 and 50 mg / ml for pupae (6.6, 12.4 mm), while for larvae (4.8, 9.4) and for BBF adult (3.5, 4.2 mm). Growth inhibition in table 1 increases by extracts prepared by chloroform at the two (10, 50 mg / ml) concentrations; 20.0 and 34.4 mm resulted by pupal extract, 18.2 and 28.2 mm by larval extract and later extract of imago 9.2 and 12.6 mm. Acetone extract at 10 and 50 mg / ml concentrations were exhibited 6.4 and 10. 6 mm for pupa, (5.0, 8.4 mm) and (3.6, 3.2 mm by larva and adult respectively .Inhibition zones by ethanol stage extracts at the two concentrations; for pupa (5.2, 6.5 mm), and the bacterium *Staph aureus* not be affected by larva and imago ethanol extract. *Staph. aureus* was weakly affected (2.2 mm) by positive control Ceftriaxone antibiotic.

By the three (larva, pupa and imago) developmental stages, extracts of the holometabolous *C. vomoria* behaved as growth inhibitor of Gram negative bacteria, *Klebsiella pneumonia* at 10 mg / ml concentration; the inhibition growth diameter (5.0, 18.2, 6.0, and 4.2 mm), (4.4, 16.4, 15.2 and 2.4 mm) and (3.2, 8.0, 3.4 and 0.0) for pupa, larva and imago extracts by the solvents hexane, chloroform, acetone and ethanol respectively. At the second (50 mg / ml) concentration, the inhibition growth diameter (10.6, 38.2, 10.0, and 8.2 mm) for larval, (9.8.29.4, 9.2 and 4.3 mm) for larva and (5.2, 13.6, 5.5 and 0.0 mm) caused by adult extract prepared by the solvents; hexane, chloroform, acetone and ethanol respectively. In comparison; the positive control (CRO) inhibited growth of *K. pneumonia* with diameter 8.5 mm (table 1).

Table 1: Antibacterial effect of extracted *Callifora vomitoria* developmental stages through growth inhibition zones Gram - positive bacteria *Staphylococcus aureus*.

Sequent organic Solvents mg/ml		**Inhibition growth zone (mm) by extract of**		
		Larva	**Pupa**	**Imago**
Hexane	10	4.8±0.8 b	6.6±1.0 a	3.5±0.6 c
	50	9.4±0.2 b	12.4±1.0 a	4.2±0.5 d
Chloroform	10	18.2±0.2 b	20.0±0.6 a	9.2±0.4 ef
	50	28.2±0.4 bc	34.4±0.5 a	12.6±1.0 e
Acetone	10	5.0±1.0 b	6.4±0.8 a	3.6±1.0 d
	50	8.4±0.5 b	10.6±0.6 a	3.2±0.8 e
Ethanol	10	3.0±0.8 bc	5.2±0.2 a	0.5±0.2 f
	50	3.3±0.4 bc	6.5±0.6 a	0.0±0.0 f
-ve (DMSO)		0.0±0.0	0.0±0.0	0.0±0.0
+ Ve control group (CRO)		2.2±0.5 d		

- Means with vertical different letters are significantly different at P≤0.05 (Duncan's test).

Table 2: Antibacterial activity of extracted *Calliphora vomitoria* immature/stages represented by growth inhibition zones of the Gram - negative bacteria, *Klebsiella pnuemuoniae*.

Sequent organic Solvents mg\ml		Inhibition growth zone (mm) by extract of		
		Larva	Pupa	Imago
Hexane	10	4. 4±0.2 b	5.6±0.8 a	3.2±0.5 d
	50	9.8±0.2 ab	10.6±0.8 a	5.2±0.5 de
Chloroform	10	16.4±1.0 b	18.2±0.8 a	8.0±0.8 g
	50	29.4±0.4 c	38.2±0.2 a	13.6±0.8 h
Acetone	10	5.2±0.5 b	6.0±0.4 a	3.4±0.2 ce
	50	9.2±0.5 ab	10.0±0.6 a	5.5±0.2 e
Ethanol	10	2.4±0.2 c	4.2±0.2 a	0.0±0.0 f
	50	4.3±0.6 c	8.2±0.2 a	0.0±0.0 e
-ve (DMSO)		0.0±0.0 f	0.0±0.0 f	0.0±0.0 f
+ Ve (CRO)		8.5±0.5 a		

- Means with vertical different letters are significantly different at P≤0.05 (Duncan's test).

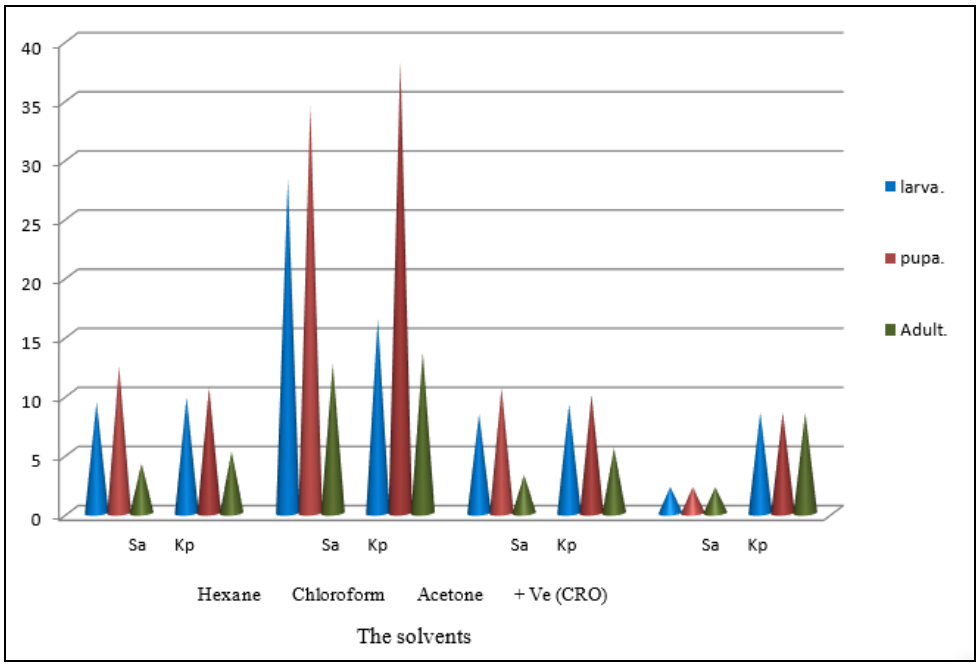

Fig 1: Variations in antibacterial activity *Calliphora vomitoria* stages as growth inhibition zones of *Staphylococcus aureus* and *Klebsiella pneumoniae.*

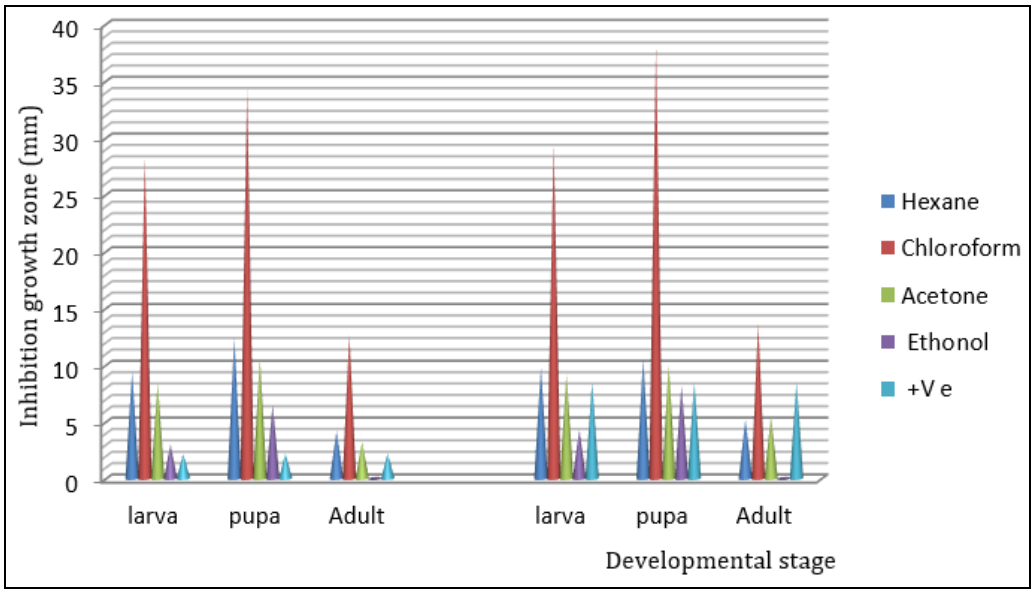

Fig 2: Antibacterial activity of BBF developmental stages had varied with the solvent polarity against *Staphylococcus aureus* and *Klebsiella pnuemuoniae*.

Discussion

Antimicrobial ingredients in insects are metabolites of peptides and lipids have been synthesized to be used against pathogenic invaders [24, 10, 25, 26, 27, 29]. In the present study, fresh extract of the metamorphic stages (larva, pupa and imago) of the BBF were prepared with sequent solvents (hexane, chloroform, acetone and ethanol) which exhibited meaningful antibacterial activity with significant variation between these stages. Fig 1 evokes that significant difference between inhibition growth zones of *Staphylococcus aureus* resulted by these stages at the concentrations 10 and 50 mg/ ml in comparison with inhibition by the standard drug antibiotic Ceftriaxone. but *Klebsiella pneumoniae* more sensitive to the standard drug than all BBF stages except extracts with chloroform pupa and larva extracted by acetone (table 2). On the other hand, pupal extract significantly more effective than the extract of larvae, and the later was more active than that of adult extract against Gram positive (*Staph. aureus*) and Game negative (*K. pneumoniae*) bacteria. Look for Fig 2 reveals variation in the antibacterial activity of each stage according to the applied solvent in the successive pattern.

For applying [23] method on the present blue bottle fly antibacterial activity. In context about comparison with other studies; present study have been given more promising in future pharmaceutical resources through active antibacterial chemical ingredients of the calliphorid, *Calliphora vomitoria* pupal stage.

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