The Journal of Medical Entomology and Parasitology is one of the series issued quarterly by the Egyptian Academic Journal of Biological Sciences. It is an important specialist journal covering the latest advances in that subject.

It publishes original research and review papers on all aspects of basic and applied medical entomology, parasitology and host-parasite relationships, including the latest discoveries in parasite biochemistry, molecular biology, genetics, ecology and epidemiology in the content of the biological, medical entomology and veterinary sciences.

In addition to that, the journal promotes research on the impact of living organisms on their environment with emphasis on subjects such a resource, depletion, pollution, biodiversity, ecosystem…..etc.

www.eajbs.eg.net

Egypt. Acad. J. Biolog. Sci., 9(1): 1 – 12 (2017)

Egyptian Academic Journal of Biological Sciences
E. Medical Entom. & Parasitology

ISSN: 2090 – 0783
www.eajbs.eg.net

Antibacterial Properties of Larval Secretions of the Blowfly, *Lucilia sericata* (Diptera: Calliphoridae)

Mohamed Elshehaby, Mahran Tony, Abdel - Rahman Sultan and Abdel baset, M. A. abd Reheem
Zoology Department, Faculty of science, Al azhar University, Assiut, Egypt.

ARTICLE INFO
Article History
Received: 15/12/2016
Accepted: 20/1/2017

Key words:
Lucilia sericata
antibacterial
larval therapy
methicillin-resistant
Staphylococcus aureus
streptococcus sp.,
secretions.

ABSTRACT

Secretions aseptically collected from larvae or maggots of the green bottle fly *Lucilia sericata* (Meigen) (Diptera: Calliphoridae) exhibit antimicrobial activity along with other activities beneficial for wound healing. With the rise of multidrug-resistant bacteria. New approaches for identifying the active compounds responsible for the antimicrobial activity were examined. Five different pathogenic bacterial strains were used in this study, Three of them are gram positive bacteria, namely: *Staphylococcus aureus*, *streptococcus sp*, and *proteus sp* and two of them are gram negative bacteria, namely: *E. coli*. and *Salmonella typhi*. Considering the activity against organisms typically associated with clinical infection, may be a source of novel antibiotic-like compounds that may be used for infection control. Therefore, the aim of this study was to use a novel approach to investigate the output of secreted proteins from the maggots under conditions mimicking clinical treatments. Results revealed that the secretions aseptically markedly inhibit the growth of both gram-positive and gram-negative bacteria.

INTRODUCTION

Maggots of the blowfly *Lucilia sericata* have been successfully used as a debridement agent for chronic and infected wounds through history. First used by ancient people, maggot debridement therapy (MDT) was introduced into Western medicine following World War I by Baer1931 Baer (1931) and extensively used to treat osteomyelitis and gas gangrenous wounds. Following the rediscovery of Alexander Fleming's penicillin from 1929 by Howard Florey and colleagues in 1939 (Pechter .and Sherman 1983), since the discovery of penicillin, numerous antibiotics have been developed, primarily to treat bacterial or fungal infections. This fact constituted an important contribution to human and animal health in the fight against infectious diseases; the use of surgical maggots was abandoned (Pechter and Sherman1983, Sherman and Pechter 1988). However, due to the appearance of antibiotic resistance and increasing problems with chronic wounds worldwide, Seeking novel antibacterial substances and antibiotic combination therapy are the strategic options to overcome the, maggot debridement resistant MDR organisms (Jaklic *et al*., 2008, Steenvoorde and Jukema 2004). Such initiative has greatly driven the search for new antimicrobial agents with novel mechanisms of action that are broadly effective and less likely to induce antimicrobial resistance. These drugs will be very important, particularly for the treatment of immune compromised patients (Guardabassi and Kruse 2003, Hujer *et al*., 2004).

A wide variety of organisms produces such bioactive compounds and some of these natural substances have been shown to be able to kill bacteria (Wenhua *et al*., 2006, Perumal *et al*., 2006). Indeed larvae from this facultative, myiasis causing fly are currently the exclusive blowfly species of choice for larval therapy (the application of maggots to wounds) the treatment has seen a renaissance in modern medicine through pioneering work by Church and other biotherapy advocates. The FDA has approved MDT as a medical device, and maggots are produced aseptically and delivered by commercial companies to wound care centers and hospitals worldwide. MDT has at least two confirmed beneficial effects when applied to wounds. These are debridement (removal of necrotic tissue) Dumville *et al*., 2009 and the removal of pathogenic bacteria. (Mumcuoglu *et al*., 2001). The maggots feed through extracorporeal digestion, by excreting a complex cocktail of enzymes in their excretions/secretions (ES), aim of the work to effect excretion/secretion (ES) products on five bacterial and determine the nature, of the antibacterial activity exhibited by maggot excretion/secretion (ES) products by analyzed (gel electrophoresis SDS, PAGE and GC MS, Gas chromatography mass spectra).

MATERIALS AND METHODS

Sample collection

The samples were caught from different places including gardens, around livestock, in Al-Azhar University Assiut and were sent to the laboratory (Zoology Department) in faculty of science, in appropriately labeled tubes. Chicken viscera, cattle liver were used as bait for collecting adult stage of the fly in open area used hand catch by net was performed in March 2015. Beginning in mornings and continued until a sufficient number of specimens for the colonization process had been captured. Information such as place and date of collection, sampling method and weather condition were recorded (Hall 1995).

The experimental study was performed from April to June 2015. The adult forms were kept in 40 × 40 × 40 cm cages. The flies were maintained in the laboratory under controlled conditions of mean temperature of 30±4°C, relative humidity of 80±10%, and daily light /dark period of 12 h. protected with an external net curtain to avoid the entry of other insect species. After laying eggs, the dead specimens were identified morphologically by using the taxonomic keys of (James 1974, Zumpt 1965, Whitworth 2006) The flies were fed on three diets (liver, meat and Beef), that were evaluated over three continuous generations and replicate of three cages for each food (F1, F2 and F3 generation). The flies fed on diets were placed in the Petri dish and a carbohydrate-rich source 30% sucrose (Sherman and Wyle 1996). Supervision of rearing cages was essential and larvae were isolated from rearing cages. Upon emerging, the adults were placed in new cages and provided with essential food (Spiller 1996). Recording the time required for eggs hatching, larval stage developments, and pupation, and total time for egg-eclosion was performed every three hours intervals for eggs and every six hours intervals for larvae and pupa. On each recording occasion, at least 10 individual of each cages, checked on a light microscope.

Extracting larval excretions and secretions (ES)

Eggs laid by the colony established with flies from the *lucilia sericata* were taken for extracting larval ES and providing continuity for the colony. *L. sericata* larvae were bred in the same conditions as described above and used as control in later assays. These larvae were taken from a previously-established colony (Rueda 2010). The ES were prepared following the methodology described by (Ely *et al*., 2004). Briefly, 200 from each first-, second- and third-instar larvae from *L. sericata* colonies

were collected and washed for 5 min with dd water then 5 min with d .d water then washed for 10 min with a 0.5% sodium hypochlorite, 5% formaldehyde solution and sterile water. The larvae were put in dark falcon tube and then add 1 mL MilliQ sterile water then incubated at 37 ◦C for 2 h.

Antibacterial activity assay

Composition of nutrient Broth medium .
- -Peptone 5 g/l
- -NaCl 5 g/l
- -Beef extract 3 g/l
- -Adjusted pH at 7.

Sterilized medium by Autoclave at 121oC for 15 min.

Composition of nutrient agar medium.
- -Peptone 5 g/l
- -NaCl 5 g/l
- -Beef extract 3 g/l
- -Agar 15 g/l
- -Adjusted pH at 7.

- Sterilized medium by Autoclave at 121oC for 15 min.

The obtained extract was tested for their antibacterial activity against five different pathogenic bacterial strains. Three of them are gram positive bacteria, namely: *Staphylococcus aureus*, *streptococcus sp*, and *proteus* sp and two of them are gram negative bacteria, namely: *E.coli.* and *Salmonella typhi*. The tested bacterial cultures were inoculated in Petri dish then poured with sterilized nutrient agar medium then allowed to solidify; the wells of 0.5cm was made in the medium by using sterilized cork borer. 120µl of crude extract was transferred in to wells. All plates were incubated at 37^{o}C, and take measure of inhibition zone around the well after 4h to all strain of bacteria, by Verneer Caliper to the nearest 0.02 mm. after incubation period was finished all plates were observed for formation of inhibition zone for 24H , (Ely *et al*., 2004; Petit *et al*., 2004). Data were included in the analysis program of SPSS for windows, version 16, and Excel program for windows to make paint statistical curve.

Separating of *Lucilia sericata* larval ES protein profile by SDS-PAGE:

The sample of ES was spun at 14,000 rpm for 5 min at 4 ◦C and the supernatant (containing the ES product) was quantified using a the Biuret method (Weichselbaum 1946, Josephson and Gyllensward 1975), and stored at −20 ◦C until use ES were run on 14% SDS-PAGE in reducing conditions. Briefly, 50 microlitre ES from larval stage fed on meat were in SDS reducing buffer (0.5M Tris–HCl pH 6.8, 25% glycerol, 10% SDS, 0.5% bromophenol blue and 5% -mercaptoethanol), heated at 60 ◦C for 5 min and then run on SDS-PAGE at 70 V. The gels were stained with Coomassie blue (0.1% Coomassie blue, 40% methanol and 10% acetic acid) or Simply Blue Safe Stain for 1 h at room temperature and then incubated with a distaining solution (40% methanol (v/v) and 5% acetic acid (v/v)) or distilled water for 1 day for visualizing the protein profile. A sample of *L. sericata* ES fed in line with the aforementioned parameters was also evaluated.

Gas chromatography-mass spectrometry (GC-MS):

Then the maggot ES was analyzed for presence of an antibacterial factor using gas chromatography-mass spectrometry (GC-MS). The extracted ES was made to undergo gas-chromatography and mass-spectrometry (GC-MS) analysis to test the presence of previously suggested antibacterial compound, phenylacetaldehyde (Nigam *et al*., 2006).

The GC/MS analysis was performed using a Thermo scientific trace GC Ultra/ISQ single Quadrupole MS, TG-5MS fused silica capillary column (30m, 0.250mm,0.25µm film thickness) for GC/MS detection an electron ionization system with ionization energy of 70 electron volt was used Helium gas was used as the carrier gas at a constant flow rate of 1ml/min. The injector and MS transfer line temperature was set at 280 ^{o} C .The oven temperature was programmed at an initial temperature at 40 ^{o} C (hold 3 min) at 280 ^{o} C as a final temperature at an increasing rate of 5 ^{o} C/min (hold 5 min).

The quantification of all the identified components was investigation using a

percent relative peak area. A tentative identification of the compound was performed based on the comparison of their relative retention time and mass spectra with those of the Nist, Willy library data of the GC/MS system.

RESULTS

Effects of Excretion and Secretion crude of larvae against different bacterial strains after 24 h, 48 h and 72 h.

We make test on different bacteria strains by ES of larvae that fed on liver, meat and beef burger for 24h, 48h and 72h we obtained on the ES of larvae that fed on meat is higher effect and the inhibition zone stop growth after 24h for three types of ES of larvae that fed on liver, meat and beef burger. According to results that we obtained when were the larvae reared on liver, meat and beef burger diets the meat food was a good media for the duration and the development of stages for this fly, and when we make test on different bacteria strains by ES of larvae that fed on liver, meat and beef burger for 24h, 48h and 72h found the ES of larvae that fed on meat is higher effect and the inhibition zone stop after 24h for three types of ES of larvae that fed on liver, meat and beef.(Tables 1, 2, 3) and Figs. (1, 2, 3 and 4) So that we measured inhibition zones every 4h for 24 hours for the ES of larvae that fed on meat. For this reasons we make Gas-chromatography and mass-spectrometry (GC-MS) analysis for larval ES that fed on meat.

Table 1: Inhibition zone (cm) of crude Excretion and Secretion of *Lucilia sericata* larvae that fed on meat against different bacteria strains after 24 h, 48 h and 72 h.

Types of bacteria	Inhibition zone (cm) ±SD of larvae ES that fed on meat diet 24H	Inhibition zone (cm) ±SD of larvae ES that fed on meat diet 48H	Inhibition zone (cm) ±SD of larvae ES that fed on meat diet 72H
E -coli	1.79±0.031[a]	1.79±0.031[a]	1.79±0.031[a]
Proteus sp	1.94±0.007[a]	1.94±0.007[a]	1.94±0.007[a]
Staphylococcus aureus	2.66±0.031[b]	2.66±0.031[b]	2.66±0.031[b]
Streptococcus sp	2.72±±0.003[b]	2.72±±0.003[b]	2.72±±0.003[b]
Salmonella typhi	2.96±0.045[c]	2.96±0.045[c]	2.96±0.045[c]

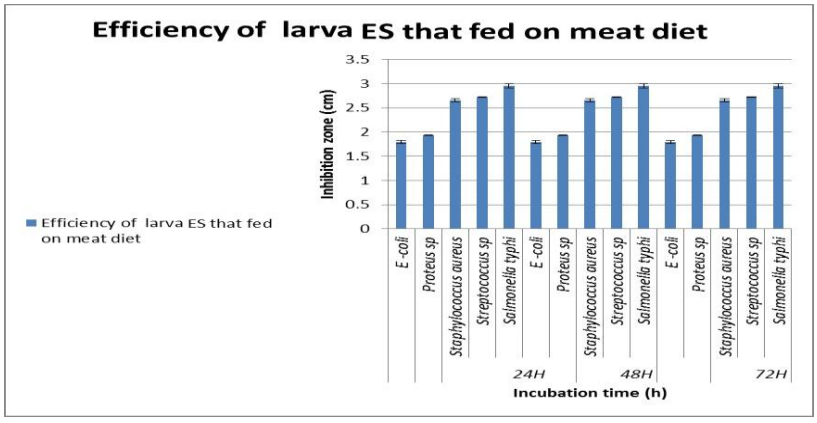

Fig. 1: Efficiency of larvae ES that fed on meat diet on different strains of bacteria after 24h, 48h and72 h.

Table 2: Inhibition zone (cm) of crude Excretion and Secretion of *Lucilia sericata* larvae that fed on liver against different bacteria strains after 24 h, 48 h and 72 h.

Types of bacteria	Inhibition zone (cm) ±SD of larvae ES that fed on liver diet 24H	Inhibition zone (cm) ±SD of larvae ES that fed on liver diet 48H	Inhibition zone (cm) ±SD of larvae ES that fed on liver diet 72H
E -coli	1.68 ±0.098[a]	1.68 ±0.098[a]	1.68 ±0.098[a]
Proteus sp	1.85±0.007[a]	1.85±0.007[a]	1.85±0.007[a]
Staphylococcus aureus	2.53±0[b]	2.53±0[b]	2.53±0[b]
Streptococcus sp	2.60±0.120[b]	2.60±0.120[b]	2.60±0.120[b]
Salmonella typhi	2.77±0[c]	2.77±0[c]	2.77±0[c]

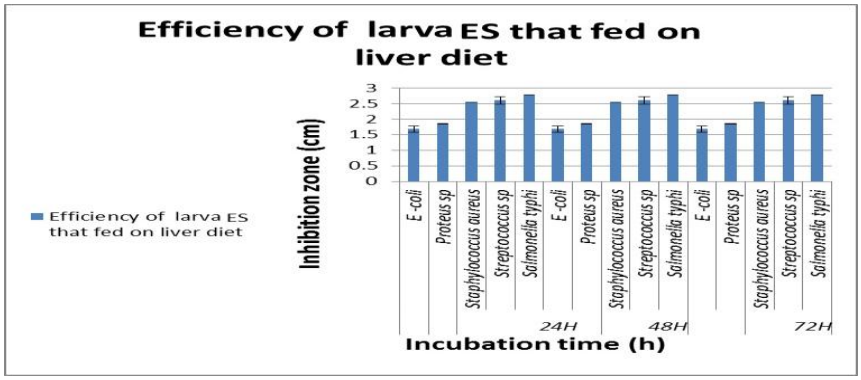

Fig. 2: Efficiency of larvae ES that fed on liver diet on different strains of bacteria after 24h, 48h and 72 h.

Table 3: Inhibition zone (cm) of crude Excretion and Secretion of *Lucilia sericata* larvae that fed on beef burger against different bacteria strains after 24 h, 48 h and 72 h.

Types of bacteria	Inhibition zone (cm) ±SD of larvae ES that fed on beef diet 24H	Inhibition zone (cm) ±SD of larvae ES that fed on beef diet 48H	Inhibition zone (cm) ±SD of larvae ES that fed on beef diet 72H
E –coli	1.62±0.031[a]	1.62±0.031[a]	1.62±0.031[a]
Proteus sp	1.83±0.031[a]	1.83±0.031[a]	1.83±0.031[a]
Staphylococcus aureus	2.47±0.14[b]	2.47±0.14[b]	2.47±0.14[b]
Streptococcus sp	2.51±0.063[b]	2.51±0.063[b]	2.51±0.063[b]
Salmonella typhi	2.70±0.084[c]	2.70±0.084[c]	2.70±0.084[c]

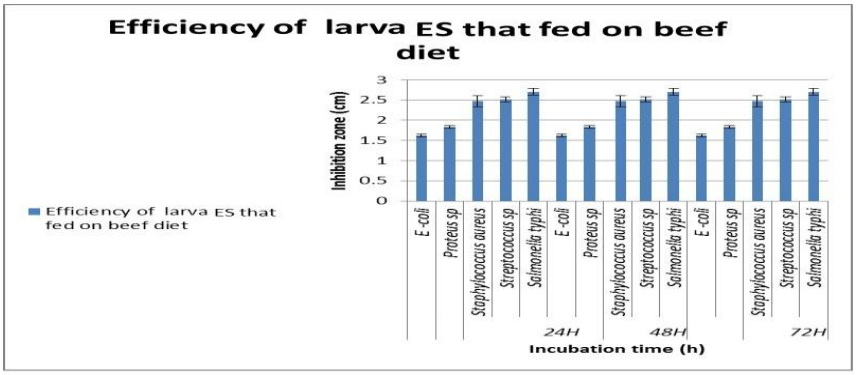

Fig. 3: Efficiency of larvae ES that fed on beef burger diet on different strains of bacteria after 24h, 48hand72 h.

Fig. 4: Inhibition zone effect of *lucilia sericata* crude SE.

Effects of Excretion and Secretion crude of larvae that fed on meat against different bacterial strains every 4h for 24h.

Crude excretion and secretion of *L. sericata* larvae from colonies that fed on meat displayed a significant effect against different gram-positive and gram-negative bacterial strains. So that we measured inhibition zones every 4h for 24 hours. All tested bacterial strains were found to be susceptible to ES. Indicating that ES was more effective against *Salmonella typhi*, *Streptococcus sp*, *Staphylococcus aureus*, *Proteus* sp and *E-coli* in descending order as shown from Table (4).

Table 4: Values of inhibition zones crude ES of *L. sericata* larvae against different bacterial strains after 4h, 8h and 12h, 6h, 20h and 24h

	E-coli	*Proteus sp*	*Staphylococcus aureus*	*Streptococcus sp*	*Salmonella typhi*
4h	1.27 ± 0.037^{a}	1.33 ± 0.014^{a}	1.71 ± 0.038^{b}	1.73 ± 0.040^{b}	1.89 ± 0.060^{c}
8h	1.36 ± 0.011^{a}	1.41 ± 0.008^{a}	1.88 ± 0.027^{b}	1.87 ± 0.10^{b}	2.17 ± 0.17^{c}
12h	1.45 ± 0.014^{a}	1.54 ± 0.029^{a}	2.11 ± 0.029^{b}	2.14 ± 0.14^{b}	2.37 ± 0.22^{b}
16h	1.66 ± 0.038^{a}	1.80 ± 0.054^{a}	2.51 ± 0.060^{b}	2.59 ± 0.020^{b}	2.73 ± 0.040^{c}
20h	1.70 ± 0.040^{a}	1.86 ± 0.066^{b}	2.58 ± 0.061^{c}	2.61 ± 0.016^{c}	2.79 ± 0.049^{d}
24h	1.79 ± 0.031^{a}	1.94 ± 0.007^{a}	2.66 ± 0.031^{b}	2.72 ± 0.003^{b}	2.96 ± 0.045^{c}

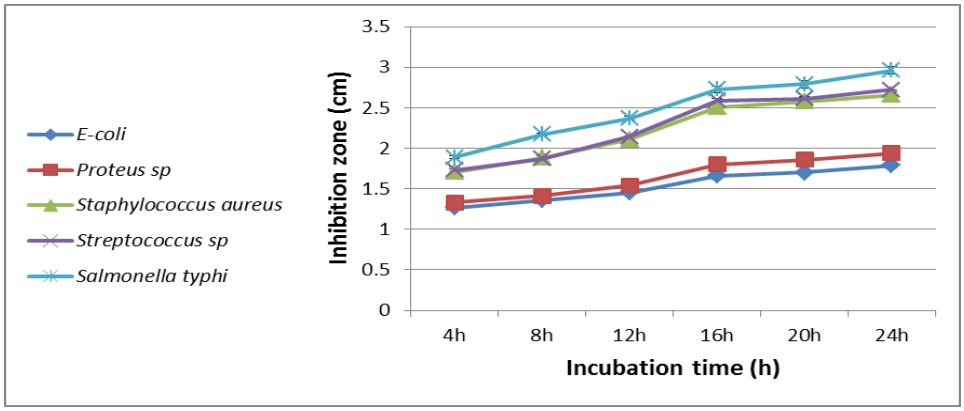

Fig. 5: Development inhibition zones Crude ES *L. sericata* larvae of against different bacterial strains after 4h, 8h and 12h, 16h, 20h and 24h.

Protein profile of crude Es of *L. sericata* larvae:

Polyacrylamide gel of 14 % was prepared to bring high and low molecular weight protein profiles under observation, and to relate the fluctuations in different protein fractions with larvae of *Lucilia sericata*. Total protein analysis involving SDS-PAGE of ES products from first-, second- and third-larval instar fed on meat diet, within investigated of protein profiles showed the expression of 11 protein fractions ranged between 7-58kDa. Bands at 58 kDa , 46 kDa, 43.09 kDa, 33.63 kDa , 30 kDa, 26.31 kDa, 21.42 kDa , 17kDa, 15.6 kDa, 8.50 kDa and 7 kDa were observed in *L. sericata*. Dominant 175 kDa, 80 kDa and 23 kDa bands for larvae ES were disappeared in the ES protein profile for larvae fed on meat diet was thus analyzed in this stage showed in (Table 5 and Fig 6).

Crude ES *Lucilia sericata* larvae have been injected into GC-MS, and the bioactive peaks were recorded for further structural identification. The results provided important leads for chemical identification the compounds which triggers the response are be identified and listed out in Table (6) and Fig. (7).

In this analysis showed a large amount of protein, lipids and smallamounts of carbohydrates contains on the chemical composition of ES.

The chromatogram of ES show peaks of the 21 substances whose share in the total ion current. The identified components in raw fully overlap because of a different number of the registered peaks.

Table 5: Separating of *Lucilia sericata* larval ES that fed on meat by SDS-PAGE protein profile.

Band No.	M0l. Wt.	Amount	ES
1	58	1.007	+
2	46	0.308	+
3	43.09	5.08	+
4	33.63	7.77	+
5	30	18.28	+
6	26.31	5.21	+
7	21.42	2.47	+
8	17	1.53	+
9	15.6	28.42	+
10	8.50	9.004	+
11	7	96.16	+

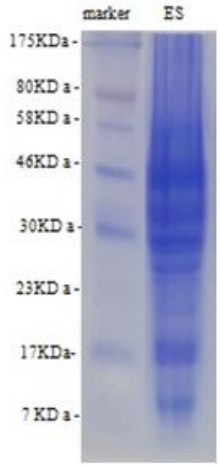

Fig. 6: Separating of *Lucilia sericata* larval ES that fed on meat by SDS-PAGE protein profile: Gas-chromatography and mass-spectrometry (GC-MS) analysis of larval ES that fed on meat.

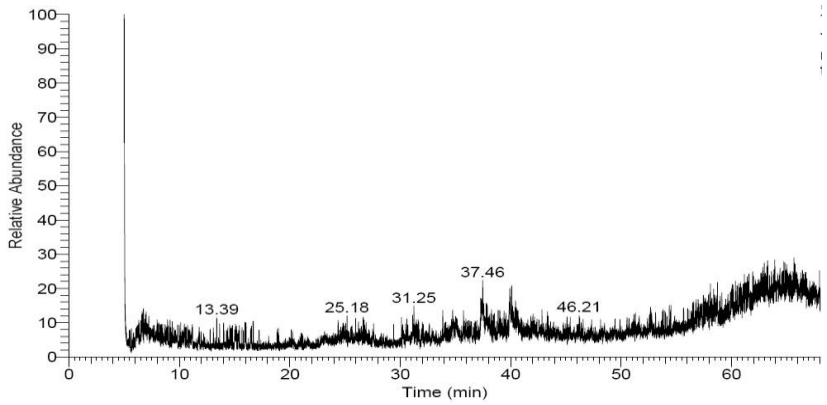

Fig. 7: Chromatogram of extracted from ES of *Lucilia sericata* larvae

Table 6: Relative chemical composition of extracts of *Lucilia sericata* larvae ES that fed on meat.

NO / RT	Compound Name	Area %	Area	Molecular Weight	Molecular Formula
1- 22.91	2,5-Diamino-6-methyl-4-pyrimidinol	1.45	11978.65	140	C5H8N4O
2- 23.24	2-Bromo-4-""[2-(1,3di oxalan2yl) ethenyl]-4,4':2',2":4",4"':2"',2""-qui Nquepyridine	1.16	9579.87	563	C30H22BrN5O2
3- 23.52	6-[Chloro(p-tolylsulfiny l) methyl]-6-undecanol	0.54	4468.50	358	C19H31ClO2S
4- 31.26	Thieno[2,3-b] pyridine-2-carboxamide,3amino	1.85	15336.64	315	C16H14FN3OS
5- 34.65	11- pheny-l2,4,6,8-tetra(2-thienyl) 11-aza5,13-dithiatetracyclo[7.3.0.1 (2,8).0(3,7)]trideca-3,6-diene-10,12,13-trione	2.07	17149.11	657	C32H19NO3S6
6- 34.76	Benzene,(1-Butylheptely)	9.16	75923.89	232	C17H28
7- 35.09	2,4-bis(áchloroethyl)-6,7-bis[Ámethoxycarbo nylethyl]-1,3,5-trimethylporphyrin	1.69	13969.08	648	C35H38Cl2N4O4
8- 37.47	(4-Bromophenyl) bis(2,4-dibromophenyl) amine	20.96	1736	635	C18H10Br5N
9- 39.91	Pentacosane,13-phenyl-(CAS)	12.85	1064	428	C31H56
10- 40.09	Pentacosane,13-phenyl	6.26	51846.01	428	C31H56
11- 40.27	3-(1-Cyclopentenyl)furan	0.60	4945.69	134	C9H10O
12- 40.46	Benzene,(1-propylnonyl)	1.43	11863	246	C18H30
13- 40.59	Pentacosane,13-phenyl-(CAS)	1.56	12921	428	C31H56
14-40.66	3,4,5,6-Tetrakis(pchlorophenoxy)1,2-dicyanobenzene	1.04	8588.19	632	C32H16Cl4N2O4
15-59.63	Pregnane-3,11,20,2-1tetrol, cyclic 20,21-(butyl boronate), (3à,5á,11á,20R)-(CAS)	0.67	5579.41	418	C25H43BO4
16-61.59	1-Monolinoleoylglycerol trimethylsilyl ether	1.08	8966.41	498	C27H54O4Si2
17- 62.89	Heptasiloxane,1,1,3,3,5,5,7,7,9,9,11,1 1,13,13tetradecamethy-l	0.93	7713.49	504	C14H44O6Si7
18- 64.53	Glycerine-1,3-dimyrist ate, 2-O-trimethylsilyl-	1.28	10596.24	584	C34H68O5Si
19- 64.67	Lucenin 2	1.05	8710.55	610	C27H30O16
20- 67.32	Lucenin 2	0.75	6202.81	610	C27H30O16
21-67.63	4,6-Dimethoxy-7-(4',6'-dimethoxyIindol-2-'yl)-2,3-diphenylindole	2.17	17942.12	504	C32H28N2O4

DISCUSSION

Antimicrobial study:

Effects of Excretion and Secretion crude of larvae that fed on meat against different bacterial strains every 4h for 24h.

Excretion and Secretion crude of larvae from *L. sericata* colonies that fed on meat displayed a significant effect against different gram-positive and gram-negative bacterial strains employed in this study. Inhibition zones were measured every 4h for 24 hours. The crude SE caused a marked inhibition in bacterial growth with inhibition zones for *E-coli* of 1.27, 1.36, 1.45, 1.66, 1.70 and 1.71 cm. for *Proteus sp* it measured 1.33, 1.41, 1.54, 1.80, 1.86 and 1.89 cm.for *Staphylococcus aureus* it measured 1.71, 1.88, 2.11, 2.51, 2.58 and 2.60 cm. for *Streptococcus sp* the inhibition zone was 1.73, 1.87, 2.14, 2.59, 2.61 and 2.66 cm. *Salmonella typhi* it measured 1.89, 2.17, 2.37, 2.73, 2.79 and 2.82 cm. Growth curves of different bacteria during the incubation period in the presence of various bacteria strains and the same value of ES injection of crude are significant. Crude ES of *Lucilia sericata* larvae displayed a significant effect against different gram-positive and gram-negative bacterial strains. The ES was more effective against *Salmonella tuphi, Streptococcus sp, Staphylococcus aureus,* and less effectives against *Proteus sp and E-coli*. More recent work, using fluorescence microscopy, monitored the death of *E- coli* during larval digestion and demonstrated that bacterial load reduction took place primarily in the midgut of *L. sericata* (Mumcuoglu *et al*., 2001). Many studies have provided the evidence that the antimicrobial action results from both larval ingestion of wound bacteria, which appears to kill the bacteria as they pass through the larval digestive tracts, and by antimicrobial activity of larval components, including salivary gland secretions and faecal waste products. ES from *Lucilia sericata* are important in the lysis of the necrotic tissue. To the best of our knowledge, only a few of its components have been purified and characterized from calliphoridae like protein, aspartyl glucosaminidase-like protein and insecticidal

toxins (Price *et al*., 2009, Quistad *et al*., 1994).

There are several different classes of antibiotics that target specific cell envelope structures or enzymatic steps of cell wall synthesis (Jordan *et al*., 2008). Results of this study revealed that crude ES is effective against gram-positive and gram-negative bacteria, which might be related to the difference in cell envelope structure. Maggot ES are differentially effective against biofilms of *S. aureus* and *P. aeruginosa* indicating that different molecules within ES are responsible for the biofilm disruption. Cell wall of bacteria comprises a complex structure that is fundamentally different between gram positive and gram-negative bacteria. Cell wall in gram-positive bacteria is thick (15–80 nm), consisting of several layers of peptidoglycans and molecules of teichoic acids. In contrast, cell wall of gram-negative bacteria is relatively thin (10 nm) in and is composed of a single layer of peptidoglycan surrounded by a membranous structure (the outer membrane) which may invariably contain lipopolysaccharides. Thus, the outer membrane is more hydrophobic in gram-negative than in gram-positive bacteria and constitutes a target for being tracked by hydrophobic agents and other antibiotic agents (Schwarz and Reiter2001, Singleton2004). It has been shown that once the larvae have been applied to a wound, they trigger processes that help to debride, eliminate bacteria and stimulate the formation of granulation tissue by means of releasing ES protein products (Horobin *et al*., 2003). It has been reported that the higher proteolytic activity in second and third larval stage could be associated with the highest increase in weight after their molting (Cepeda-Palacios *et al*., 1999). In the same way, (Figueroa *et al*., 2006) reported the application of sterile larvae of *Lucilia sericata* Meigen (Diptera: Calliphoridae) in patients with chronic ulcers. The results obtained in this study shows that disinfection of the larvae of *Lucilia sericata* made with NaClO is effective for obtaining sterile larvae for use in larval therapy. We suggest the treatment with NaClO at 0.5%, because it uses enough chemicals and amount of time for the safe cleaning of the larvae. We caution though that microbiological testing should be part of the routine investigation of sterilization so the larval therapy can be used safely.

Protein profile of *Lucilia sericata* larvae ES that fed on meat:

The Coomassie -stained larval ES gels demonstrated that the second and third larval stage had higher protein production .On the other hand, the proteolytic profile from all analyzed stages showed that had a greater number of bands .It has been reported that the higher proteolytic activity in second and third larval stage could be associated with the highest increase in weight after their molting.

The ES from *L. sericata* larval stage has been shown to display significant antibacterial activity in larval therapy in several disease. The *Lucilia sericata* larvae ES protein profile was characterized by having bands from 7kDa to 58 kDa it was found that ES from those fed on a natural diet contained a greater amount of proteins there by coinciding with previous reports where it was described that larvae fed on a natural diet (especially decomposing tissue or bacteria-rich environments) secrete a greater amount of proteins (Anderson 2000). The main proteases detected in the larvae from other dipteral species playing a very important role in healing wounds.

Gas-chromatography and mass-spectrometry (GC-MS) analysis of larval ES that fed on meat.

As can be seen from data of ES larvae the energy value is explained by its high content of protein, (Bogdanov 2015). The energy value of ES is even somewhat higher owing to a higher content of lipids and sugars. As can be seen, this is very much in agreement with the literature data results, as regards the crude homogenate (Bărnuţiu *et al*., 2013).

However, a marked difference can be observed between our data concerning the total protein and carbohydrate content and

those reported by (Narumi 2004). The determination of the vitamin and mineral content in the ES was beyond our research scope, however the data presented by other authors (Burmistrova 1999, Narumi, 2004) demonstrated a high content of water soluble B complex vitamins such as thiamine, riboflavin, niacin, pyridoxine, biotin, cobalamine, folic and pantothenic acids. These larvae are usually treated as unwanted material or as a waste product to be thrown away. One of the most numerous groups of components identified in ES, consists of free amino acids the count included all essential amino acids. The relative content of free amino acids in the ES of larvae turned out to be higher. These data are well in agreement with previously obtained results (Lazaryan 2002).

Noticeable differences in the relative content also apply to the other multi-component group of the homogenate, namely sugars. However, neither of them contains any significant quantities of substances with considerable antibacterial properties. So that we can state that ES is a valuable product and also a prospective pharmaceutical raw material to be used for medical purposes.

In particular, it is necessary to investigate the content and dynamics of ES changes in the development process of the larvae. Thus larvae have a variety of biological and health-promoting properties which make it an ideal additive to be used for medical purposes.

REFERENCES

Anderson, G.S. (2000). Minimum and maximum development rates of some forensically important Calliphoridae (Diptera). J. *Forensic Sci.* 45: 824-832.

Baer, W.S. (1931). The treatment of chronic osteomyelitis with the maggot (larva of the blow fly). J Bone Joint Surg; 13: 438.

Bărnuţiu, L.I.; Mărghitaş, L.; Dezmirean, D.; Bobiş, O.; Cristina Mihai, C.; and Crenguţa Pavel, C. (2013). Physicochemical composition of *Apilarnil* (bee drone larvae).

Bogdanov, S. (2015). Royal Jelly, Bee Brood: Composition, Health, Medicine: A Review. Bee Product Science, from www.bee-hexagon.net. April 2015.

Burmistrova, L.(1999). Physico-chemical analysis and biochemical appreciation of drone brood. PhD Thesis, Ryazan Medical University (in Russian).

Cepeda-Palacios, R.; Avila, A.; Ramirez-Orduna, R.; and Dorchies, P. (1999). Estimation of the growth patterns of *Oestrus ovis L.* larvae hosted by goats in Baja California Sur. *Mexico Vet. Parasitol*. 86: 119–126.

Dumville, J. C.; Worthy, G.and Bland, J. M. (2009). Larval therapy for leg ulcers (VenUS II): randomised controlled trial. BMJ; 338: b773.

El-Ebiarie, A. and Taha, N. (2012). Molecular characterization of serine proteases from both first and third larval instars of Chrysomya megacephala. Life Sci. J. 9, 2086–2093.

Ely, R.; Supriya, T.; and Naik, C. G. (2004). Antimicrobial activity of marine organisms collected from the coast of South East India. Journal of Experimental Marine Biology and Ecologe, 304: 121-127.

Figueroa, L.; Uherek, F.; Yusef, P.; López, L.; and Flores, J. (2006). Maggot therapy in patients with chronic skin ulcers. *Parasitol Latinoam* 61: 160-164.

Guardabassi, L. and Kruse H. (2003). Overlooked aspects concerning development and spread of antimicrobial resistance. Central European symposium on antimicrobial resistance, Brijuni, Croatia, 4–7 July, 2003. Expert Rev Anti Infect Ther, 1(3):359–362.

Hall, M. J. (1995). Trapping the flies that cause myiasis :their responses to host stimli. Ann. Trop. Med. Parasitol., 89(4):333-357.

Horobin, A. J.; Shakesheff, K. M.; Woodrow, S.; Robinson, C. and Pritchard, D. I. (2003). Maggots and wound healing: an investigation of the effects of secretions from *Lucilia sericata* larvae upon interactions between human dermal fibroblasts and extracellular matrix components. *Br. J. Dermatol*., 148: 923–933.

Hujer, A.M.; Bethel, C.R.; Hujer, K. M. and Bonomo, R. A. (2004). Antibiotic resistance in the institutionalized elderly. Clin Lab Med., 24(2):343–361.

Jaklic, D.; Lapanje, A. and Zupancic, K. (2008). Selective antimicrobial activity of maggots

against pathogenic bacteria. J Med Microbiol., 57: 617–25.

James, M.T.(1974). The flies that cause myiasis in man. United States Department of Agriculture Miscellaneous Publication; 631: 1–175.

Jordan, S.; Hutchings, M.I. and Mascher, T. (2008). Cell envelope stress response in Gram-positive bacteria. FEMS Microbiol Rev., 32(1):107–146.

Josephson, B. and Gyllensward, A. S. (1975) J. Clin. Lab. Invest., 9:29.

Lazaryan, D. S. (2002). Comparative amino acids analysis in bee brood.Pharma. Chem. J., 36: 680–682.

Mumcuoglu, K.Y.; Miller, J. and Mumcuoglu, M. (2001). Destruction of bacteria in the digestive tract of the maggot of Lucilia sericata (Diptera: Calliphoridae). J Med Entomol, 38: 161–6.

Mumcuoglu, K.Y.; Miller, J. and Mumcuoglu, M. (2001). Destruction of bacteria in the digestive tract of the maggot of Lucilia sericata (Diptera: Calliphoridae). J Med Entomol, 38: 161–6.

Narumi, S. (2004). Honeybee brood as a nutritional food. Mitsubachi Kagaku (Honeybee Science), 25:119–124.

Nigam, Y.; Bexfield, A.; Thomas, S. and Ratcliffe, N. A. (2006). Maggot therapy: The science and implication for CAM part Ⅱ-Maggots combat infection. Evidence-based Complementary and Alternative Medicine, 3:303-308.

Pechter, E. A. and Sherman, R. A. (1983) Maggot therapy: the surgical metamorphosis. Plast Reconstr Surg, 72: 567–70.

Perumal Samy, R.; Pachiappan, A.; Gopalakrishnakone, P.; Thwin, M. M; Hian, Y. E.; Chow, V. T.; Bow, H. and Weng, J. T. (2006). In vitro antibacterial activity of natural toxins and animal venoms tested against *Burkholderia pseudomallei*. BMC Infect Dis., 6:1–16.

Petit, K. E.; Mondeguer, F.; Roquebert, M.F.; Biard, J.F.; and Pouchus, Y. F. (2004). Detection of griseofulvin in a marine strain of *Penicillium waksmanii* by ion trab mass spectrometry. J. Microbiol. Methods, 58: 59-65.

Price, D. R.; Bell, H. A.; Hinchliffe, G.; Fitches, E.; Weaver, R.; and Gatehouse, J. A. (2009). Avenom metalloproteinase from the parasitic wasp *Eulophus pennicornis* is toxic towards its host, tomato moth (*Lacanobia oleracae*). Insect Mol. Biol., 18(2):195–202.

Quistad, G.B.; Nguyen, Q.; Bernasconi, P. and Leisy, D. J. (1994). Purification and characterization of insecticidal toxins from venom glands of the parasitic wasp, *Bracon hebetor*. Insect *Biochem Mol Biol*., 24(10): 955–961.

Rueda, L.C.; Ortega. L.G.; Segura, N.A.; Acero, V.M. ; and Bello, F. (2010). *Lucilia sericata* strain from Colombia: Experimental colonization, life tables and evaluation of two artificial diets of the blowfly *Lucilia* sericata (Meigen) (Diptera: calliphoridae), Bogota, Colombia strain. Biological Research, 43:197-203.

Schwarz, G. and Reiter, R. (2001). Negative cooperativity and aggregation in biphasic binding of mastoparan X peptide to membranes with acidic lipids. *Biophys Chem*., 90 (3):269–277.

Sherman, R. and Wyle, F. (1996). Low-cost, low-maintenance rearing of maggot in hospitals,clinics and schools. Am J Trop Med Hyg, 54: 38–41.

Sherman, R.A. and Pechter, E.A. (1988) Maggot therapy: a review of the therapeutic applications of fly larvae in human medicine, especially for treating osteomyelitis. Med Vet Entomol, 2: 225–30.

Singleton, P. (2004). Bacteria in Biology, Biotechnology and Medicine. Chichester: John Wiley and Sons.

Spiller, D. (1996). Insect colonization and mass production. In: Smith CN, editor .*Houseflies* :Chapter 14.New York and London: Academic Press., 203-225.

Steenvoorde, P. and Jukema, G. N. (2004). The antimicrobial activity of maggots: in-vivo results. J Tissue Viability, 14: 97–101.

Weichselbaum, T. E. (1946). Amer. Clin. Path., 16: 40-48.

Wenhua, R.; Shuangquan, Z.; Daxiang, S.; Kaiya, Z and Guang, Y. (2006). Induction, purification and characterization of an antibacterial peptide scolopendrin I from the venom of centipede *Scolopendra subspinipes multilans*. Indian J Biochem Biophys, 43:88–93.

Whitworth, T. (2006). Keys to the genera and species of blow flies (Diptera: Calliphoridae) of America north of Mexico. Proceedings of the Entomological Society of Washington, 108: 689-725.

Zumpt, F. (1965). Myiasis in man and animals in the Old World. A textbook for physicians, veterinarians and zoologists. Butterworth & Co., London.

ARABIC SUMMERY

مميزات مضادات البكتريا لإفرازات يرقات الذباب الأخضر ليوسيليا سريكاتا (رتبة ذات الجناحين- عائلة كاليفوريدى)

محمد الشهابي , مهران تونى , عبد الرحمن سلطان , عبد الباسط محمد أحمد عبد الرحيم
قسم علم الحيوان, كلية العلوم بنين, جامعة الازهر, أسيوط , مصر

الإفرازات التي تم جمعها في جو معقم و مطهر من يرقات أو ديدان الذبابة الخضراء (لرتبة ذات الجناحين :عائلة كاليفوريدى) ميجان تظهر نشاط مضادات الميكروبات جنبا إلى جنب مع غيرها من الأنشطة المفيدة لالتئام الجروح .مع ظهور البكتيريا المقاومة للأدوية المتعددة. تم فحص الإفرازات الجد يدة لتحديد المركبات النشطة المسئولة عن النشاط المضادة للميكروبات. استخدمت خمسة سلالات بكتيرية مختلفة المسببة للأمراض في هذه الدراسة، ثلاثة منهم بكتيريا إيجابية الجرام، وهما: المكورات العنقودية الذهبية، نوع العقدية، نوع المتقلبة واثنان منهم بكتيريا سالبة الجرام، وهي: إشرشيا كولاي. والسالمونيلا التيفود. وبالنظر إلى النشاط ضد الكائنات التي ترتبط عادة مع العدوى السريرية، قد تكون مصدرا للمركبات مثل المضادات الحيوية الجديدة التي يمكن استخدامها لمكافحة العدوى. لذلك، كان الهدف من هذه الدراسة هو استخدام نهجا جديدا للتحقيق في إنتاج بروتينات يفرز من اليرقات في ظل ظروف محاكاة العلاجات السريرية وكشفت النتائج أن إفرازات التي تم جمعها في جو معقم و مطهر من يرقات أو ديدان الذبابة الخضراء تمنع بشكل ملحوظ من نمو البكتيريا إيجابية الجرام على حد سواء وسلبية الجرام