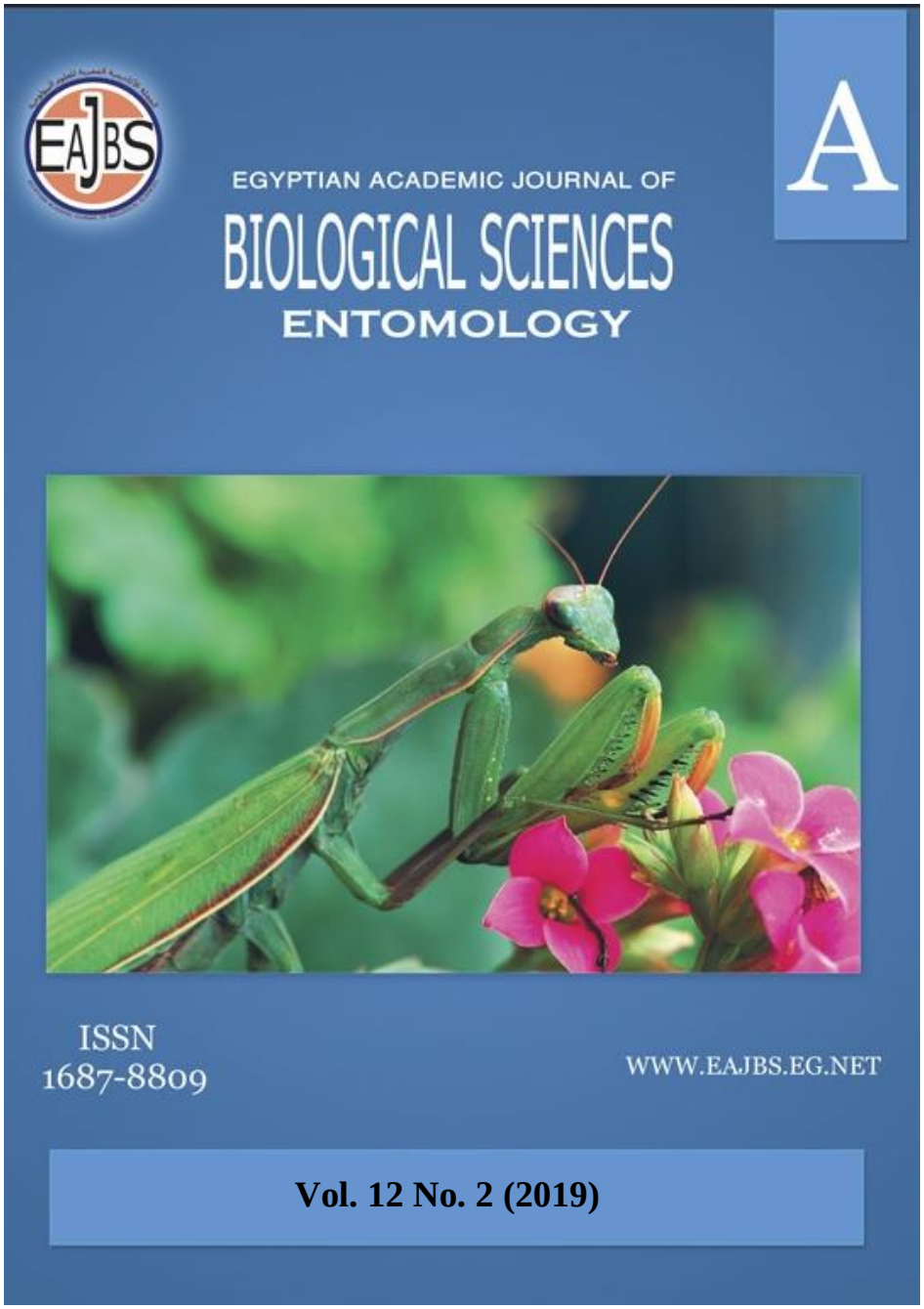

Egyptian Academic Journal of Biological Sciences is the official English language journal of the Egyptian Society for Biological Sciences, Department of Entomology, Faculty of Sciences Ain Shams University.

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www.eajbs.eg.net

Citation: *Egypt. Acad. J. Biolog. Sci. (A. Entomology) Vol. 12(2) pp: 19- 33 (2019)*

Egypt. Acad. J. Biolog. Sci., 12(2):19– 33 (2019)

Egyptian Academic Journal of Biological Sciences
A. Entomology

ISSN 1687- 8809
http://eajbsa.journals.ekb.eg/

Antimicrobial and Antiviral Activity of *Lucilia sericata, Chrysomya albiceps* (Diptera: Calliphoridae) And *Musca domestica* (Diptera: Muscidae) Whole Body Extract

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ARTICLE INFO

Article History
Received:29/1/2019
Accepted:2/3/2019

Keywords:
Antimicrobial activity; Antifungal activity and Antiviral activity; whole body extracts; *Lucilia sericata*; *Chrysomya albiceps*; *Musca domestica*;; cytotoxic; Vero cell line; MTT; hepatitis A virus.

ABSTRACT

The present study dealt with evaluating the effect of whole body extracted by ethyle acetate of *L. sericata, C. albiceps* and *M. domestica* as exhibited antibacterial activity against some species of bacteria. They were shown to inhibit the growth of Gram–positive bacteria *Staphylococcus aureus* and *Staphylococcus epidermidis*, Gram–negative bacteria, *Escherichia coli* and *Klebsiella pneumoniae* and Fungi, *Aspergillus fumigatus* thus exhibited limited inhibitory effect towards Gram–positive bacteria *Bacillus subtilis* and Gram–negative *Pseudomonas aeruginosa* and Fungi *Syncephalastrum racemosum* and *Candida albicans*, this effect was slowed down when challenged with secretion on a solid media but no zone of complete inhibition was detected. Growth inhibiting activity was determined in liquid growth media using the Gram–positive, Gram–negative bacterial and fungal strains as indicator organisms. The antiviral activity of whole body extracted by ethyle acetate of *L. sericata, C. albiceps and M. domestica* nES were tested against Vero cell line was developed for isolation and propagation of many enteroviruses and hepatitis A and average values were considered.

INTRODUCTION

The antibacterial properties of whole body extracted by ethyle acetate that has been studied most extensively. Although in vitro studies of the antibacterial activities have revealed that they are potent against many pathogenic species of bacteria (Pavillard & Wright, 1957; Robinson & Norwood, 1933 ; Simmons, 1935 ; Vistnes et al., 1981), the effects of whole body extracts have not been determined for the diverse groups of micro-organisms that can infect chronic wounds. Body extracts fulfilled the required definitions of an antiseptic. In addition, the larval extracts ability to ingest bacteria was also evaluated. Body extracts contained viable bacteria after 48 h of contact with the respective organisms. These extracts also continued excreting bacteria. Therefore, extracts should be disposed of after use as they must be regarded as medical waste. Antimicrobial peptides have been shown to be an essential element of the insect's innate defense system,

providing powerful resistance to a wide range of harmful pathogens and parasites (Boman, 1995, 2000). Insects constitute the largest group of extant organisms and perhaps their individual number account for as much as 80% of all known fauna (Leem et al., 1999). In addition, the presence of insects in any ecological system, from waterway to extremely septic environments, has stimulated scientists to look for new therapeutic agents in this class of arthropods (Chernysh et al., 2002). A great part of efforts has been achieved for the investigation and re-examination of insect sources to obtain compounds that may have antimicrobial and anticancer activities (Shehata et al., 2016). Many antibacterial proteins have been isolated from different species of insects (Cociancich et al., 1994) and can be classified into five major groups: cecropins, insect defensins, attacin-like (glycine- rich) proteins, proline- rich peptides and lysozymes (Hultmark 1993). However, only a small number of antifungal peptides/ polypeptides have been reported from insects, for example, drosomycin (Fehlbaum et al., 1994), metchinikowin, cecropin A and B from Hyalophora cecropia (Levashina et al., 1995) and heliomicin from Hellothis virescens (Lamberty et al., 1999). Vero cell line was developed for isolation and propagation of many enteroviruses and hepatitis A. In the current study, Vero cells from passage number 76 grown and maintained in Dulbecco's Modified Eagle's Medium (DMEM) with Hanks salt base, supplemented with 10% fetal calf serum and 50 µg/ml gentamycin antibiotic solution. Rapidly growing virus strains producing a cytopathic effect (CPE) in Vero cell cultures within 3 days was used during this study, Cytotoxicity of crude extracts of *L. sericata C. albiceps M. domestica* were determined through morphological changes in Vero cells treated with different extracts in comparison with untreated control one.

MATERIALS AND METHODS

Insect Used:

The 3rd larval instar of *L. sericata* collected from Tonamel- Village, Aga Center, Al-Dakahlya Governorate, *C. albiceps* and *Musca domesica* were collected from Animal House, Al-Azhar University Cairo Governorate, Egypt and maintained at Medical Entomology Laboratory, Animal House, Zoology Department, Faculty of Science, Al-Azhar University, Cairo, under controlled laboratory conditions of 27 ± 2 ºC for all insect used and 70 ± 5 % RH for just *L. sericata* and *Musca domesica* and 60±10% RH for *C. albiceps* and 12-12 light-dark photoperiod.

Preparation of Whole Body Extracts:

The extraction was performed using a standard procedure described by **Ahn *et al.*, (2000)** and **Meylears *et al.*, (2002)** with small modifications. Third instar larvae of *L. sericata, C. albiceps* and *M. domestica* were washed five times with distilled water at room temperature and then dried with air on a clean bench at room temperature for 6h. Fifty grams of each insect species were thoroughly homogenized in 50ml of 40 mM tris-HCl (pH 7.4), the homogenate was centrifuged at 10,000 rpm for 30 min. at 4°C and the supernatant was used as buffer extract. The residue was soaked in pure methanol or hexane then centrifuged again. The supernatant was considered as methanolic or hexane extract.

Antimicrobial Bioassay:

1. **Antibacterial bioassay:**

Agar well method was performed using Gram-positive, Gram-negative bacterial and fungal strains as indicator organisms.

2. **Microorganisms:**

Six pathogenic bacterial strains were used for the antibacterial assay:

A.The Gram-Positive Bacterial Strains:

1.Staphylococcus aureus (RCMB 29213)

2.Bacillus subtilis (RCMB 29218)

3.Staphylococcus epidermidis (RCMB 29216)

B.The Gram-Negative Bacterial Strains:

1.Pseudomonas aeruginosa (RCMB 25910)

2.Escherichia coli (RCMB 25922)

3.Klebsiella pneumoniae (RCMB 25916)

All tested strains were obtained from The Regional Center for Mycology and Biotechnology Antimicrobial Unit Test Organism, Al-Azhar University, Cairo, Egypt.

3. Evaluation of Antibacterial Activity:

The antibacterial assay employed was broadly based on the standard agar diffusion assay, where by a colony of the target microorganism was picked off a stock plate and suspended in Ringer's solution (10mL). An aliquot of microorganism suspension (100mL) was swabbed onto iso-sensitise agar plates (10mL agar) containing prebored wells (4mm). Secretory products and extracts (25mL) were dispensed into the wells and the plates incubated at 35 ºC for 24h. Radial zones of inhibition (mm) of bacterial growth around the sample wells were noted and used as an arbitrary measure of antibacterial activity.

4. Growth Medium Used for Bioassay Test:

Nutrient agar medium, gm/L **(Tadashi, 1975)**, consisted of: peptone, 5.0; beef extract, 3.0; and agar-agar, 15.0 the pH was adjusted to 7.0 before sterilization.

Procedure:

1. A 24h old culture of each the test microbial strains used.
2. Five ml of sterile distilled water were added to the culture tube and mixed by a vortex mixer.
3. Five drops of the suspension were added to 100 ml of nutrient agar medium at 45 ºC. This was dispensed among petri dishes, 10 ml of each.
4. The insect secretions were dissolved in 1% ethanol in a concentration of 1.0 mg/ml.
5. Analytical paper disks 740-E/2 "Diam" were loaded with insect secretions and aseptically put on the surface of seeded plates with the different test organisms.
6. The plates were left for 2 hours in a refrigerator for diffusion after which the plates were incubated at 30 ºC for 24h.

The detection of an inhibitory clear zone around the paper disks is an indication of the antagonistic properties of the tested larval ES and crude extracts under study.

Antifungal Bioassay:

A.Microorganisms:

Three pathogenic fungi were used for the antifungal assay:

1. ***Aspergillus fumigatus*** (RCMB 02451)
2. ***Syncephalastrum racemosum*** (RCMB 02621)
3. ***Candida albicans*** (RCMB 02833)

All fungi strains were obtained from The Regional Center for Mycology and Biotechnology Antimicrobial unit test organism, Al-Azhar University, Cairo, Egypt.

Growth Medium Used For Bioassay Test:

Sucrose-Nitrate agar medium gm/L **(Tadashi, 1975)**, consisted of sucrose, 30; $NaNO_3$, 2.0; K_2HPO_4, 1.0; $mgSO_4$. $7H_2O$, O.5; agar, 15.0 and distilled water, 1000ml. The pH value was adjusted at 7-7.3 before sterilization.

Procedure:

1- A 48h old culture of each of the test microbial strains was used.
2- Five ml of sterile distilled water were added to the culture tube and mixed by a vortex mixer.
3- Certain filamentous fungi were singly streaked aseptically on the surface of the solid sucrose of the solid Sucrose-Nitrate medium, whereas the unicellular ones (yeast) were seeded into the medium while it is in a semi-solid state.
4- The insect extracts were dissolved in 1% ethanol (just one drop) in a concentration of 1.0 mg/ml.
5- Analytical paper disks 740-E/2 ″Diam″ were loaded with the insect extracts and aseptically put on the surface of the seeded plates with the different test organisms.
6- The plates were left for 2 hours in a refrigerator for diffusion after which the plates were incubated at 30ºC for 48 hours. The detection of an inhibitory clear zone of growth around the paper disks is an indication of the antagonistic properties of the larval secretion and excretion.

Determination of the Growth-Inhibition Zone:

The occurrence of microbial growth inhibition was assessed using a classical diffusion method. In general, this method is based on the visual observation of microbial growth inhibition on agarized media and by determining the diameter of growth-inhibition zone in mm (Mean±SD) (Bulet *et al*., 1991).

Antiviral Bioassay:

Vero cells (derived from the kidney of normal African green monkey) were obtained from American Type Culture Collection (ATCC) continuous cell line established by (Yasumura and kawakita, 1963). Vero cell line was developed for isolation and propagation of many enteroviruses and hepatitis A. In the current study, Vero cells from passage number 76 grown and maintained in Dulbecco's Modified Eagle's Medium (DMEM) with Hanks salt base, supplemented with 10% fetal calf serum and 50 µg/ml gentamycin antibiotic solution. Rapidly growing virus strains producing a cytopathic effect (CPE) in Vero cell cultures within 3 days was used during this study.

Cytotoxicity of crude extracts of *L. sericata C. albiceps M. domestica* were determined through morphological changes in Vero cells treated with different extracts in comparison with untreated control one. While anti-proliferative activity was measured using MTT assay. The MTT kit was based on dehydrogenase in a viable cell to determine cell viability with a colorimetric method that reduced the coloring reagent. Vero cells were grown as a monolayer in media supplemented with 10% inactivated fetal bovine serum. The monolayers of (10,000) cells were plated (10^4 cells/well) in 96-well tissue culture plate and incubated for 24 h at 37°C in a humidified incubator with 5% CO_2 before treatment with the extracts to allow attachment of the cell to the plate except three well without cells as blank. The plates were incubated for 48 h into CO_2 incubator at 37°C and 5% CO_2. After 48h the cells were observed under an inverted microscope before completing the assay to observe the difference in morphology between cell controls and treated one. The cell culture media containing different concentrations of tested extracts and dead cells was decanted and viable attached cells into the tissue culture plate were left. The plate containing viable cells was washed twice with PBS. Fifty microliters of MTT reagent was added to each well including blank and negative control wells. After the addition of MTT reagent, the plates were incubated in dark for 4 h for the reduction of MTT into formazan (purple needle color) by dehydrogenase activity in mitochondria of viable cells. One hundred microliters of DMSO was added to each well to solubilize

the purple crystals of form azan. Absorbance was measured at (570 nm) with microplate reader. The maximum non-toxic concentration (MNTC) was determined. The percentage of cell survival was calculated by the following equation:

$$\text{Survival rate \%} = \frac{A\ \text{sample} - A_b}{A_c - A_b} \times 100$$

A_c=Negative control

A_b=Blank

RESULTS

Determination of Antimicrobial Activity of Whole Body Extracted by Ethyl Acetate on *L. sericata, C. albiceps and M. domestica*.:

The determination of antibacterial activity of whole body extracted by ethyl acetate against bacteria studied. Were shown in tables (1, 2) and indicated by growth inhibition zone in figure (1). They seem to inhibit the growth of gram–positive bacteria, *S. aureus* and *S. epidermidis* and *B. subtilis* for all species, gram–negative bacteria *E. coli* for *L. sericata* and *C. albiceps* and *K. pneumonia* for *L. sericata* and *M. domestica*, *P. aeruginosa* for all tested species. Fungi, *A. fumigatus* for just *L. sericata*. But exhibited limited inhibitory activity towards gram–negative bacteria *E. coli* for *M. domestica* and *K. pneumoniae* for just *C. albiceps* and fungi *S. racemosum* and *C. albicans* for all tested species, was slowed down when challenged with whole body extracted by ethyl acetate on a solid media no complete inhibition zone was detected. Growth-inhibiting activity was determined in liquid growth media using the gram–positive and gram–negative bacteria and fungal strains as indicator organisms.

Antibacterial Activity of Whole Body Extracted by Ethyl Acetate against Gram-Positive Bacteria on *L. sericata C. albiceps* and *M. domestica*:

The obtained results in table (1) and figure (1) showed antibacterial activity (growth–inhibitory activity) for *L. sericata, C. albiceps and M. domestica*, detected against gram–positive bacteria and represented by *B. subtilis* that recorded the highest antibacterial activity (growth–inhibitory activity) for *L. sericata* 26±2.1 mm, *C. albiceps* 23±1.2 mm *and M. domestica* 22±0.85 mm followed by *S. aureus*, where recorded mean growth-inhibition zone for *L. sericata* 22±1.8 mm, *C. albiceps* 12±1.5 mm *and M. domestica* 18±0.75 mm followed by *S. epidermidis* was recorded mean growth-inhibition zone for *L. sericata* 20±0.42 mm, *C. albiceps* 10±0.65 mm *and M. domestica* 6±0.48 mm.

Antibacterial Activity of Whole Body Extracted by Ethyl Acetate against Gram-Negative Bacteria on *L. sericata C. albiceps* and *M. domestica*:

Escherichia coli recorded (growth–inhibitory activity) for *L. sericata, C. albiceps and M. domestica*, detected against gram–negative bacteria. The mean growth–inhibition zone of *E. coli* that recorded for *L. sericata* 31±1.8 mm, *C. albiceps* 19±1.5 mm followed by *K. pneumoniae*, was recorded mean growth-inhibition zone for *L. sericata* 17±0.85 mm and *M. domestica* 20±0.78 mm followed by *P. aeruginosa* was recorded mean growth-inhibition zone for *L. sericata* 15±1.4 mm, *C. albiceps* 15±1.9 mm and *M. domestica* 7±0.58 mm, showed in Table (1) and Figure (1).

Table 1: Growth-inhibition zone of different bacterial strains caused by *L. Sericata, C. albiceps and M. domestica* larval excretion/secretion.

Bacterial strain	Gram	Growth-inhibition zone in mm		
	+/-	*L. Sericata*	*C. albiceps*	*M. domestica*
Bacillus subtilis	+ve	15±2.1	12±1.2	13.5±0.44
Staphylococcus aureus	+ve	12±1.5	9±0.85	4.5±0.58
Staphylococcus epidermidis	+ve	13±0.42	8±0.61	3±0.65
Pseudomonas aeruginosa	-ve	10±1.8	-	10±0.58
Escherichia coli	-ve	19±1.5	12±1.2	-
Klebsiella pneumoniae	-ve	6±0.85	9±0.75	-

NA: No activity.

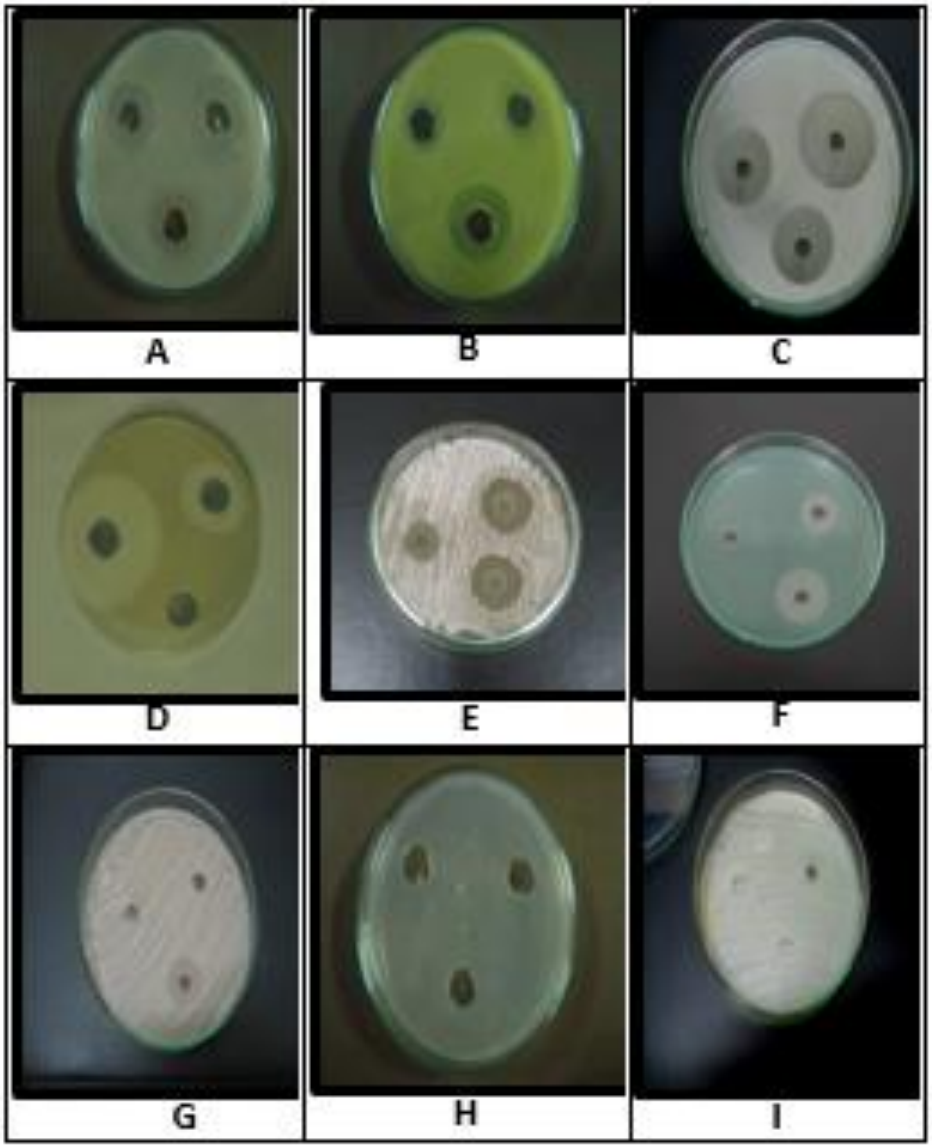

Fig. (1): Effect of whole body extracted by ethyl acetate of *L. Sericata, C. albiceps and M. domestica* against Gram–positive (A) *Staphylococcus aureus*. (B) *Staphylococcus epidermidis*. (C) *Bacillus subtilis*, Gram–negative bacteria (D) *Escherichia coli*. (E) *Klebsiella pneumonia*. (F) *Pseudomonas aeruginosa*. and Fungi (G) *Aspergillus fumigates*. (H) *Candida albicans.* (I) *Syncephalastrum racemosum* represented by inhibition zone (mm).

Antifungal Activity of Whole Body Extracted by Ethyl Acetate on *L. sericata C. albiceps* and *M. domestica*:

The obtained results in table (2) and figure (1), showed antifungal activity (growth–inhibitory activity) for just *L. sericata*, represented by *A. fumigatus* that recorded mean growth-inhibition zone for *L. sericata* 13±0.35 mm and no inhibition zone detected by others.

Table 2: Growth-inhibition zone of different fungal strains caused by *L. Sericata, C. albiceps and M. domestica* of larval excretion/secretion.

Fungal strain	Growth-inhibition zone in mm		
	L. Sericata	***C. albiceps***	***M. domestica***
Aspergillus fumigates	-	-	-
Candida albicans	7±0.45	-	-
Syncephalastrum racemosum	-	-	-

Antiviral Activity of Whole Body Extracted by Ethyl Acetate on *L. sericata, C. albiceps* and *M. domestica*.

1. **Antiviral Activity of Whole Body Extracted by Ethyl Acetate on *L. sericata:***

Data in the table (3) revealed that the whole body extracted by ethyl acetate of *L. sericata* showed the cytotoxic effect to Vero cell in all dilutions except the last one; cytotoxic effect of *L. sericata* on Vero exhibits that MIC is 9.765µg/ml, also the incubation period increases the effect of the extract on Vero cell. Finally, MNTC was detected as 4.882µg/ml for further studies by MTT assay.

According to table (4). The maximum non-toxic concentration of this extract was 4.9 µg/ml it subsequently investigated to evaluate their potentially against HAV (Hepatitis A Virus), so, according to the method applied, HAV was injected to Vero cell causing toxicity for 32 of Vero cell which represents 100% of its actual virulent power. By application of whole body extracted by ethyl acetate of *L. sericata* the toxicity of virus to Vero cell became 4.6 which represent 10.6% of viral activity so, whole body extracted by ethyl acetate of *L. sericata* exhibited antiviral activity in percentage of 89.4%.

2. **Antiviral Activity of Whole Body Extracted by Ethyl Acetate on *C. albiceps:***

Data in table (5) revealed that the whole body extracted by ethyl acetate of *C. albiceps* showed the cytotoxic effect to Vero cell in all dilutions except the last two ones; its cytotoxic effect on Vero exhibits that MIC was 9.765µg/ml. also, the incubation period increases the effect of the extract on Vero cell. MNTC was detected as 4.882µg/ml for it at further studies by MTT assay.

According to table (6) the maximum non-toxic concentrations of whole body extracted by ethyl acetate of C. albiceps was 4.9µg/ml, it subsequently investigated to evaluate their potentially against HAV (Hepatitis A Virus), so, according to the method applied, HAV was injected to Vero cell causing toxicity for 37 of them which represents 100% of its actual virulent power. While, by using whole body extracted by ethyl acetate of C. albiceps the toxicity of virus to Vero cell became 7 which represent 19.6% of viral activity so, whole body extracted by ethyl acetate of *C. albiceps* exhibited antiviral activity in percentage of 80.4%.

Table (3): Examination of antiviral activity of whole body extracted by ethyl acetate of *L. sericata* on Vero cell during the present study.

					Continue....				
Sample code		*L. sericata*			Sample code		*L. sericata*		
Dilution µg/ml	Days	Effect of samples on Vero cells			Dilution µg/ml	Days	Effect of samples on Vero cells		
2500	1st	+4	+4	+4	39.062	1st	+4	+4	+4
	2nd	+4	+4	+4		2nd	+4	+4	+4
	3rd	+4	+4	+4		3rd	+4	+4	+4
1250	1st	+4	+4	+4	19.531	1st	+3	+2	+3
	2nd	+4	+4	+4		2nd	+4	+3	+4
	3rd	+4	+4	+4		3rd	+4	+4	+4
625	1st	+4	+4	+4	9.765	1st	+3	+2	+3
	2nd	+4	+4	+4		2nd	+3	+2	+3
	3rd	+4	+4	+4		3rd	+4	+4	+4
312.5	1st	+4	+4	+4	4.882	1st	-	-	-
	2nd	+4	+4	+4		2nd	-	-	-
	3rd	+4	+4	+4		3rd	-	-	-
156.25	1st	+4	+4	+4	2.441	1st	-	-	-
	2nd	+4	+4	+4		2nd	-	-	-
	3rd	+4	+4	+4		3rd	-	-	-
78.125	1st	+4	+4	+4	1.22	1st	-	-	-
	2nd	+4	+4	+4		2nd	-	-	-
	3rd	+4	+4	+4		3rd	-	-	-

Table (4): Determination of antiviral effect of whole body extracted by ethyl acetate of *L. sericata* on HAV during the present study

Test	Dilution extract µg/ml	Viability	Toxicity	Viral activity %	Anti-viral effect %
Control Vero		100	0		
HAV		68.25568	31.74432	100	0
L. sericata	4.882	95.41339	4.58661	10.644	89.356

Table (5): Examination of antiviral activity of whole body extracted by ethyl acetate of *C. albiceps* on Vero cell during the present study.

		Continue.....							
Sample code		*C. albiceps*			Sample code		*C. albiceps*		
Dilution µg/ml	Days	Effect of samples on Vero cells			Dilution µg/ml	Days	Effect of samples on Vero cells		
2500	1st	+4	+4	+4	39.062	1st	+4	+4	+4
	2nd	+4	+4	+4		2nd	+4	+4	+4
	3rd	+4	+4	+4		3rd	+4	+4	+4
1250	1st	+4	+4	+4	19.531	1st	+4	+4	+4
	2nd	+4	+4	+4		2nd	+4	+4	+4
	3rd	+4	+4	+4		3rd	+4	+4	+4
625	1st	+4	+4	+4	9.765	1st	+3	+3	+3
	2nd	+4	+4	+4		2nd	+4	+4	+4
	3rd	+4	+4	+4		3rd	+4	+4	+4
312.5	1st	+4	+4	+4	4.882	1st	-	-	-
	2nd	+4	+4	+4		2nd	-	-	-
	3rd	+4	+4	+4		3rd	-	-	-
156.25	1st	+4	+4	+4	2.441	1st	-	-	-
	2nd	+4	+4	+4		2nd	-	-	-
	3rd	+4	+4	+4		3rd	-	-	-
78.125	1st	+4	+4	+4	1.22	1st	-	-	-
	2nd	+4	+4	+4		2nd	-	-	-
	3rd	+4	+4	+4		3rd	-	-	-

Table (6): Determination of antiviral effect of whole body extracted by ethyl acetate of *C. albiceps* on HAV during the present study.

Test	Dilution extract µg/ml	Viability	Toxicity	Viral activity %	Anti-viral effect %
Control Vero		100	0		
HAV		65.3478	34.6522	100	0
C. albiceps	4.882	92.9708	7.0292	17.584	82.416

3. Antiviral Activity of Whole Body Extracted by Ethyl Acetate on *M. domestica:*

Data in table (7) revealed that the whole body extracted by ethyl acetate of *M. domestica* showed the cytotoxic effect to Vero cell in all dilutions except the last two ones; its cytotoxic effect on Vero exhibits that MIC was 4.882µg/ml. also, the incubation period increases the effect of the extract on Vero cell. MNTC was detected as 2.441µg/ml for it at further studies by MTT assay.

According to table (8). The maximum non-toxic concentrations of whole body extracted by ethyl acetate of *M. domestica* was 2.4µg/ml, it subsequently investigated to evaluate their potentially against HAV (Hepatitis A Virus), so, according to method applied, HAV was injected to Vero cell causing toxicity for 37 of them which represents 100% of its actual virulent power. While, by using whole body extracted by ethyl acetate of *M. domestica* the toxicity of virus to Vero cell became 10 which represent 22.8% of viral activity so, whole body extracted of *M. domestica* exhibited antiviral activity in percentage of 78%.

Table (7): Examination of antiviral activity of whole body extracted by ethyl acetate of *M. domestica* on Vero cell during the present study.

		Continue.....							
Sample code		***C. albiceps***			**Sample code**		*C. albiceps*		
Dilution µg/ml	**Days**	**Effect of samples on Vero cells**			**Dilution µg/ml**	**Days**	Effect of samples on Vero cells		
2500	1st	+4	+4	+4	39.062	1st	+4	+4	**+4**
	2nd	+4	+4	+4		2nd	+4	+4	**+4**
	3rd	+4	+4	+4		3rd	+4	+4	**+4**
1250	1st	+4	+4	+4	19.531	1st	+4	+4	**+4**
	2nd	+4	+4	+4		2nd	+4	+4	**+4**
	3rd	+4	+4	+4		3rd	+4	+4	**+4**
625	1st	+4	+4	+4	9.765	1st	+3	+3	**+3**
	2nd	+4	+4	+4		2nd	+4	+4	**+4**
	3rd	+4	+4	+4		3rd	+4	+4	**+4**
312.5	1st	+4	+4	+4	4.882	1st	+2	+2	**+1**
	2nd	+4	+4	+4		2nd	+1	+2	**+2**
	3rd	+4	+4	+4		3rd	+2	+2	**+2**
156.25	1st	+4	+4	+4	2.441	1st	-	-	-
	2nd	+4	+4	+4		2nd	-	-	-
	3rd	+4	+4	+4		3rd	-	-	-
78.125	1st	+4	+4	+4	1.22	1st	-	-	-
	2nd	+4	+4	+4		2nd	-	-	-
	3rd	**+4**	**+4**	**+4**		**3rd**	-	-	-

Table (8): Determination of antiviral effect of whole body extracted by ethyl acetate of *M. domestica* on HAV during the present study.

Test	Dilution extract µg/ml	Viability	Toxicity	Viral activity %	Anti-viral effect %
Control Vero		100	0		
HAV		62.8934	37.1066	100	0
C. albiceps	2.441	89.9813	10.0187	22.804	78.196

DISCUSSION

To control pathogens, insects have a complex immune system including the responses of hemocytes in hemolymph, and physical barriers, such as the integument and gut (Boman 1995; Gillespie and Kanost, 1997); antimicrobial agents (peptides are synthesized as effector molecules in the epithelial and midgut tissues (Brey *et al*., 1993; Lehane *et al*., 1997; Ferrandon *et al*., 1998). The presence of polar compounds with antimicrobial activity has already been detected on the epicuticular layer of arthropods (Kuhn-Nentwig 2003), including social insects (Hölldobler and Wilson, 1990; Turillazzi *et al.*, 2006). Although fat bodies are the main sources of the inducible anti-pathogenic peptides in insects (Gillespie and Kanost, 1997), the

epidermis may also produce antibacterial and antifungal peptides in response to local infections (Brey *et al.,* 1993; Ferrandon *et al.,* 1998).

In the present study, different larval extracts of *Lucilia sericata*, *Chrysomya albiceps* and *Musca domestica* were tested for evaluating their antibacterial (using agar well diffusion and microdilution methods), antifungal and antiviral activity.

1. Antibacterial Activity of Tested Insect Extracts:

The present study showed that, the different whole body extracts from of *L. sericata*, *C. albiceps* and *M. domestica* exhibited a variable antibacterial activity against both Gram-positive namely; *Staphylococcus epidermidis* and *Staphylococcus aureus* and Gram-negative namely; *Escherichia coli* and *Klebsiella pneumoniae*.

The obtained results showed that, whole body extracts from *L. sericata*, *C. albiceps* and *M. domestica* caused the highest antibacterial activity against the different bacterial strains tested. In general, Gram-positive bacteria strains were affected by tested extracts more than the Gram-negative bacteria strains. These results are similar to results recorded by Leem *et al*., (1999) for the saw fly, *Acantholyda parki* extract which have a broad antibacterial spectrum against both Gram- negative and Gram-positive bacteria; Hou *et al*., (2007) as they found that the extract of the housefly larvae at high concentration showed higher antibacterial activity against both Gram- positive and Gram- negative bacteria. Also, they concluded that, the extract of the housefly generally possess wide broad antibacterial activity against both Gram- positive and Gram-negative bacteria, this conclusion was in consistency with the present study. In agreement with these results, the wide broad antibacterial spectrum caused by the different whole body extracts of *L. sericata*, *C. albiceps* and *M. domestica* larvae was similar to that recorded by Zeariya (2009) using the darkling beetle, *Ocnera hispida* whole body extracts against different Gram-positive and Gram-negative bacteria.

It is suggested that insect bodies produce combinations of antibacterial peptides in response to natural infection leading to a broad spectrum of activity against micro-organisms. In spite of such a response, the susceptible insects within the host range of a given pathogen are successfully killed by the pathogen. In contrast, the insects resistant against the pathogen appear to be out of the host range (Yamauchi 2001). In agreement with this suggestion, the present results of *L. sericata*, *C. albiceps* and *M. domestica* whole body extracts showed a variable activity against both Gram-positive and Gram-negative bacteria tested. Similarly, Zeariya (2009) showed that, whole body extracts of *Musca domestica* larvae caused variable activity against different Gram-positive and Gram-negative bacterial strains.

Generally, most of insect extracts exhibit antibacterial activity against both Gram-positive and Gram-negative bacteria, for example, the silkworm, *Bombyx mori* (Hara and Yamakawa, 1995a&b), the European bumblebee, *Bombus pascuorum* (Rees *et al*., 1997) and larvae of *Tenebrio molitor* (Lee *et al*., 1998). However, some insect species show activity against only Gram-positive bacteria; for example; *Aedes aegypti* (Lowenberger *et al*., 1995), *Chironomus plumosus* (Lauth *et al*., 1998) and *Anopheles gambiae* (Vizioli *et al*., 2001a&b).

2. Antifungal Activity of Tested Insect Extracts:

The present study revealed that, the bacterial strains tested were more sensitive to the different *L. sericata*, *C. albiceps* and *M. domestica* larval extracts used than the fungal strains tested. These results agree with results obtained by Hou *et al*., (2007) who reported that, the extract of the housefly larvae showed higher activity against Gram- positive bacteria than Gram- negative bacteria and did not show any antifungal activity; Zeariya (2009) showed that, the different whole body extracts

from *H. ephippiger* (nymph), *O. hispida* (adult) and *M. domestica* (3rd instar larvae and adult) induced antibacterial activity more than antifungal activity.

The present study and results are comparable to those obtained by Jianwei *et al.*, (2009), where they concluded that the antifungal activity of *M. domestica* hemolymph inhibits the growth of *Candida albicans* (both sensitive and resistant strains).

3. Antiviral Activity of Tested Insect Extracts:

Ethyl acetate extracts of *L. sericata*, *C. albiceps* and *M. domestica* larvae displayed a variable effect on Hepatitis A virus (HAV) Vero cell lines. Meanwhile, the present study and the results are in agreement with such results recorded by many authors for using different insect materials against different Vero cell lines, for example, Esser *et al.*, (1979) proved that up on treatment of murine virus capsid with melittin, the viral membrane peeled off the surface, generating membrane "edges" which could be stabilized by melittin and prevent virus cell entry; Baier *et al.*, (2000) proved that, the nasal application of lipopeptide increased protection against a lethal infection of influenza; Wachinger *et al.*, (1998) who stated that, the reduction of viral infectivity is not due to an effect of melittin on the virus particles but to an intracellular action of the peptide, which is readily taken up into the cells. Simultaneously Fenard *et al.*, (2001) stated that, the honeybee venom phospholipase A_2 (p3bv) peptide (amino acids 21 to 35 of bvPLA$_2$) was found to inhibit the replication of T-lymphotropic (T-tropic) HIV-1 isolates (ID_{50}= 2 mM) but had no effect on monocytotropic (M-tropic) HIV-1 isolates. P3bv was also found to be capable of preventing the cell-cell fusion process mediated by T-tropic HIV-1 envelope and Chernysh *et al.*, (2002) isolated alloferon from wild-type strain of the blowfly, *C. vicina* and they reported that, that the activity spectrum of alloferon as an antiviral agent is not restricted to influenza viruses. In fact, alloferon-based therapy of herpes simplex and hepatitis B and C infections is now under extensive preclinical and clinical study. However, it has been shown that preventive and/or therapeutic administration of alloferon essentially increased survival rate and suppressed virus reproduction in mice intracerebral infected with type 2 herpes simplex viruses.

Conclusion

From the obtained results, it is concluded that, the whole body extracted by ethyle acetate from the larvae of *L. sericata*, *C. albiceps* and *M. domestica* have a variable antibacterial activities against both Gram-positive and Gram-negative bacterial strains.

The tested *L. sericata*, *C. albiceps* and *M. domestica* extracts did not show any antifungal activity against tested fungi species; *Candida albicans*, *Syncephalastrum racemosum*, *Aspergillus fumigates* for just *C. albiceps* and *M. domestica*.

Ethyl acetate extract of *L. sericata*, *C. albiceps* and *M. domestica* larvae displayed a variable effect on Hepatitis A virus (HAV).

So, the tested extracts used may be considered as new antimicrobial and antifungal agents.

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