



## Antimicrobial and antiviral activity of maggots extracts of *Lucilia sericata* (Diptera: Calliphoridae)

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### ABSTRACT

This study was conducted as an attempt to investigate the antimicrobial activity of *Lucilia sericata* (Diptera: Calliphoridae) maggots whole body extracts produced by different solvents against some bacterial and fungal strains, and also to study the role of *L. sericata* maggot extracts as antiviral therapeutic agents. Results showed that the highest antibacterial activity was attained by petroleum ether extract 24h post treatment for both Gram-positive and Gram-negative bacteria either by Microbial Growth Inhibition method or by Minimum Inhibitory Concentration method. Regarding the antifungal activity, all tested extracts showed antifungal activity against tested fungal strains and it may be arranged in descending order as the following: Petroleum Ether > Hexane > Ethyl Acetate > acetone. In addition, the antiviral activity was determined and the maximum non-toxic concentration was 39.06µg/ml. The obtained results explained that petroleum ether extract was effective as anti-HSV with anti-viral activity percent of 92.9. Generally, the available results indicate that *L. sericata* maggot extracts induced remarkable effects on both antimicrobial and antiviral activities.

### INTRODUCTION

Natural products represent a large family of diverse chemical entities with a wide variety of biological activities that have found multiple uses notably in human and veterinary medicine and in agriculture. The field of natural product discovery has undergone a tremendous development over the past few decades due to the consequence of several new and revolutionizing drug discovery and development techniques (Hasaballah, 2015 and Wohlleben *et al.* 2016).

Insects are the largest group of the still existing organisms, their individual number account for as much as 80% of all known fauna and considered a large, unexplored and unexploited source of potentially useful compounds for modern medicine due to their mode of action of non-selective interaction with microbes' cell surface membranes (Leem *et al.* 1999; Pemberton 1999; Hancock and Rozek, 2002; Zasloff, 2002; Boman, 2003; Bulet *et al.* 2004; Hassan *et al.* 2013 and Hasaballah 2018).

Antimicrobial peptides consider important factors of the innate immunity system in many organisms, as they are small; they usually possess multi-functional peptides whose fundamental biological role proposed to the elimination of

pathogenic microorganisms, including Gram-positive and Gram-negative bacteria, fungi, and viruses. Based on the amino acid sequence and biochemical properties, antimicrobial peptides are divided into three classes; linear peptides without cysteine, peptides with stabilized structure by disulfide bridges and peptides with over-representation of one amino acid (Januszanis *et al.* 2012).

In insects, antimicrobial peptides/polypeptides are mainly synthesized in the fat body and released into hemolymph where they play the crucial role in innate immune system and host defence mechanisms (Fouda *et al.* 2013), with a broad-spectrum activity against both Gram-positive and Gram-negative bacteria and fungi (Hoffmann 1995; Hoffmann *et al.* 1996; Januszanis *et al.* 2012). Hou *et al.* (2007) reported the possibility of antimicrobial activity of insects' body extracts to ascertain phylogenetic patterns among insect species. In the same context, a great part of efforts have been achieved for the investigation and re-examination of insect sources to obtain compounds that may have antimicrobial activity (Shehata *et al.* 2016; Amer *et al.* 2019 a, b, c).

This study aimed to investigate the antimicrobial activity of *Lucilia sericata* (Diptera: Calliphoridae) maggots petroleum ether, hexane, acetone and ethyl acetate extracts against different bacterial and fungi strains. In addition, to study the role of *Lucilia sericata* maggot extracts as anti-HSV therapeutic agents.

## MATERIALS AND METHODS

### Colonization of tested flies

*Lucilia sericata* maggots were collected and transferred to Medical Entomology Insectary, Biology Department, Faculty of Science, Jazan University (KSA) and maintained for several generations under controlled conditions, at temperature of (27±2°C), relative humidity (70±5%) and photoperiods (12h light: 12h dark). Adults were reared in mesh cages (30×30×30cm) with three sides of wire, maggots were feed on an artificial diet (liver), and the emerged flies were feed on milk powder and sucrose solution.

### Preparation of maggots' extracts

The extraction was performed according to the methods of Ahn *et al.* (2000) and Meylears *et al.* (2002). The extraction was carried out using petroleum ether, hexane, acetone and ethyl acetate solvents.

### Antimicrobial bioassay

#### Antibacterial activity of tested extracts

Six pathogenic bacterial strains were used for the antibacterial assay; *Staphylococcus aureus* (ATCC25923), *Staphylococcus pyogenes* (ATCC12344) and *Bacillus subtilis* (ATCC6051) as Gram-positive bacterial strains; whereas, *Escherichia coli* (ATCC25922DQ), *Klebsiella pneumoniae* (ATCC11296) and *Pseudomonas aeruginosa* (ATCC10145) were used as Gram-negative bacterial strains. Microbial growth inhibition was tested using agar well diffusion method (Valgas *et al.* 2007; Hasaballah & Elnaggar, 2017). Also, Minimum Inhibitory Concentration (MIC) was determined based on the microdilution method by broth microdilution method using 96-well micro-plates (Irith *et al.* 2008).

#### Antifungal activity of tested extracts

The fungi *Aspergillus flavus*, *Aspergillus fumigatus*, *Candida albicans*, *Geotricum candidum* and *Penicillium* sp. strains were used for *in vitro* evaluation of the antimicrobial activity. All tested microorganisms were supplied by the Microbiology Department, Faculty of Science, Jazan University, KSA. Sucrose-

Nitrate agar medium gm/L consisted of: Sucrose, NaNO<sub>3</sub>, K<sub>2</sub>HPO<sub>4</sub>, 1.0 mgSO<sub>4</sub>, 7H<sub>2</sub>O and distilled water, 1000 ml was used in this test. The pH value was adjusted to 7-7.3 before sterilization (Tadashi, 1975). The detection of inhibitory clear zone around the paper disks is an indication of the antagonistic properties of the extracts under evaluation.

### **Antiviral assay**

#### **Determination of samples cytotoxicity on VERO cell**

Petroleum ether extract was used to determine the anti-HSV activity. Growth medium was decanted from 96 well micro titer plates after confluent sheet of VERO cell formed. Plate was incubated at 37°C and examined frequently for up to 2days. Cells were checked for any physical signs of toxicity. A 20µl of MTT solution was added to each well. Incubation for 1-5h was done to allow the MTT metabolism. The media dumped off. Plate dried on paper towels to remove residue. Formazan (MTT metabolic product) re-suspend in 200µl DMSO, Placed on a shaking table, 150 rpm for 5 minutes, to thoroughly mix formazan into the solvent. The optical density was read at 560nm and subtracts background at 620nm. The maximum non-toxic concentration of each extract was determined and was used for further biological studies.

#### **Antiviral assay (MTT Assay Protocol)**

Equal volume (1:1 v/v) of non-lethal dilution of tested extracts was incubated and the virus suspended for 1h. A 100µl from viral/ sample suspension was added, placed on a shaking table, 150rpm for 5 minutes. Incubation at (37°C & 5% CO<sub>2</sub>) was done for 1day to allow the virus to take effect. A 2ml of MTT solution per 96 well plates was prepared at 5mg/ml in PBS. A 20µl MTT solution was added to each well, placed on a shaking table, 150rpm for 5 minutes, to thoroughly mix the MTT into the media. The media dumped off. Plate dried on paper towels to remove residue. Formazan (MTT metabolic product) re-suspend in 200µl DMSO, placed on a shaking table, 150rpm for 5 minutes, to thoroughly mix Formazan into the solvent. The optical density was read at 560nm and subtracts background at 620nm. Optical density should be directly correlated with cell quantity.

#### **Statistical analysis**

The statistical analysis of the data obtained was done according to Armitage, (1974) and Lentner *et al.* (1982) and the analysis was revised by Quattro-pro for windows program version 2.0 Microsoft, windows version 7.0, graphics were drawn using Harvard Graphics program version 4.0. The obtained data were assessed by calculation of mean (M), standard deviation (SD) and student t-test.

## **RESULTS**

### **Antimicrobial activity using well diffusion method antibacterial activity**

The activity of whole body of *Lucilia sericata* maggots' against different pathogenic bacterial strains was evaluated. Results in Table (1) and Fig. (1A-1C) show the antibacterial activity against Gram-positive bacterial strains tested. The obtained results revealed that the highest antibacterial activity 24 hrs post treatment was attained by petroleum ether extract for *S. aureus* and *B. subtilis* with mean growth-inhibition zone of 19.0±0.36mm and 20.5±0.40mm; respectively, compared to the standard antibiotic used (Ampicillin). While, acetone and ethyl acetate extracts exhibited no activity against *S. pyogenes* bacterial strains.

Table 1: Antibacterial activity of *Lucilia sericata* maggots' different crude extracts against different strains of Gram-positive bacteria.

| Bacteria                       | Petroleum ether        | Hexane                 | Acetone                | Ethyl acetate          | Standard ( <i>Ampicillin</i> ) |
|--------------------------------|------------------------|------------------------|------------------------|------------------------|--------------------------------|
| <i>Staphylococcus aureus</i>   | 19.0±0.36 <sup>d</sup> | 17.4±0.30 <sup>d</sup> | 16.5±0.68 <sup>d</sup> | 17.9±0.45 <sup>d</sup> | 27.6±0.22 <sup>a</sup>         |
| <i>Staphylococcus pyogenes</i> | 17.7±0.32 <sup>d</sup> | 16.7±0.52 <sup>d</sup> | NA                     | NA                     | 25.8±0.14 <sup>a</sup>         |
| <i>Bacillus subtilis</i>       | 20.5±0.40 <sup>d</sup> | 19.8±0.47 <sup>d</sup> | NA                     | 18.0±0.34 <sup>d</sup> | 28.2±0.33 <sup>a</sup>         |

All data represented as Mean ± SD; NA: No Activity; Means followed by the same letters aren't statistically significant (P>0.05)

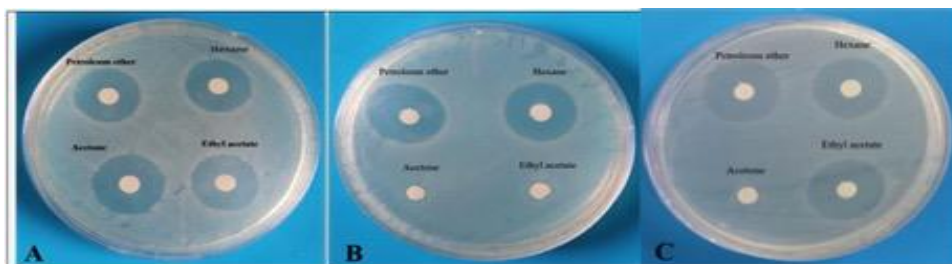


Fig. 1: Antibacterial activity indicated by growth-inhibition zone of *Lucilia sericata* maggots' crude extracts against Gram-positive bacteria. (A: *Staphylococcus aureus*; B: *Staphylococcus pyogenes*; C: *Bacillus subtilis*).

On the other hand, the whole body extracts of different solvents of *L. sericata* maggots' exhibited antibacterial activity against Gram-negative bacterial strains as given in table (2) and Figs. (2A-2C). The highest antibacterial activity was recorded with petroleum ether extract against *E. coli* with mean growth-inhibition zone of 21.0±0.51mm, compared with 27.6±0.10mm for the standard antibiotic (Gentamycin).

Table 2: Antibacterial activity of *Lucilia sericata* maggots' crude extracts against different strains of Gram-negative bacteria.

| Bacteria                      | Petroleum ether        | Hexane                 | Acetone                | Ethyl acetate          | Standard ( <i>Gentamycin</i> ) |
|-------------------------------|------------------------|------------------------|------------------------|------------------------|--------------------------------|
| <i>Escherichia coli</i>       | 21.0±0.51 <sup>d</sup> | 19.8±0.35 <sup>d</sup> | 19.4±0.30 <sup>d</sup> | 18.2±0.40 <sup>d</sup> | 27.6±0.10 <sup>a</sup>         |
| <i>Klebsiella pneumoniae</i>  | 17.7±0.32 <sup>d</sup> | 16.8±0.75 <sup>d</sup> | NA                     | 16.0±0.63 <sup>d</sup> | 25.2±0.12 <sup>a</sup>         |
| <i>Pseudomonas aeruginosa</i> | 20.5±0.4 <sup>c</sup>  | 19.8±0.47 <sup>d</sup> | NA                     | NA                     | 22.3±0.16 <sup>a</sup>         |

All data represented as Mean ± SD; NA: No Activity; Means followed by the same letters aren't statistically significant (P>0.05).

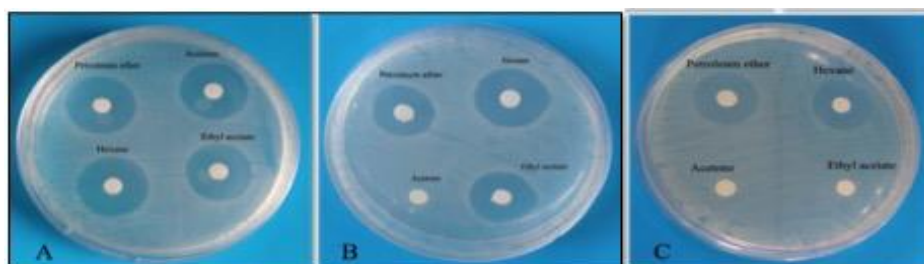


Fig. 2: Antibacterial activity of *Lucilia sericata* maggots' crude extracts against Gram-negative bacteria. (A: *Escherichia coli*; B: *Klebsiella pneumoniae*; C: *Pseudomonas aeruginosa*)

**Antifungal activity**Table 3: Antifungal activity of *Lucilia sericata* maggots' crude extracts against different fungal strains.

| Fungi                        | Petroleum ether        | Hexane                 | Acetone                | Ethyl acetate          | Standard<br>(Amphotericin B) |
|------------------------------|------------------------|------------------------|------------------------|------------------------|------------------------------|
| <i>Aspergillus flavus</i>    | 17.9±0.66 <sup>d</sup> | 17.1±0.38 <sup>d</sup> | NA                     | NA                     | 24.6±0.29 <sup>a</sup>       |
| <i>Aspergillus fumigatus</i> | 17.8±0.44 <sup>d</sup> | 16.5±0.50 <sup>d</sup> | NA                     | 15.0±0.36 <sup>d</sup> | 25.8±0.17 <sup>a</sup>       |
| <i>Candida albicans</i>      | 19.6±0.33 <sup>d</sup> | 18.8±0.46 <sup>d</sup> | 17.2±0.55 <sup>d</sup> | 16.3±0.40 <sup>d</sup> | 21.6±0.14 <sup>a</sup>       |
| <i>Geotricum candidum</i>    | 20.3±0.53 <sup>d</sup> | 19.5±0.62 <sup>d</sup> | 16.4±0.50 <sup>d</sup> | 18.0±0.53 <sup>d</sup> | 23.0±0.10 <sup>a</sup>       |
| <i>Penicillium sp.</i>       | NA                     | NA                     | NA                     | NA                     | 24.0±0.20 <sup>a</sup>       |

All data represented as Mean ± SD; NA: No Activity; Means followed by the same letters aren't statistically significant (P>0.05)

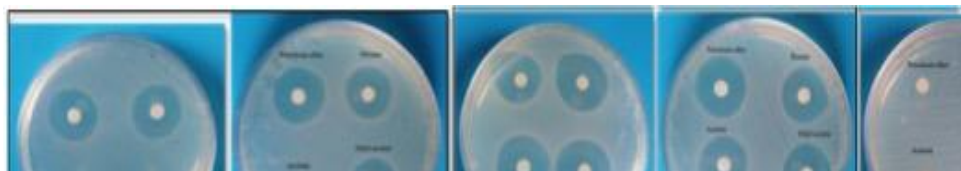


Fig. 3: Antifungal activity indicated by growth-inhibition zone of *Lucilia sericata* maggots' crude extracts against fungal strains. (A: *Aspergillus flavus*; B: *Aspergillus fumigatus*; C: *Candida albicans*; D: *Geotricum candidum*; E: *Penicillium sp.*).

Regarding the antifungal activity, data given in Table (3) and illustrated in Fig. (3A-3E) indicated that all tested extracts showed antifungal activity against *A. flavus*, *A. fumigatus*, *C. albicans* and *G. candidum* fungal strains, while acetone extract showed no activity against *A. flavus* and *A. fumigatus*. Ethyl acetate extract showed no activity against only *A. flavus*. In addition, none of the tested extracts showed activity against *Penicillium* fungal strain.

In general, petroleum ether extract was more effective against all fungal strains tested than those of hexane, acetone and ethyl acetate. The growth-inhibition zones recorded by petroleum ether extract were 17.9±0.66, 17.8±0.44, 19.6±0.33 and 20.3±0.53mm for *A. flavus*, *A. fumigatus*, *C. albicans* and *G. candidum*; respectively, compared with the standard (Amphotericin B).

#### Determination of Minimum Inhibitory Concentration (MIC) by Microdilution Method:

Determination of Minimum Inhibitory Concentrations (MICs) of *L. sericata* maggots' crude extracts against tested bacterial strains was investigated. The obtained results revealed the effectiveness of *L. sericata* maggots' crude extracts against tested Gram-positive bacteria. Petroleum ether extract recorded the highest antibacterial activity against *S. aureus* as compared with other tested extracts (Table 4).

Table 4: Antibacterial activity of *Lucilia sericata* maggots' crude extracts against Gram-positive bacteria by Microdilution plate at 480nm.

| Bacterial strains              | Conc.<br>(mg/ml) | <i>Lucilia sericata</i> maggots' different extracts |                      |                      |                      |
|--------------------------------|------------------|-----------------------------------------------------|----------------------|----------------------|----------------------|
|                                |                  | Petroleum ether                                     | Hexane               | Acetone              | Ethyl acetate        |
| <i>Staphylococcus aureus</i>   | Control          | 4.7±0.3 <sup>a</sup>                                | 4.4±0.5 <sup>a</sup> | 4.3±0.6 <sup>a</sup> | 4.0±0.3 <sup>a</sup> |
|                                | 50.0             | 1.1±0.4 <sup>d</sup>                                | 1.2±0.2 <sup>d</sup> | 1.2±0.2 <sup>d</sup> | 1.3±0.3 <sup>d</sup> |
|                                | 25.0             | 0.9±0.4 <sup>d</sup>                                | 1.2±0.5 <sup>d</sup> | 1.1±0.1 <sup>d</sup> | 1.2±0.5 <sup>d</sup> |
|                                | 12.5             | 0.9±0.2 <sup>d</sup>                                | 1.0±0.2 <sup>d</sup> | 1.0±0.4 <sup>d</sup> | 1.2±0.1 <sup>d</sup> |
| <i>Staphylococcus pyogenes</i> | Control          | 4.7±0.3a                                            | 4.4±0.5a             | 4.3±0.6a             | 4.0±0.3a             |
|                                | 50.0             | 1.3±0.2 <sup>d</sup>                                | 1.1±0.1 <sup>d</sup> | NA                   | NA                   |
|                                | 25.0             | 1.3±0.2 <sup>d</sup>                                | 1.2±0.5 <sup>d</sup> | NA                   | NA                   |
|                                | 12.5             | 1.0±0.6 <sup>d</sup>                                | 1.0±0.3 <sup>d</sup> | NA                   | NA                   |
| <i>Bacillus subtilis</i>       | Control          | 4.7±0.3a                                            | 4.4±0.5a             | 4.3±0.6a             | 4.0±0.3a             |
|                                | 50.0             | 1.2±0.4 <sup>d</sup>                                | 1.2±0.3 <sup>d</sup> | NA                   | 1.1±0.1              |
|                                | 25.0             | 1.0±0.2 <sup>d</sup>                                | 1.1±0.1 <sup>d</sup> | NA                   | 1.0±0.2              |
|                                | 12.5             | 1.0±0.2 <sup>d</sup>                                | 1.1±0.2 <sup>d</sup> | NA                   | 1.0±0.3              |

On the other hand, acetone and ethyl acetate extracts didn't show any activity against *S. pyogenes* at all tested concentrations. In addition, acetone extract showed no activity against *B. subtilis*. Data given in table (5) indicated that, the MIC values ranged from 12.25 to 50.0mg/ml depending on tested bacterial strain. Bacterial strain *B. subtilis* was severely affected by petroleum ether and hexane fractions whereas only ethyl acetate fraction presented MIC of 25.0mg/ml.

Table 5: MIC values of *Lucilia sericata* maggots' crude extracts against different strains of Gram-positive bacteria.

| Bacterial strains              | <i>Lucilia sericata</i> maggots' different extracts |        |         |               |
|--------------------------------|-----------------------------------------------------|--------|---------|---------------|
|                                | Petroleum ether                                     | Hexane | Acetone | Ethyl acetate |
| <i>Staphylococcus aureus</i>   | 12.5                                                | 25.0   | 50.0    | 25.0          |
| <i>Staphylococcus pyogenes</i> | 12.5                                                | 25.0   | NA      | NA            |
| <i>Bacillus subtilis</i>       | 12.5                                                | 12.5   | NA      | 25.0          |

On the other side, for Gram-negative bacteria, acetone and ethyl acetate extracts observed no activity against *P. aeruginosa*, while only acetone extract showed no activity against *K. pneumoniae*. Also, petroleum ether and hexane recorded a discrete activity against *E. coli* with MICs of 12.5 and 25.0mg/ml; respectively. While, the crude extracts of petroleum ether recorded MIC of 12.5mg/ml against *k. pneumoniae*, and petroleum ether and hexane extracts registered MIC of 12.5mg/ml against *P. aeruginosa* (Tables 6 & 7).

Table 6: Antibacterial activity of *Lucilia sericata* maggots' crude extracts against Gram-negative bacteria by Microdilution plate at 480nm.

| Bacterial strains             | Conc. (mg/ml) | <i>Lucilia sericata</i> maggots' different extracts |                      |                      |                      |
|-------------------------------|---------------|-----------------------------------------------------|----------------------|----------------------|----------------------|
|                               |               | Petroleum ether                                     | Hexane               | Acetone              | Ethyl acetate        |
| <i>Escherichia coli</i>       | Control       | 4.1±0.7 <sup>a</sup>                                | 3.9±0.5 <sup>a</sup> | 3.7±0.6 <sup>a</sup> | 4.2±0.4 <sup>a</sup> |
|                               | 50.0          | 1.4±0.4 <sup>d</sup>                                | 1.2±0.3 <sup>d</sup> | 1.2±0.3 <sup>d</sup> | 1.4±0.6 <sup>d</sup> |
|                               | 25.0          | 1.2±0.1 <sup>d</sup>                                | 1.0±0.3 <sup>d</sup> | 1.2±0.4 <sup>d</sup> | 1.3±0.1 <sup>d</sup> |
|                               | 12.5          | 1.1±0.2 <sup>d</sup>                                | 1.1±0.4 <sup>d</sup> | 1.3±0.4 <sup>d</sup> | 1.2±0.2 <sup>d</sup> |
| <i>Klebsiella pneumoniae</i>  | Control       | 4.1±0.7 <sup>a</sup>                                | 3.9±0.5 <sup>a</sup> | 3.7±0.6 <sup>a</sup> | 4.2±0.4 <sup>a</sup> |
|                               | 50.0          | 1.3±0.1 <sup>d</sup>                                | 1.4±0.2 <sup>d</sup> | NA                   | 1.4±0.4 <sup>d</sup> |
|                               | 25.0          | 1.3±0.2 <sup>d</sup>                                | 1.4±0.1 <sup>d</sup> | NA                   | 1.3±0.2 <sup>d</sup> |
|                               | 12.5          | 1.2±0.7 <sup>d</sup>                                | 1.3±0.6 <sup>d</sup> | NA                   | 1.4±0.1 <sup>d</sup> |
| <i>Pseudomonas aeruginosa</i> | Control       | 4.1±0.7 <sup>a</sup>                                | 3.9±0.5 <sup>a</sup> | 3.7±0.6 <sup>a</sup> | 4.2±0.4 <sup>a</sup> |
|                               | 50.0          | 1.4±0.3 <sup>d</sup>                                | 1.4±0.2 <sup>d</sup> | NA                   | NA                   |
|                               | 25.0          | 1.3±0.1 <sup>d</sup>                                | 1.4±0.4 <sup>d</sup> | NA                   | NA                   |
|                               | 12.5          | 1.3±0.1 <sup>d</sup>                                | 1.4±0.7 <sup>d</sup> | NA                   | NA                   |

Table 7: MIC values of *Lucilia sericata* maggots' crude extracts against different strains of Gram-negative bacteria.

| Bacterial strains             | <i>Lucilia sericata</i> maggots' different extracts |        |         |               |
|-------------------------------|-----------------------------------------------------|--------|---------|---------------|
|                               | Petroleum ether                                     | Hexane | Acetone | Ethyl acetate |
| <i>Escherichia coli</i>       | 12.5                                                | 25.0   | 25.0    | 50            |
| <i>Klebsiella pneumoniae</i>  | 12.5                                                | 25.0   | NA      | 25.0          |
| <i>Pseudomonas aeruginosa</i> | 12.5                                                | 12.5   | NA      | NA            |

### Antiviral Assay

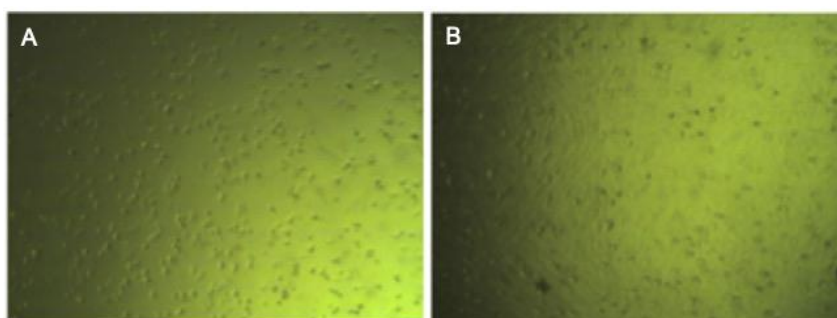
The antiviral activity of *L. sericata* maggots' petroleum ether extracts against Herpes simplex virus (HSV-1) was tested and the maximum non-toxic concentration (MNTC) determined. The MNTC of tested *L. sericata* extracts recorded 39.06µg/ml (table 8). The obtained results revealed that, the petroleum ether extracts of *L. sericata* was effective as anti-HSV. The antiviral activity recorded 92.9 (Tables 8, 9 and Fig.4 A&B).

Table 8: Effect of *Lucilia sericata* maggots' petroleum ether extracts against Vero cell.

| ID                                                | Dilution (µg/ml) | O.D          |              |              | Mean O.D     | Viability % | Toxicity % | SE              | CC <sub>50</sub> (µg/ml) |
|---------------------------------------------------|------------------|--------------|--------------|--------------|--------------|-------------|------------|-----------------|--------------------------|
| vero                                              |                  | <b>0.284</b> | <b>0.269</b> | <b>0.275</b> | <b>0.276</b> | <b>100</b>  | <b>0</b>   | <b>0.004359</b> |                          |
| <i>L. sericata</i> maggots'<br>Pet. ether extract | 10000            | 0.023        | 0.015        | 0.02         | 0.019333     | 7.004831    | 92.99517   | 0.002333        | 136.841                  |
|                                                   | 5000             | 0.024        | 0.026        | 0.023        | 0.024333     | 8.816425    | 91.18357   | 0.000882        |                          |
|                                                   | 2500             | 0.034        | 0.023        | 0.035        | 0.030667     | 11.11111    | 88.88889   | 0.003844        |                          |
|                                                   | 1250             | 0.03         | 0.04         | 0.036        | 0.035333     | 12.80193    | 87.19807   | 0.002906        |                          |
|                                                   | 625              | 0.035        | 0.042        | 0.054        | 0.043667     | 15.82126    | 84.17874   | 0.005548        |                          |
|                                                   | 312.5            | 0.067        | 0.0744       | 0.059        | 0.0668       | 24.2029     | 75.7971    | 0.004447        |                          |
|                                                   | 156.25           | 0.103        | 0.112        | 0.11         | 0.108333     | 39.25121    | 60.74879   | 0.002728        |                          |
|                                                   | 78.12            | 0.215        | 0.226        | 0.224        | 0.221667     | 80.31401    | 19.68599   | 0.003383        |                          |
|                                                   | 39.06            | 0.277        | 0.269        | 0.278        | 0.274667     | 99.51691    | 0.483092   | 0.002848        |                          |
| 19.53                                             | 0.284            | 0.277        | 0.276        | 0.279        | 101.087      | 0           | 0.002517   |                 |                          |

Table 9: Antiviral activity of tested maggots' extracts against Herpes simplex virus (HSV-1) using methyl thiazolyltetrazolium (MTT) assay protocol.

| Test                                              | Conc. (µg/ml) | O.D   |       |       | Mean O.D | Viability | Toxicity | Viral activity % | Anti-viral effect % |
|---------------------------------------------------|---------------|-------|-------|-------|----------|-----------|----------|------------------|---------------------|
| Control Vero                                      | --            | 0.272 | 0.295 | 0.282 | 0.283    | 100       | 0        | --               | --                  |
| HSV 1                                             | --            | 0.112 | 0.124 | 0.123 | 0.119667 | 42.28504  | 57.71496 | 100              | 0                   |
| <i>L. sericata</i> maggots'<br>Pet. ether extract | 39.06         | 0.277 | 0.269 | 0.268 | 0.271333 | 95.8775   | 4.122497 | 7.142852         | 92.85714796         |

Fig. 4: (A) effect of HSV-1 on Vero cells, (B) Activity of *L. sericata* extract against HSV-1 at 39.06µg/ml.

## DISCUSSION

Insects are known to have both cellular and humoral immune systems that together form a potent defense against invading bacteria (Gotz and Boman, 1985; kimbrell, 1991). In cellular immunity, mechanisms such as phagocytosis and encapsulation are operative (Boman and Hultmark, 1987), while humoral responses mainly involve the production of a variety of antibacterial and antifungal proteins that are induced or increased in response to infection (Abraham *et al.* 1995).

In addition, use of insect extracts in Folk Medicine encourage the scientists to develop potential new medicines for treating serious diseases such as viral infections and problems associated with the newly emerging antibiotic-resistance. There is already a long history of the use of these insect extracts in Folk Medicine (Ratcliffe *et al.* 2014). The present study aimed at evaluate the antimicrobial activity of *Lucilia sericata* maggots' whole body extracts using Well diffusion and Microdilution methods, and to determine the activity of maggots' petroleum ether extract as anti-HSV-1.

Furthermore, the antibacterial activity results showed that, tested extracts evoked a variable activity against both Gram-positive bacteria and Gram-negative

bacteria depending on the solvent used in extraction. Generally, petroleum ether was the most effective against different bacteria species followed by hexane, ethyl acetate and acetone extract. Also, Gram-positive bacterial strains were more sensitive to the tested maggots' extracts than Gram-negative bacterial strains. Similar results were observed by Leem *et al.* (1999) using, *Acantholyda parki* isolates as a broad antibacterial spectrum against both Gram-negative and Gram-positive bacteria; Yamauchi, (2001) who suggested that insect bodies produce combinations of antibacterial peptides in response to natural infection leading to a broad spectrum 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 and in contrast, the insects resistant against the pathogen appear to be out of the host range. *Lucilia sericata* maggots' extracts was more effective than those of *C. albiceps*, *S. carnaria* and *M. domestica* as recorded by Thomas *et al.* (1999), where *L. sericata* larvae were able to decrease the total bacterial count of *S. aureus* in vitro and to combat clinical infections in a variety of wound types including those caused by antibiotic-resistant strains. Such findings may be due to the presence of antibacterial agents in their secretion/excretion (Amer *et al.* 2019b).

On the other hand, tested extracts showed a variable antifungal activity against *A. flavus*, *A. fumigatus*, *C. albicans* and *G. candidum* fungal strains with no activity of any tested extracts against *Penicillium*. In general, petroleum ether was more effective against tested strains than those of hexane, ethyl acetate and acetone. However, the present study has shown that the bacterial strains tested were more sensitive to tested extracts used than the fungal strains. In agreement with these results, Meylaers *et al.* (2004) observed that beside the antibacterial activity, methanolic whole body extract of un-infected last instar larvae of *M. domestica* displayed antifungal activity against *Saccharomyces cerevisiae*. Hou *et al.* (2007) reported that the housefly larvae showed higher activity against Gram-positive bacteria than Gram-negative bacteria and did not show any antifungal activity. Also, the activity of some insect body extracts against bacteria and fungi was also recorded by Yamada and Natori, (1994) using the flesh fly, *S. peregrine*; Rees *et al.* (1997) using the European bumble bee, *B. pascuorum*, Leem *et al.* (1999) using the sawfly, *A. parki*; Vizioli *et al.* (2001) using the mosquito vector, *A. gambiae*; Cytrynska *et al.* (2007) using the wax moth, *Galleria mellonella*.

The obtained results revealed also that petroleum ether extract of *L. sericata* recorded promising activity as antiHSV-1. The same results were reported by Esser *et al.* (1979), for treatment of murine virus capsid with melittin; 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 that readily taken up into the cells; Baier *et al.* (2000) who found that, nasal application of lipopeptide increased protection against the lethal infection of influenza; Fenard *et al.*, (2001) who found that, the honeybee venom inhibits the replication of T-lymphotropic HIV-1 isolates; Chernysh *et al.* (2002) who tested the activity of alloferon against Herpes Simplex and Hepatitis B and C infection; Slocinska *et al.* (2008) who stated that, insects originally owned Cecropins and melittin peptides that able to cause antiviral action against HSV; Ai *et al.* (2008) who found that, the protein fractions extracted and purified from the larvae of the housefly possess antiviral activity; Also, Amer *et al.* (2019b) reported that, *L. sericata*, *C. albiceps* and *M. domestica* secretion/excretion and maggots' whole body extracts recorded antiviral activity against Hepatitis A Virus (HAV).



## CONCLUSION

From the aforementioned results it could be concluded that, petroleum ether, hexane, acetone and ethyl acetate extracts of *L. sericata* maggots' possess a variable antibacterial activities against both Gram-positive and Gram-negative bacterial strains. All tested extracts showed also variable antifungal activity against tested strains except for *Penicillium*. In addition, petroleum ether extract recorded potent antiviral activity against Herpes Simplex Virus (HSV-1), concluding that it may play a role as a potential anti-HSV agent.

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