ABSTRACT

Maggot therapy's success is partly due to the ingestion and killing of living microorganisms. The aim of this study was to investigate the antibacterial activities of maggots’ crude extracts (DMSO and PBS extracts) and excretion/secretion of Chrysomya albiceps (Diptera: Calliphoridae). The excretion/secretion (E/S) and crude extracts were collected from third larval instars of C. albiceps. The extracts were tested against Escherichia coli and Pseudomonous aeruginosa, as Gram-negative bacteria and Staphylococcus aureus and Bacillus subtilis as Gram-positive bacteria. Antimicrobial activity was done by calculating the reduction percentage of the colony's growth. The E/S was appeared the highest antibacterial activities against Gram-positive and Gram-negative bacteria, while the PBS extract has appeared the lower antibacterial activities. Generally, the E/S of C. albiceps maggot was found to be more effective as antibacterial agents than crude extracts (DMSO and PBS extracts).

INTRODUCTION

Dipterous larvae can feed on the host's living or dead tissues, liquid body substance, or ingested food and can cause a broad range of infestations, depending on the body location and the relationship of the larvae with the host (Noutsis and Millikan, 1994).

In traditional medical practice, the maggots of some Calliphoridae species such as Lucilia sericata (Meigen) has been employed for maggot therapy. This mode of treatment remains appropriate for cases where antibiotics are ineffective and surgery impracticable. (Sherman and Pechter 1988)

Maggot therapy is a relatively rapid and effective treatment, particularly in large necrotic wounds requiring debridement and resistant to conventional treatment and conservative surgical intervention, maggot debridement is safe, effective, and acceptable to most patients, even when administered by nonphysicians (Mumcuoglu et al., 1999, Sherman et al., 1995, Sherman et al., 2001).

Maggot of the C. albiceps failed in maggot therapy because these maggots can cause primary and secondary cutaneous myiasis in mammals (Adham et al., 2001 and Madeira, 2001).

Insect natural products were isolated from ants, bees, wasps, beetles, cockroaches, termites, flies, true bugs, moths and more. Biological activities of these products include

Keywords: Antibacterial activity, Excretion/Secretion, crude extracts maggot, Chrysomya albiceps
antimicrobial, antifungal, antiviral, anticancer, antioxidant, anti-inflammatory and immunomodulatory effects. (Seabrooks et al., 2017). The crude extract exerts antibacterial effects by changing the bacterial membrane permeability and inhibiting plasmid DNA replication (Ge et al., 2015)

The present study aimed to evaluate the effect of C. albiceps crude extract and excretion/secretion on some pathogenic bacteria; Escherichia coli and Pseudomonous aeruginosa as Gram-negative bacteria and Staphylococcus aureus and Bacillus subtilis as Gram-positive bacteria.

**MATERIALS AND METHODS**

### Bacterial Strains:

The antibacterial activities of C. albiceps maggots’ crude extracts and excretion/secretion were tested against Escherichia coli (ATCC-11775), Pseudomonous aeruginosa (ATCC-10145), Staphylococcus aureus (ATCC-12600) Bacillus subtilis (ATCC-6051).

### Collection of Maggots’ Excretion and Secretion:

The 3rd larval instar of C. albiceps maggots was collected from the maintained culture at the Laboratory of Medical Entomology, Animal House, Faculty of Science, Al-Azhar University. The collected maggots were washed with ethanol 70 % followed by sterile distilled water and then incubated with phosphate-buffered saline (PBS) for 6 h with ratio 100 maggots/200 μL PBS at 25 °C in darkness. After that, the E/S was collected and centrifuged at 10000 rpm for 10 min at 4 °C. The supernatant was sterilized by filtration through a 0.2 μm Millipore bacterial filter and stored at -20 °C (Abdel-Samad, 2019).

### Preparation of Maggots’ Extracts:

Chrysomya albiceps maggots were collected and washed 3 times with sterilized water and stored at -80 °C. The freeze-dried maggots (25 g) were homogenized before the addition of 250 mL of solvent (in this study use tow solvents; DMSO and PBS). The mixture was stirred by using a magnetic stirrer at 4 °C for 12 hours and filtered with filter paper. The mixture was centrifuged at 13,000 × g for 20 minutes. The supernatant was sterilized and stored at 20°C (Park et al., 2015).

### Colony-forming unit (CFU) Assay:

Bacteria were grown for 17 h in 20 ml of tryptic soy broth at 30 °C with oscillation (90 oscillations per min). One millilitre of culture was washed twice in PBS and adjusted to a concentration of 2 × 10^5 bacteria per ml in fresh PBS. Fifty microliters of extracts were incubated with 10 μl of bacterial suspension for 30 min or 4 h at 37 °C. Samples were then immediately transferred onto the ice, diluted 1:4 or 1:9, respectively, with PBS; 50-μl aliquots were spread onto nutrient agar plates in triplicate before incubation overnight at 30 °C and subsequent counting of CFUs. In controls, extracts were replaced with sterile PBS, pH 8.3, or heat-inactivated extracts (100 °C for 8 min to denature proteins) (Bexfield et al., 2004).

### RESULTS

The total bacterial counts of B. subtilis, S. aureus, P. aeruginosa, and E. coli as control were 1.731×10^5, 1.548×10^5, 1.688×10^5 and1.693×10^5 CFU/ml, respectively. After treatment with E/S, a total bacterial count of B. subtilis was 0.515×10^5 CFU/ml with a bacterial growth reduction of 70.24 %. While, the reduction percent of S. aureus bacterial growth was 62.34 %, where the total bacterial count of S. aureus became 0.583×10^5 CFU/ml after treatment with E/S. The E/S reduced the total bacterial count of P. aeruginosa and E.
coli to 0.276×10^5 and 0.412×10^5 CFU/ml, respectively, with reduction percent of 83.65 and 75.66 %, respectively (Table. 1 and Fig. 1).

The highest antibacterial activity for DMSO extract against Gram-positive bacteria was recorded by *B. subtilis*, where the total bacterial count attained 1.031×10^5 CFU/ml. The reduction percent of *B. subtilis* growth was 40.44%; whereas, *S. aureus* recorded a total bacterial count of 1.013×10^5 CFU/ml after DMSO extract treatment with a reduction percent of 34.56 % (Table 2 and Fig. 1).

Results represented in table (2) and Figure (1) showed the highest antibacterial activity for DMSO extract against Gram-negative bacteria (*P. aeruginosa*). It recorded a total bacterial count of 1.008×10^5 CFU/ml after DMSO extract treatment. The reduction percent of *P. aeruginosa* growth recorded 40.28%, followed by *E. coli* (38.39 %) where a total bacterial count was 1.043×10^5 CFU/ml.

Data represented in table (3) and Figure (1) showed limited antibacterial activity for PBS extract against the tested Gram-positive and Gram-negative bacteria. The total bacterial count of *B. subtilis*, *P. aeruginosa*, and *E. coli* was 1.603×10^5, 1.574×10^5 and 1.489×10^5 CFU/ml with reduction percent 7.39, 6.75 and 12.05 %, respectively. Whereas the effect of PBS extract against *S. aureus* was not detected.

**Table (1):** Antibacterial activity of *Chrysomya albiceps* maggot’s excretion/secretion.

<table>
<thead>
<tr>
<th>Bacteria</th>
<th>Survival cells (CFU/ml)</th>
<th>Reduction (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Treated</td>
</tr>
<tr>
<td><em>Bacillus subtilis</em></td>
<td>1.731×10^5</td>
<td>0.515×10^5</td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em></td>
<td>1.548×10^5</td>
<td>0.583×10^5</td>
</tr>
<tr>
<td><em>Pseudomonas aeruginosa</em></td>
<td>1.688×10^5</td>
<td>0.276×10^5</td>
</tr>
<tr>
<td><em>Escherichia coli</em></td>
<td>1.693×10^5</td>
<td>0.412×10^5</td>
</tr>
</tbody>
</table>

**Table (2):** Antibacterial activity of *Chrysomya albiceps* maggot’s DMSO extract.

<table>
<thead>
<tr>
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<th>Survival cells (CFU/ml)</th>
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</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Treated</td>
</tr>
<tr>
<td><em>Bacillus subtilis</em></td>
<td>1.731×10^5</td>
<td>1.031×10^5</td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em></td>
<td>1.548×10^5</td>
<td>1.013×10^5</td>
</tr>
<tr>
<td><em>Pseudomonas aeruginosa</em></td>
<td>1.688×10^5</td>
<td>1.008×10^5</td>
</tr>
<tr>
<td><em>Escherichia coli</em></td>
<td>1.693×10^5</td>
<td>1.043×10^5</td>
</tr>
</tbody>
</table>

**Table (3):** Antibacterial activity of *Chrysomya albiceps* maggot’s PBS extract.

<table>
<thead>
<tr>
<th>Bacteria</th>
<th>Survival cells (CFU/ml)</th>
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</tr>
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<tbody>
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<td><em>Pseudomonas aeruginosa</em></td>
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<td>1.574×10^5</td>
</tr>
<tr>
<td><em>Escherichia coli</em></td>
<td>1.693×10^5</td>
<td>1.489×10^5</td>
</tr>
</tbody>
</table>
Fig. 1: Antibacterial activities of Chrysomya albiceps maggots’ excretion/secretion and crude extracts (DMSO and PBS extracts).

DISCUSSION

The pronounced positive result of antibacterial activity of E/S against different bacterial strains in this study was in complete agreement with the results of Thomas et al. (1999), where the E/S was able to kill or prevent the growth of a wide range of potentially pathogenic bacteria.

The present study proved potent antibacterial activity of Chrysomya albiceps maggots’ E/S against both Gram-negative bacteria (Escherichia coli and Pseudomonas aeruginosa) and Gram-positive bacteria (Staphylococcus aureus and Bacillus subtilis). This activity was similar to that recorded by Barnes et al., (2010), using the maggot E/S of blowflies which were to be able to inhibit or reduce the bacterial growth of S. aureus, E. coli, and P. aeruginosa.

Hassan et al. (2016) recorded that E/S inhibits the growth of Gram-positive bacteria (S. aureus and B. subtilis) Gram–negative bacteria (P. aeruginosa and E. coli), these findings were hassling with the antibacterial activity of E/S against S. aureus, B. subtilis, P. aeruginosa and E. coli obtained in the present study. Also, E/S showed antibacterial activity against S. aureus and P. aeruginosa, this result was similar to that of Kerridge et al., 2005 (using S. aureus and P. aeruginosa) and Harris et al., 2013 (using S. aureus).

The effect of E/S against different bacterial strains may be resulting from biofilm disruption of bacteria, as referred by Valachova et al. (2014) who stated that the E/S of the maggot is differentially effective against biofilms of S. aureus and P. aeruginosa.

The natural compounds of crude extracts obtained from different insect sources can inhibit the growth of various bacterial species such as P. aeruginosa, E. coli, and S. aureus (Abdu-Allah et al., 2019). In agreement with the results of Teh et al. (2017), the larval extract of L. cuprina exerted a broad-spectrum antibacterial activity against S. aureus, methicillin resistant S. aureus (MRSA), P. aeruginosa and E. coli.

The positive results of maggot extracts in this study compatible with the notes of Hou et al. (2007), where the extracts of dipterous larvae possess a broad antibacterial activity against both Gram-negative bacteria and Gram-positive bacteria.

Conclusion

Generally, the E/S of C. albiceps maggots were found to be more effective as antibacterial agents than DMSO and PBS extracts, whereas DMSO extract was found to be more effective than PBS extract against the studied bacterial pathogens.
REFERENCES


