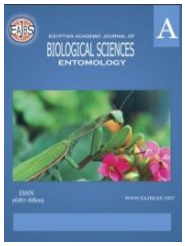
Egypt. Acad. J. Biolog. Sci., 13(1):99-104 (2020)

Egyptian Academic Journal of Biological Sciences
A. Entomology

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



Antibacterial Activities of *Chrysomya albiceps* Maggots' Extracts (Diptera: Calliphoridae)

Reda H. Elmorsy; Ahmed S. Bream, and Mohammad R. K. Abdel-Samad*
Department of Zoology and Entomology, Faculty of Science, Al- Azhar University, Cairo, Egypt
Email: mrk1323@azhar.edu.eg

ARTICLE INFO
Article History
Received:9/1/2020
Accepted:12/2/2020

Keywords:
Antibacterial activity, Excretion/Secretion, crude extracts maggot, *Chrysomya albiceps*

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 th*e 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

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.

Bacteria	Survival cells (CFU/ml)		Reduction (%)
	Control	Treated	
Bacillus subtilis	1.731×10^5	0.515×10^5	70.24
Staphylococcus aureus	1.548×10^5	0.583×10^5	62.34
Pseudomonas aeruginosa	1.688×10^5	0.276×10^5	83.65
Escherichia coli	1.693×10^5	0.412×10^5	75.66

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

Bacteria	Survival cells (CFU/ml)		Reduction (%)
	Control	Treated	
Bacillus subtilis	1.731×10^5	1.031×10^5	40.44
Staphylococcus aureus	1.548×10^5	1.013×10^5	34. 56
Pseudomonas aeruginosa	1.688×10^5	1.008×10^5	40.28
Escherichia coli	1.693×10^5	1.043×10^5	38.39

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

Bacteria	Survival cells (CFU/ml)		Reduction (%)
	Control	Treated	
Bacillus subtilis	1.731×10^5	1.603×10^5	7.39
Staphylococcus aureus	1.548×10^5	1.552×10^5	Not detected
Pseudomonas aeruginosa	1.688×10^5	1.574×10^5	6.75
Escherichia coli	1.693×10^5	1.489×10^5	12. 05

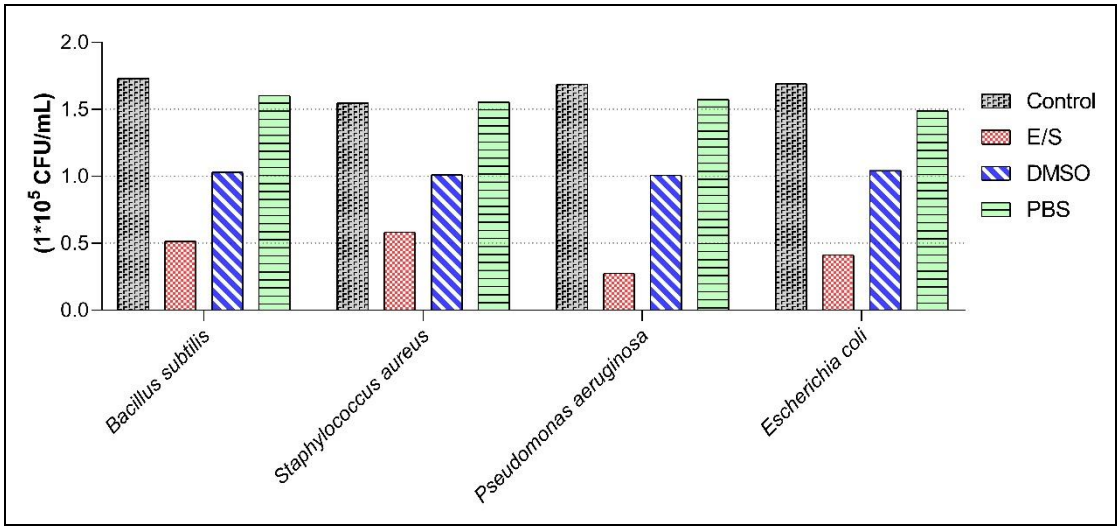


Fig. 1: Antibacterial activities of *Chrysomya albiceps* maggots' excretion/secretion and crude extracts (DMSO and PBS extracts).

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 (*Staphylococcu 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

Abdel-Samad, M. R. (2019). Antiviral and virucidal activities of *Lucilia cuprina* maggots' excretion/secretion (Diptera: Calliphoridae): first work. Heliyon, 5(11), e02791.

Abdu-Allah, S. N., Mzhr, N. N., Alubadi, A. E. M., & Shanyoor, G. J. (2019). Effect of Crude Extracts of Natural Compounds from local Iraqi insects of Worker bees and Ladybirds as Antimicrobial Activity on Pathogens. Journal of Pharmaceutical Sciences and Research, 11(2), 371-374.

Adham, F. K., Abdel-Meguid, A., Tawfik, M. A. A., & El-Khateeb, R. M. (2003). Seasonal incidence of the carrion breeding blowflies *Lucilia sericata* (Meigen) and *Chrysomya albiceps* (Wied.) (Diptera: Calliphoridae) in Abu-Rawash Farm, Giza, Egypt. Veterinary Medical Journal, 49: 377-383.

Barnes, K. M., Gennard, D. E., & Dixon, R. A. (2010). An assessment of the antibacterial activity in larval excretion/secretion of four species of insects recorded in association with corpses, using *Lucilia sericata* Meigen as the marker species. Bulletin of entomological research, 100(6), 635-640.

Bexfield, A., Nigam, Y., Thomas, S. & Ratcliffe, N. A. (2004). Detection and partial characterisation of two antibacterial factors from the excretions/secretions of the medicinal maggot *Lucilia sericata* and their activity against methicillin-resistant *Staphylococcus aureus* (MRSA). Microbes and infection, 6(14), 1297-1304.

Ge, Q. S., Zhang, H. M., Liu, X., Wang, S. Y., Lv, D. C., & Li, X. D. (2015). Crude extract of maggots: Antibacterial effects against *Escherichia coli*, underlying mechanisms, separation and purification. World Journal of Gastroenterology: WJG, 21(5), 1510.

Harris, L. G., Nigam, Y., Sawyer, J., Mack, D., & Pritchard, D. I. (2013). *Lucilia sericata* chymotrypsin disrupts protein adhesin-mediated *staphylococcal* biofilm formation. Appl. Environ. Microbiol., 79(4), 1393-1395.

Hassan, M. I., amer, M. S., hammad, K. M., & Zidan, M. M. (2016). antimicrobial activity for excretion and secretion of the greenbottle fly larvae *Lucilia sericata* (meigen)(diptera: calliphoridae). journal of the egyptian society of parasitology, 46(1), 179-184.

Hou, L., Shi, Y., Zhai, P., & Le, G. (2007). Antibacterial activity and in vitro anti-tumor activity of the extract of the larvae of the housefly (*Musca domestica*). Journal of Ethnopharmacology, 111(2), 227-231.

Kerridge, A., Lappin-Scott, H., & Stevens, J. R. (2005). Antibacterial properties of larval secretions of the blowfly, *Lucilia sericata*. Medical and veterinary entomology, 19(3), 333-337.

Madeira, N. G. (2001). Would *Chrysomya albiceps* (Diptera: Calliphoridae) be a beneficial species? Arquivo Brasileiro de Medicina Veterinária e Zootecnia, 53(2), 1-5.

Mumcuoglu, K. Y., Ingber, A., Gilead, L., Stessman, J., Friedmann, R., Schulman, H., & Bichucher, H. (1999). Maggot therapy for the treatment of intractable wounds. International journal of dermatology, 38(8), 623-627.

Noutsis, C., & Millikan, L. E. (1994). Myiasis. Dermatologic clinics, 12(4), 729-736.

Park, K. H., Kwak, K. W., Nam, S. H., Choi, J. Y., Lee, S. H., Kim, H. G. and Kim, S. H. (2015). Antibacterial activity of larval extract from the black soldier fly *Hermetia illucens* (diptera: Stratiomyidae) against plant pathogens. J. Entomol. Zool. Stud, 3, 176-179.

Seabrooks, L., & Hu, L. (2017). Insects: an underrepresented resource for the discovery of biologically active natural products. Acta Pharmaceutica Sinica B, 7(4), 409-426.

Sherman, R. A., & Pechter, E. A. (1988). Maggot therapy: a review of the therapeutic applications of fly larvae in human medicine, especially for treating

osteomyelitis. Medical and veterinary entomology, 2(3), 225-230.

Sherman, R. A., Sherman, J., Gilead, L., Lipo, M., & Mumcuoglu, K. Y. (2001). Maggot debridement therapy in outpatients. Archives of physical medicine and rehabilitation, 82(9), 1226-1229.

Sherman, R. A., Wyle, F., & Vulpe, M. (1995). Maggot therapy for treating pressure ulcers in spinal cord injury patients. The journal of spinal cord medicine, 18(2), 71-74.

Teh, C. H., Nazni, W. A., Norazah, A., & Lee, H. L. (2017). Determination of antibacterial activity and minimum inhibitory concentration of larval extract of fly via resazurin-based turbidometric assay. BMC microbiology, 17(1), 36.

Thomas, S., Andrews, A. M., Hay, N. P., & Bourgoise, S. (1999). The anti-microbial activity of maggot secretions: results of a preliminary study. Journal of Tissue Viability, 9(4), 127-132.

Valachova, I., Bohová, J., Kozanek, M., Takac, P., & Majtan, J. (2014). *Lucilia sericata* medicinal maggots: A new source of antimicrobial compounds. Microbial Pathogens and Strategies for Combating Them: Science, Technology and Education. Spain: Formatex Research Center, 1745-53.