



Research article

Antiviral and virucidal activities of *Lucilia cuprina* maggots' excretion/secretion (Diptera: Calliphoridae): first work



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ABSTRACT

Maggots of *Lucilia sericata* and *L. cuprina* are a backbone of the maggot debridement therapy. Further, the excretion/secretion (E/S) of these maggots has antibacterial and antifungal activities, nevertheless the antiviral activity of E/S for these maggots still out the focus. This study aimed to evaluate the E/S of *L. cuprina* maggots against the Rift Valley Fever (RVF) and Coxsackie B4 (CB4) viruses for first time. After collection of the E/S, its cytotoxicity on Vero cells was evaluated and the safe concentration was determined which used to investigate the antiviral and virucidal effect of E/S on the selected viruses. The E/S decreased the titers of the tested viruses compared with that of untreated viruses. The outcome data refer to that the E/S of *L. cuprina* consider as a promising antiviral and virucidal agent.

1. Introduction

Maggot debridement therapy (MDT) is an expression referring to the treatment of wounds by using of maggots (Larvae of flies). The surgeon Ambroise Paré was the first person noted the beneficial effects of maggots on the soldiers' wounds on the battlefield of Saint Quentin (1557) (Goldstein, 1931). The maggots of *Lucilia sericata* and *L. cuprina* are considered as a core of the MDT. The process of MDT done through three stages: debridement (Cleaning the necrotic tissues), disinfection (Antimicrobial effects) and stimulation of wound healing (Erdmann and Khalil, 1986; Chan et al., 2007; Sherman, 2014).

Excretion/secretion (E/S) produced by maggots possesses proteolytic enzymes and contains collagenase, allantoïn, sulphydryl radicals, serine proteases, calcium, cysteine, glutathione, growth stimulating factors, leucine aminopeptidase, carboxypeptidases A & B and embryonic growth stimulating substance (Vistnes et al., 1981; Casu et al., 1994; Chambers et al., 2003; Nigam et al., 2006; Gupta, 2008). These proteolytic enzymes can dissolve the necrotic and infected tissues on the wound.

Despite the significance of medical uses of *L. sericata* and *L. cuprina* maggots or their E/S, the antiviral activity of E/S for these maggots still out the focus despite viruses such as Rift Valley fever virus, Dengue virus, Coxsackie virus...etc. represent dangerous pathogens worldwide.

According to World Health Organization (World Health Organization, 2018), severe form of RVF in humans was occurred in many countries

such as; Republic of Niger (2016), Republic of Mauritania (2012), Republic of South Africa (2010), Madagascar (2008–2009), Sudan (2007), Kenya, Somalia & Tanzania (2006), Egypt (2003) and Saudi Arabia and Yemen (2000). The availability and abundance of the potential vectors, suitability of environmental conditions, importation of livestock's from infected countries and the close association of susceptible domestic animals with humans, the RVF virus could possibly occur and circulate (Kenawy et al., 2018).

In this study, depending on the significant effects of maggots' E/S against bacteria, fungi and *Leishmania*, the effect of *Lucilia cuprina* maggots' E/S on Rift Valley Fever (RVF) and Coxsackie B4 (CB4) viruses was investigated for first time.

2. Materials and methods

2.1. Collection of E/S

The 3rd larval instar of *Lucilia cuprina* maggots was collected from 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

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supernatant was sterilized by filtration through a 0.2 µm Millipore bacterial filter and stored at -20 °C.

2.2. Cells and viruses

Vero cells of adult African green monkey kidney (American tissue culture collection, USA) were supplied by Tissue Culture Department, VACSERA. The Vero cells were maintained in MEME media supplemented with fetal calf serum (10 %), penicillin (100 µg/ml) and streptomycin 10 (10 µg/ml) in CO₂ incubator at 37 °C. Pantropic Rift Valley fever (RVF) virus Menya strain [Menya/Sheep/258] and Cocksackie B4 virus (CB4) were used to investigate the antiviral and virucidal activities of E/S on Vero cells using cytopathic effect inhibition assay. The viruses kindly provided by laboratory of Virology at VACSERA Company, Dokki, Giza.

2.3. Cell viability

Cytotoxicity of the E/S was assessed on Vero cells by the MTT assay (Bahuguna et al., 2017). Briefly; the Vero cells were seeded in a 96-well microtiter plate and incubated for 24 h at 37 °C in a 5 % CO₂ incubator. The cells were divided in to sets, one treated with two-fold serial dilution from the E/S which diluted with culture media, while in other set, the culture media were added to cells and considered as control. The cells were re-incubated for 24 h in the same conditions. After that, MTT was added (10 µl/well) and incubated for 4 h at 37 °C in a CO₂ incubator. Then DMSO (50 µl/well) was added for 5 min at 37 °C in a CO₂ incubator. Finally, the optical density was measured with ELISA reader at 570 nm for treated cells at each dilution and cells without treatment as control. Cell viability % was calculated by the following equation:

$$\text{Cell viability \%} = (\text{mean OD of tested cells}/\text{mean OD of control cells}) \times 100$$

where OD is optical density.

2.4. Antiviral activity assay

Vero cells were seeded in tissue culture plate 96-well and divided into 2 sets. The 1st set was treated with the safe concentration of E/S (the safe concentration was adjusted by dilute E/S with culture media) as treated group, while the other set was treated with the free media of E/S as control group. Both groups were incubated at 37 °C for 24 h. After incubation, the media were discarded, and D-PBS was used for washing. The virus (multiplicity of infection (MOI) = 0.1) was added into both sets to obtain the control and treated groups. After that, both groups were incubated at 37 °C for 24 h. Titers of control and treated RVF and CB4 viruses were determined according to the standard TCID₅₀ method (Reed and Muench, 1938).

2.5. Virucidal activity assay

Virucidal activity of E/S against RVF and CB4 viruses (MOI = 0.1) was conducted on monolayer of Vero cells. The virus was mixed with equal volume from the safe concentration of E/S and incubated at 37 °C for 1, 3, 6 and 24 h to obtain treated group. The negative control group was obtained by mixing the virus with equal volume from the E/S' free media and incubated at 37 °C for the same time intervals. Both groups were added into previously cultured Vero cells and incubated at 37 °C for 24 h. Titers of control and treated viruses were determined according to the standard TCID₅₀ method (Reed and Muench, 1938).

2.6. Statistical analysis

Data were expressed as the mean ± standard error of the mean (S.E.M). The significance of differences between the values of control and treated groups was assessed using a two-tailed independent Student's t-

tests and performed using GraphPad Prism version 8.0.0 for Windows, GraphPad Software, San Diego, California USA. Statistically significant was set at $P \leq 0.05$.

3. Results

3.1. Cell viability

Regarding the Cytotoxicity evaluation of the E/S, the viability of Vero cells is inversely proportional with the E/S concentration. The concentration of E/S (100 %) induced a decrease in cell viability and showed approximately 30 % cell viability. While concentrations of 50, 25, 12.5, 6.25 and 3.13 % showed 34, 48, 78, 99 and 100 % cell viability, respectively (Fig. 1). The safe concentration of the E/S, which used in the antiviral and virucidal investigations, was considered as 5 %.

3.2. Titers of control viruses

Infectivity titers of control RVF and CB4 viruses were 5.8 and 4.7 log (10)/0.1 ml, respectively.

3.3. Antiviral activity

Safe concentration of E/S (5 %) significantly reduced the infectivity titers of RVF and CB4 viruses. This concentration significantly reduced the infectivity titer of RVF virus to 5.5 log (10)/0.1 ml ($P < 0.05$) and CB4 virus to 3.5 log (10)/0.1 ml ($P < 0.01$) as shown in Fig. 2. The reduction percent was approximately 5 % and 26 % for RVF and CB4 viruses, respectively.

3.4. Virucidal activity

The E/S (5 %) showed a significant reduction in infectivity titer of RVF virus. The virus titer became 4.3 log (10)/0.1 ml ($P < 0.01$) after incubation with the concentration of E/S for 1 h. While the titer of RVF virus was reduced to 3.8, 3.3 and 2.5 log (10)/0.1 ml ($P < 0.001$) after incubation with E/S concentration for 3, 6 and 24 h, respectively (Fig. 3). Actually, the reduction percent of infectivity titer was approximately 26, 35, 44 and 57% for 1, 3, 6 and 24 h, respectively.

In the same time, the infectivity titer of CB4 virus was significantly reduced after intervals times' incubation with the 5 % E/S ($P < 0.001$). After 1 h incubation, the infectivity titer was 2.25 log (10)/0.1 ml with reduction nearly 53 %, while, it became 1.75 and 1.25 log (10)/0.1 ml with reduction nearly 63% and 74% after incubation for 3 and 6 h, respectively. The infectivity titer disappeared after incubation for 24 h,

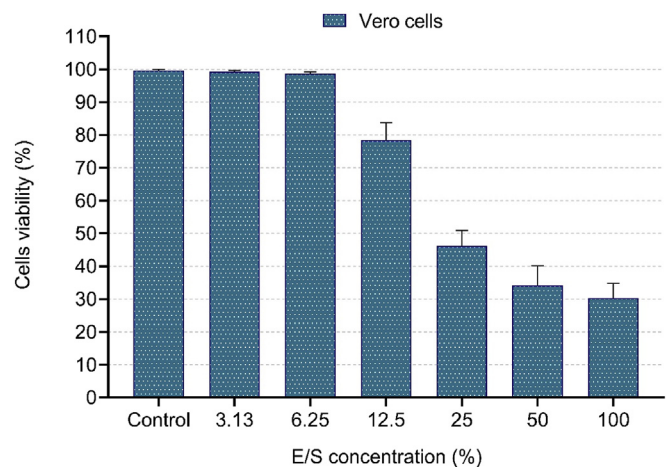


Fig. 1. Cytotoxicity of the *Lucilia cuprina* maggots' excretion/secretion on the Vero cells.

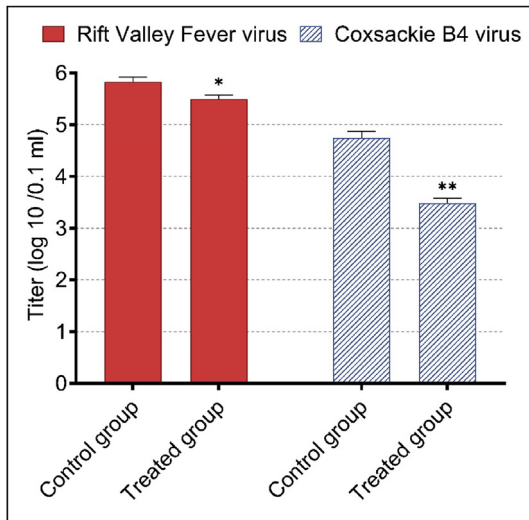


Fig. 2. Antiviral activity of *Lucilia cuprina* maggots' excretion/secretion against Rift Valley Fever and Coxsackie B4 viruses. The statistical significance is analyzed by independent t-test (* $P < 0.05$ and ** $P < 0.01$).

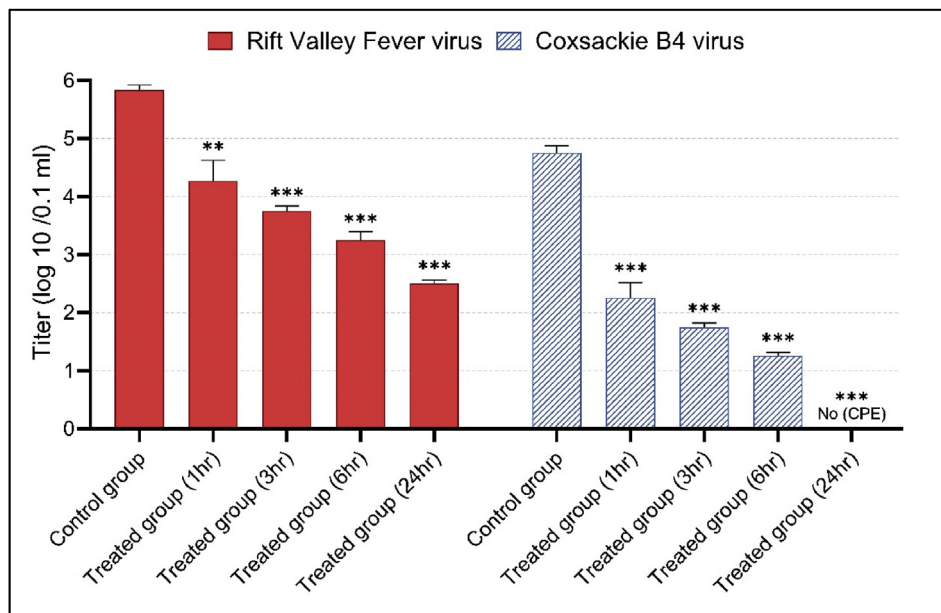


Fig. 3. Virucidal activity of *Lucilia cuprina* maggots' excretion/secretion against Rift Valley Fever and Coxsackie B4 viruses, CPE = Cytopathic effect. The statistical significance is analyzed by independent t-test (* $P < 0.05$, ** $P < 0.01$ and *** $P < 0.001$).

where, no cytopathic effect (CPE) was detected (Fig. 3).

4. Discussion

Globally, the insects and their extracts play a great role in the traditional medicine, where, natural components from insect bodies, secretions and excretions showed an activity against bacteria, fungi, viruses and parasites (Wachinger et al., 1998; De Clercq, 2000; Muharsini et al., 2000; Meylaers et al., 2004; Huberman et al., 2007; Slocinska et al., 2008; Meenakshisundaram et al., 2009; Lu et al., 2014; Coelho et al., 2015; Faruck et al., 2016). Broad spectrum antimicrobial peptides produced from most insects during innate immune responses and have a great antimicrobial activity (Bulet and Stocklin, 2005; Poppel et al., 2015; Xu et al., 2006).

Maggot therapy, whether the uses of *Lucilia sericata* and/or *L. cuprina* maggots itself or its excretion/secretion, is considered as a modern

technique in managements of the wound and infection. The effect of E/S of *L. cuprina* maggots gave antimicrobial effects, where, the E/S of *L. sericata* and/or *L. cuprina* maggots have antibacterial activity (Bexfield et al., 2004; Kerridge et al., 2005; van der Plas et al., 2007; Jiang et al., 2012; Valachova et al., 2014; Hassan et al., 2016), antifungal activity (Poppel et al., 2014; Hassan et al., 2016) and anti-leishmanial activity (Polat et al., 2012; Sanei-Dehkordi et al., 2016; Laverde-Paz et al., 2018).

The relationship between cell viability and concentration E/S of *L. cuprina* in this study is similar with the findings of Sanei-Dehkordi et al. (2016), where the cell viability decreased with increasing the concentration of E/S from *L. sericata*.

In this study, RVF virus was affected by the E/S of *L. cuprina* maggots which showed antiviral and virucidal activities, where, the virus titer was decreased. Also, the same finding was observed with CB4 virus. The antiviral and virucidal effects of E/S might be due to the presence of some constituent compounds such as serine proteases, glutathione and cysteine. As published in literatures, these compounds have remarkable effects on some viruses; serine proteases limit the Dengue virus type 2 infection (Brackney et al., 2008). While, glutathione inhibits the viral production of dengue virus infection (Tian et al., 2010) and may be has a value in human immunodeficiency virus (HIV) treatment (Kalebic et al., 1991). In addition, cysteine derivative inhibits the H5N1 replication (Geiler et al., 2010).

5. Conclusion

In conclusion, the effect of *Lucilia cuprina* maggots' excretion/secretion (E/S) on viruses was investigated for first time. The E/S of *L. cuprina* maggots reduced the infectivity titer of Rift Valley fever (RVF) and Coxsackie B4 (CB4) viruses. Therefore, this study recommended that E/S of *L. cuprina* maggots may serve as additional therapeutic options affecting on viral diseases. Future studies should focus on the role of E/S of *L. cuprina* maggots in the treatment of viral diseases and mode of action in vitro and in vivo levels.

Declarations

Author contribution statement

Mohammad R. K. Abdel-Samad: Conceived and designed the

experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

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Competing interest statement

The authors declare no conflict of interest.

Additional information

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References

- Bahuguna, A., Khan, I., Bajpai, V., Kang, S., 2017. MTT assay to evaluate the cytotoxic potential of a drug. *Bangladesh J. Pharmacol.* 12, 115–118.
- 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). *Microb. Infect.* 6, 1297–1304.
- Brackney, D.E., Foy, B.D., Olson, K.E., 2008. The effects of midgut serine proteases on dengue virus type 2 infectivity of *Aedes aegypti*. *Am. J. Trop. Med. Hyg.* 79, 267–274.
- Bulet, P., Stocklin, R., 2005. Insect antimicrobial peptides: structures, properties and gene regulation. *Protein Pept. Lett.* 12, 3–11.
- Casu, R.E., Pearson, R.D., Jarmey, J.M., Cadogan, L.C., Riding, G.A., Tellam, R.L., 1994. Excretory/secretory chymotrypsin from *Lucilia cuprina*: purification, enzymatic specificity and amino acid sequence deduced from mRNA. *Insect Mol. Biol.* 3, 201–211.
- Chambers, L., Woodrow, S., Brown, A.P., Harris, P.D., Phillips, D., Hall, M., Church, J.C.T., Pritchard, D.I., 2003. Degradation of extracellular matrix components by defined proteinases from the greenbottle larva *Lucilia sericata* used for the clinical debridement of non-healing wounds. *Br. J. Dermatol.* 148, 14–23.
- Chan, D.C.W., Fong, D.H.F., Leung, J.Y.Y., Patil, N.G., Leung, G.K.K., 2007. Maggot debridement therapy in chronic wound care. *Hong Kong Med. J.* 13, 382–386.
- Coelho, G., Mendonça, R., Vilar, K., Figueiredo, C., Badari, J., Taniwaki, N., Namiyama, G., 2015. Antiviral action of hydromethanolic extract of geoprotopolis from *Scaptotrigona postica* against antiherpex simplex virus (HSV-1). Evidence-based Complement. Altern. Med. eCAM2015 .
- De Clercq, E., 2000. Current lead natural products for the chemotherapy of human immunodeficiency virus (HIV) infection. *Med. Res. Rev.* 20, 323–349.
- Erdmann, G.R., Khalil, S.K., 1986. Isolation and identification of two antibacterial agents produced by a strain of *Proteus mirabilis* isolated from larvae of the screwworm (*Cochliomyia hominivorax*) (Diptera: Calliphoridae). *J. Med. Entomol.* 23, 208–211.
- Faruck, M.O., Yusof, F., Chowdhury, S., 2016. An overview of antifungal peptides derived from insect. *Peptides* 80, 80–88.
- Geiler, J., Michaelis, M., Naczki, P., Leutz, A., Langer, K., Doerr, H.-W., Cinatl, J.J., 2010. N-acetyl-L-cysteine (NAC) inhibits virus replication and expression of pro-inflammatory molecules in A549 cells infected with highly pathogenic H5N1 influenza A virus. *Biochem. Pharmacol.* 79, 413–420.
- Goldstein, H.I., 1931. Maggots in the treatment of wound and bone infections. *J. Bone Jt. Surg.* 13, 476–478.
- Gupta, A., 2008. A review of the use of maggots in wound therapy. *Ann. Plast. Surg.* 60, 224–227.
- Hassan, M.I., Amer, M.S., Hammad, K.M., Zidan, M.M., 2016. Antimicrobial activity for excretion and secretion of the green bottle fly larvae *Lucilia sericata* (Meigen) (Diptera: Calliphoridae). *J. Egypt. Soc. Parasitol.* 46, 179–184.
- Huberman, L., Gollop, N., Mumcuoglu, K.Y., Block, C., Galun, R., 2007. Antibacterial properties of whole body extracts and haemolymph of *Lucilia sericata* maggots. *J. Wound Care* 16, 123–127.
- Jiang, K., Sun, X., Wang, W., Liu, L., Cai, Y., Chen, Y., Luo, N., Yu, J., Cai, D., Wang, A., 2012. Excretions/secretions from bacteria-pretreated maggot are more effective against *Pseudomonas aeruginosa* biofilms. *PLoS One* 7, e49815.
- Kalebic, T., Kinter, A., Poli, G., Anderson, M.E., Meister, A., Fauci, A.S., 1991. Suppression of human immunodeficiency virus expression in chronically infected monocytic cells by glutathione, glutathione ester, and N-acetylcysteine. *Proc. Natl. Acad. Sci. U. S. A.* 88, 986–990.
- Kenawy, M.A., Abdel-Hamid, Y.M., Beier, J.C., 2018. Rift Valley Fever in Egypt and other African countries: historical review, recent outbreaks and possibility of disease occurrence in Egypt. *Acta Trop.* 181, 40–49.
- Kerridge, A., Lappin-Scott, H., Stevens, J.R., 2005. Antibacterial properties of larval secretions of the blowfly, *Lucilia sericata*. *Med. Vet. Entomol.* 19, 333–337.
- Laverde-Paz, M.J., Echeverry, M.C., Patarroyo, M.A., Bello, F.J., 2018. Evaluating the anti-leishmania activity of *Lucilia sericata* and *Sarconesiopsis magellanica* blowfly larval excretions/secretions in an in vitro model. *Acta Trop.* 177, 44–50.
- Lu, X., Jin, X., Wang, J., Chu, F., Zhu, J., 2014. Antihepatitis B virus activity of a protein-enriched fraction from housefly (*Musca domestica*) in a stable HBV-producing cell line. *Sci. World J.*
- Meenakshisundaram, R., Sweni, S., Thirumalaikulundusubramanian, P., 2009. Hypothesis of snake and insect venoms against Human Immunodeficiency Virus: a review. *AIDS Res. Ther.* 6, 25.
- Meylaers, K., Clynen, E., Daloz, D., DeLoof, A., Schoofs, L., 2004. Identification of 1-lysophosphatidylethanolamine (C(16:1)) as an antimicrobial compound in the housefly, *Musca domestica*. *Insect Biochem. Mol. Biol.* 34, 43–49.
- Muharsini, S., Sukarsih, Riding, G., Partoutomo, S., Hamilton, S., Willadsen, P., Wijffels, G., 2000. Identification and characterisation of the excreted/secreted serine proteases of larvae of the old world screwworm fly, *Chrysomya bezziana*. *Int. J. Parasitol.* 30, 705–714.
- Nigam, Y., Bexfield, A., Thomas, S., 2006. Maggot therapy: the science and implication for CAM Part I—history and bacterial resistance. Evidence-based Complement. Altern. Med. eCAM223–227 ,3 .
- Polat, E., Cakan, H., Aslan, M., Sirekbasan, S., Kutlubay, Z., Ipek, T., Ozbilgin, A., 2012. Detection of anti-leishmanial effect of the *Lucilia sericata* larval secretions in vitro and in vivo on *Leishmania tropica*: first work. *Exp. Parasitol.* 132, 129–134.
- Poppel, A.-K., Koch, A., Kogel, K.-H., Vogel, H., Kollwe, C., Wiesner, J., Vilcinskas, A., 2014. Lucimycin, an antifungal peptide from the therapeutic maggot of the common green bottle fly *Lucilia sericata*. *Biol. Chem.* 395, 649–656.
- Poppel, A.-K., Vogel, H., Wiesner, J., Vilcinskas, A., 2015. Antimicrobial peptides expressed in medicinal maggots of the blow fly *Lucilia sericata* show combinatorial activity against bacteria. *Antimicrob. Agents Chemother.* 59, 2508–2514.
- Reed, L.J., Muench, H., 1938. A simple method of estimating fifty per cent endpoints. *J. Epidemiol.* 27, 493–497.
- Sanei-Dehkordi, A., Khamesipour, A., Akbarzadeh, K., Akhavan, A.A., Mir Amin Mohammadi, A., Mohammadi, Y., Rassi, Y., Oshaghi, M.A., Alebrahim, Z., Eskandari, S.E., Rafinejad, J., 2016. Anti Leishmania activity of *Lucilia sericata* and *Calliphora vicina* maggots in laboratory models. *Exp. Parasitol.* 170, 59–65.
- Sherman, R.A., 2014. Mechanisms of maggot-induced wound healing: what do we know, and where do we go from here? *Evid. Based. Complement. Alternat. Med.* 592419, 2014.
- Slocinka, M., Marciniak, P., Rosinski, G., 2008. Insects antiviral and anticancer peptides: new leads for the future? *Protein Pept. Lett.* 15, 578–585.
- Tian, Y., Jiang, W., Gao, N., Zhang, J., Chen, W., Fan, D., Zhou, D., An, J., 2010. Inhibitory effects of glutathione on dengue virus production. *Biochem. Biophys. Res. Commun.* 397, 420–424.
- Valachova, I., Prochazka, E., Bohova, J., Novak, P., Takac, P., Majtan, J., 2014. Antibacterial properties of lucifensin in *Lucilia sericata* maggots after septic injury. *Asian Pac. J. Trop. Biomed.* 4, 358–361.
- van der Plas, M.J.A., Jukema, G.N., Wai, S.-W., Dogterom-Ballering, H.C.M., Lagendijk, E.L., van Gulpen, C., van Dissel, J.T., Bloemberg, G.V., Nibbering, P.H., 2007. Maggot excretions/secretions are differentially effective against biofilms of *Staphylococcus aureus* and *Pseudomonas aeruginosa*. *J. Antimicrob. Chemother.* 61, 117–122.
- Vistnes, L.M., Lee, R., Ksander, G.A., 1981. Proteolytic activity of blowfly larvae secretions in experimental burns. *Surgery* 90, 835–841.
- Wachinger, M., Kleinschmidt, A., Winder, D., von Pechmann, N., Ludvigsen, A., Neumann, M., Holle, R., Salmons, B., Erfle, V., Brack-Werner, R., 1998. Antimicrobial peptides melittin and cecropin inhibit replication of human immunodeficiency virus 1 by suppressing viral gene expression. *J. Gen. Virol.* 79 (Pt 4), 731–740.
- World Health Organization, 2018. Rift Valley Fever [WWW Document]. (Accessed 13 October 2018).
- Xu, X., Li, J., Lu, Q., Yang, H., Zhang, Y., Lai, R., 2006. Two families of antimicrobial peptides from wasp (*Vespa magnifica*) venom. *Toxicon* 47, 249–253.