Assessment Of The Antimicrobial Activity Of Maggots Extract Of Calliphora Vicina (Diptera: Calliphoridae)

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Abstract

Background: Maggot therapy is an old method used for chronic wound healing before the Penicillin discovery. In literature, maggots of blowflies are successfully employed to clean plenty of chronic necrotic wounds and reduce infecting microorganisms. Therefore this study was conducted to analyze the *Calliphora vicina* excretions/ secretions and determine their antimicrobial activity against different microorganisms.

Methods: For this purpose, early third instar Larvae of *Calliphora vicina* were used to collect excretions/secretions extract. Gas chromatography/ mass spectrometry technique was utilized to analyze and determine the active compounds of the extract.

Results: Extract was applied on *Bacillus cereus*, *Escherichia coli*, *Staphylococcus aureus*, *Salmonella typhi*, and *Pseudomonas aeruginosa (*P36 & P7) bacteria, and *Candida albicans* fungus. Analysis of *Calliphora. vicina* excretions/secretions showed the presence of many proteins and fatty acids playing a role as antimicrobial agents against *Staphylococcus aureus*, *Escherichia coli*, *Salmonella typhi*, Pseudomonas *aeruginosa (*P36 and P7), and *Candida albicans*, but have no effect against *Bacillus cereus.*

Conclusion: Numerous antimicrobial compounds found in *Calliphora* excretions/secretions elucidate a precise strategy helping maggots to combat various microorganisms especially bacteria.

Keywords: Dipteran maggots. Antimicrobial activity. Excretions/ Secretions. *Calliphora vicina*.

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Background

Maggots are fly larvae that are members of the family Calliphoridae, Diptera. One of the old herbal cures in China was the use of maggots. In maggot debridement therapy (MDT), live maggots had been used to clean wounds by liquifying, and feeding on only dead tissues (**Sherman, 2014**). The basic process of wound healing is carried out by 3 main steps; debridement, disinfection and simulation of wound repairing (**Rayner, 1999; Richardson, 2004**). The antiseptic activity of maggots during the disinfection process led many researchers to investigate their antimicrobial properties. The demonstration of antimicrobial activity of maggot extract was dated back to the 1930s by Simmons et al., followed by Pavillard et al., who described the techniques of extraction and microbial activity of Excretion/ secretion (ES) extract of maggots (**Simmons, 1935; Pavillard and Wright, 1957**). The first to apply maggots to the wounds were Zacharias and Jones during the American Civil War. However, Food and Drug Administration (FDA) did not give the permission for using medical maggots until 2004 (**Sherman, 2005**).

MDT depends on two different techniques, mechanical and biochemical techniques. The former relies on the mandibles and rough body of the maggot which contain lipids consisting of free fatty acids (FFAs), hydrocarbons, esters, aldehydes, alcohols, ketones, and acylglycerols. These compounds were known to possess antimicrobial activity against various microorganisms (**Urbanek *et al.*, 2012**). Also, FFAs were reported in particular to resist against fungal infection (**Golébiowski *et al.*, 2008**). The latter depends on proteolytic enzymes and antimicrobial peptides (AMPs) that are present in larval excretions/secretions (ES) (**Chambers *et al.*, 2003**). Metalloproteinases, aspartyl proteases, and serine with two different subclasses (trypsin & chymotrypsin-like) are the three classes of proteolytic enzymes (**Telford *et al.*, 2011**).

Antimicrobial peptides (AMPs) serve as the primary defensive tool for various living organisms and are present in all kinds of life, from bacteria to humans. They are evolutionarily conserved components of the innate immune response (**Zasloff, 2002**). In insects, AMPs synthesis is carried out by the fat bodies and then discharged into the hemolymph (**Gunne & Steiner, 1993**). They were found to be effective against positive and negative Gram bacteria, as well as fungi (**Hoffmann, 1995; Hoffmann *et al.*, 1996**). According to their structures or distinctive sequences, insect AMPs can be divided into 4 families: α helical peptides (moricin & cecropin), cysteine-rich peptides (defensin & drosomycin), proline-rich peptides (lebocin, apidecin & drosocin), and glycine-rich peptides/proteins (gloverin and attacin) (**Yi *et al.*, 2014**). Sapecins were reported with six cysteines as the first antimicrobial defensins extracted from *Sarcophaga peregrine* (**Matsuyama and Natori, 1988 a, b**). In MDT, Calliphorid larvae were mentioned to have a critical importance for varoius chronic wounds healing such as abscesses, gangrenous wounds, diabetic foot ulcers, and pressure sores (**Wollina *et al.*, 2002**). Therefore, the present study was conducted to analyze the hydrocarbons composition of *Calliphora vicina* maggot's extract and evaluate the antimicrobial activities of these compounds against some pathogenic microbial strains.

Methods

Stock Colony

Stock colony of *C. vicina* (*C. vicina*) was established from adults initially collected from Cairo, Egypt during 2019 and maintained for one year under laboratory conditions, 27 ± 2 ˚C, and 55 – 70 % RH and LD 12:12 h photoperiod. They were maintained in rearing cages (35x35x35 cm), and provided with granulated sugar and water as a food source. Fresh beef meat was used as the food supply for the larval development and as an oviposition media for females. Newly hatched maggots were then transferred to new jars, in small numbers, to prevent intraspecific competition and heat that may influence growth rates (**Goodbrod & Goff, 1990**).

Species Identification

Adults were morphologically identified using the identification key of adult Calliphoridae, Lutz et al. (2018). For molecular identification,

Gene JET Genomic DNA purification Kit (Thermo Scientific #K0721) was used for DNA extraction from 3rd instar larvae according to the manufacturer protocol. Primer pairs (C1-J-2183a: 5′CAACATTTATTTTGATTTTTTGG3′ & TL2-N-3014: 5′TCCAATGCACTAATCTGCCATATTA3′) (**Simon *et al.*, 1994**) were used for mitochondrial COI amplification and sequencing. PCR analysis was performed using Dream Taq Green Master Mix (2X) (Thermo Scientific #K1081) according to the manufacturer's instructions. The product was visualized on 1.5% agarose gel electrophoresis photographed using Gel documentation (G: BOX) (SYNGENE model 680XHR) made in the UK. Finally, sequencing was monitored using ABI Prism Big Dye Terminator V3.1 Sequencing Kit and purified using Centri-Sep Kit according to manufacturer's protocol.

Maggot ES Extraction for Antimicrobial Bioassay

Maggot's extract was prepare according to **Arora *et al.*, (2010)** modified method with phosphate buffer saline solution (PBS) consisting of (0.24 g. KH2PO4, 1.44 g. Na2HPO4, 8 g. Na CL and 0.2 g. K CL all dissolved in 1000 ml. dist. H_2O) (**Sambrook *et al.*, 1989**). Briefly, 200 early third instar larvae were used, and they were given a thorough cleaning with ethanol, three rinses with sterile distilled water, and sterile filter paper drying. After that, each 100 larvae were suspended in 200 µl PBS in sterile tubes, incubated in dark at room temperature (25°C) for 1 hr. and then homogenized carefully. With using Eppendorf® Centrifuge 5810R, the homogenate was placed into 50 ml centrifuge tubes and spun at 4000 x g for 30 min. The supernatant was sterilized through 0.2 µm membrane (Xi'an Zenlab) syringe filter and stored at -20°C for antimicrobial screening.

Microbial Cultures

For evaluation of the antimicrobial activity, *C. vicina* maggots extract was tested against 2 Gram +ve bacterial strains [*Staphylococcus aureus* (*S. aureus*) & *Bacillus cereus* (*B. cereus*)], 3 Gram-ve bacteria [*Salmonella typhi* (*S. typhi*), *Escherichia coli* (*E. coli*), and *Pseudomonas aeruginos*a (P36 and P7)], as well as, one fungus, *Candida albicans*. These microorganisms were provided from the Botany and Microbiology Department, Faculty of Science, Zagazig University, Egypt.

Antimicrobial Bioassay

The susceptibility of fungi and bacteria to the ES extract was assessed using the modified Kirby-Bauer method (**Bauer *et al.*, 1966**). On Mueller-Hinton agar plates (BDH Laboratory Supplies, England), 50 µl of each microbial culture solution were applied. A blank paper disc (6.0 mm) was given 10 µl of the ES extract, and then the discs were allowed 3 hours at room temperature for complete dryness. After that, discs were put on agar, and incubation of the plates at 37°C was carried out for 24 hours. Then, to determine the antimicrobial activity, the growth inhibition zones were measured (mm).

Sodium dodecyl sulfate (SDS- PAGE) analysis

For the separation of the complex mixtures of maggots extract proteins, SDS- PAGE analysis was performed. The maggot extract (8 µl /20µ l) was mixed with SDS sample buffer, which was subsequently denatured for around 7 minutes in a boiling water bath. The BIO-RAD small protein tetra-vertical cell protein system was used for electrophoresis with a discontinuous buffer system. For this analysis, a molecular weight standard marker SC-2361 (SANTA CRUZ) was used (6-200 KDa). The gel was run at a constant current 40 A until the stained marker reached the bottom. Coomassie blue dye was then used to stain the gel. Finally, the gel was scanned and the picture of the gel was analyzed using Gel analyzer2010a.

Gas chromatography–mass spectrometry (GC-MS)

For GC/MS analysis, larvae of the tested fly were washed by alcohol and distilled water 3 times for complete sterilization. Larvae were ground in 20ml ethyl alcohol and filtrated by filter paper. The extract was moved to a falcon tube and covered with ethyl alcohol (1:1). This analysis was carried out on Trace GC1310-ISQ mass spectrometer (Thermo Scientific, Austin, TX, USA) using a direct capillary column TG-

5MS (30 m x 0.25 mm x 0.25 Mm film thickness) according to manufacturer protocol.

Statistical analysis

Statistically, data were analyzed and a one-way ANOVA test was used to compute the mean value and standard error (Mean ± SE) and compared using LSD test. The computer program SPSS (Statistical Package for Social Sciences) software was used for this purpose (version 14.0 for windows, SPSS Inc.).

Results

Species Identification

Morphological identification of *C. vicina* was confirmed by molecular analysis. After amplification, sequencing of approximately 800bp. of the COI gene was performed. Alignment by Basic Local Alignment Search Tool (BLAST) illustrated that the sequences of the tested species was 99% similar to previously published sequences of *Calliphora sp.* with accession number DQ295313 (**Juen & Traugott, 2005**).

Antimicrobial activity

Data in Table 1 represent the antimicrobial activity of *C. vicina* ES maggot extract on the tested microorganisms. It was clear that the extract is effective against all the strains, except the *B.* cereus. When zones of growth inhibition (mm) of the tested bacteria and fungus stains caused by ES extract were compared, results showed that, the fungus *C. albicans* produced the highest zone of growth inhibition (4.25± 0.25 mm). Of all tested bacterial strains, the *S. aureus* showed the highest zone of growth inhibition (2.75 ± 0.25mm) and the *P. aeruginosa* (P7) showed the lowest one (1.00 ± 0.40mm); while the *B. cereus* showed negative response. The difference of zones of inhibition in the other bacteria assays, *E. coli* (2.25 ± 0.25), *S. typhi* (2.25 ± 0.47) and *P. aeruginosa* (P36) (2.25 ± 0.47) were almost similar and no-significant differences (P > 0.05) in their inhibition zones.

Table 1: Antimicrobial activity of *Calliphora vicina* maggot excretions/secretions (ES) on different microorganisms

Microorganism	**Inhibition area (mm)** Mean ± SE
E. coli	2.25 ± 0.25 [a]
S. typhi	2.25 ± 0.47 [a]
S. aureus	2.75 ± 0.25 [a]
P. aeruginosa (P36)	2.25 ± 0.47 [a]
P. aeruginosa (P7)	1.00 ± 0.40 [b]
Candida albicans	4.25 ± 0.25 [c]
B. cereus	-ve

Similar letters mean non-significance (P > 0.05)
Three replicates were performed for each experiment.

Protein profile

SDS-PAGE of *C. vicina* maggot extract revealed the presence of proteins weighing from 7 to 133 K Da (Fig.1). From these proteins, the molecular weight of 103 KDa was the highest concentration (44.81%). Mean while, the lowest concentration (1.48 %) appeared at 9 KDa. The percentage of each molecular weight was shown in table 2.

Fig.1: SDS-PAGE for maggot's ES of *C. vicina* (M) represents the marker used in analysis.

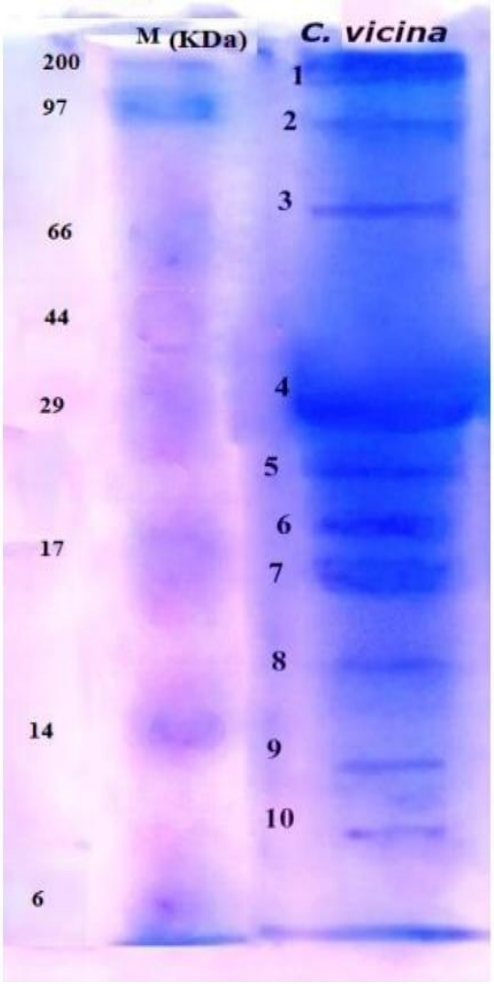

Table 2: The molecular weight (MW) in KDa and relative concentration of proteins (%) of *C. vicina* maggot extract.

No. of bands	MW	Percentage (%)
1	**133**	**2.60**
2	**103**	**44.81**
3	**80**	**8.13**
4	**34**	**2.47**
5	**28**	**3.14**
6	**22**	**3.41**
7	**18**	**16.77**
8	**13**	**7.52**
9	**9**	**1.48**
10	**7**	**9.67**

Hydrocarbon composition of *C. vicina* maggot extract

Gas chromatographic analysis of *C. vicina* maggot extract revealed the presence of fourteen hydrocarbon composition. As illustrated in table 3, *C. vicina* has a mixture of different chain lengths hydrocarbons ranging from C11 to C20. These hydrocarbons were classified in three categories namely, alkane [1component (0.82%)], saturated fatty acids [7 components (29.59%)] and unsaturated compounds [6 components (59.04%)]. From these compounds, Undecane, Methyl palmitate, Ethyl 9-hexa-decanoate and Ethyl hexa-decanoate were found to have antimicrobial activities against certain pathogens. Also, Methyl penta-decanoate, Ethyl tetra-decanoate, Methyl elaidioate and Methyl octa-decenoate were mentioned to poses anti-inflammatory effects.

Table 3: Relative chemical composition of *C. vicina* ES by GC- mass.

No.	Retention time (RT)	Compound name	Peak area percentage (PA%)	Molecular weight	Molecular formula
1	8.80	Undecane	0.82	156.0	$C_{11}H_{24}$
2	24.67	Methyl tetra-decanoate	1.67	242.0	$C_{15}H_{30}O_2$
3	26.04	Methyl penta-decanoate	0.80	256.0	$C_{16}H_{32}O_2$
4	26.17	Ethyl tetra-decanoate	1.82	256.0	$C_{16}H_{32}O_2$
5	28.33	Methyl palmitoleate	1.49	268.0	$C_{17}H_{32}O_2$
6	28.43	Methyl(9Z)-9-hexa-decenoate	10.61	268.0	$C_{17}H_{32}O_2$
7	28.87	Methyl palmitate	13.72	270.0	$C_{17}H_{34}O_2$
8	29.68	Ethyl 9-hexa-decanoate	0.95	282.0	$C_{18}H_{34}O_2$
9	30.22	Ethyl hexa-decanoate	8.41	284.0	$C_{18}H_{36}O_2$
10	32.08	Methyl-(9Z,12Z)-9,12-octa-decanoate	0.87	294.0	$C_{19}H_{34}O_2$
11	32.20	Methyl elaidioate	28.58	296.0	$C_{19}H_{36}O_2$
12	32.70	Methyl octa-decenoate	2.12	298.0	$C_{19}H_{38}O_2$
13	33.43	Ethyl Oleate	16.54	310.0	$C_{20}H_{38}O_2$
14	33.92	Ethyl octa-decenoate	1.05	312.0	$C_{20}H_{40}O_2$

Discussion

Since, accurate taxonomic identification of fly species is essential before establishing a colony in the laboratory; adults collected for this study were subjected to DNA identification after morphological identification using key of adult Calliphoridae, **Lutz *et al.* (2018)**. Particularly in forensic medicine and criminal investigation, DNA is the most potent technique for potential insect identification (**Benecke, 1998)**. Mitochondrial COI gene is the most ubiquitous barcode for all animals (**Hebert *et al.*, 2003**) as it is well recognized among numerous species and its information is consistent throughout life stages (**Wells & Williams, 2007; Nelson *et al.*, 2007**). Among the mitochondrial protein-coding genes, COI region has been chosen as a primary genetic diagnostic technique for animal species identification (**Hebert *et al.*, 2003; Luo *et al.*, 2011; Samarakoon, 2012**). During this study, it was observed that a region of 800 bp of the COI gene was sufficient to confirm *C. vicina* identification. This observation was in agreement with **Limsopatham *et al.*, (2018)** who used a sequence of 784 bp to differentiate between *C. vicina* species in Germany and England. On the other hand, **Ames *et al.*, (2006)** illustrated that approximately a region of 400 bp of COI gene was sufficient to differentiate *C. vicina* from *C. vomitoria* in the US and **Schroeder *et al.*, (2003)** used about 1300 bp. of COI & COII regions to differentiate among *C. vomitoria, C. vicina and L. sericata* in Germany.

Different species of the family Calliphoridae have variable activities against microorganisms. One of these species is *Calliphora vicina* which was observed during this study to have a great activity against *S. typhi, S. aureus, E. coli*, *P. aeruginosa* (P36), *P. aeruginosa* (P7), and *Candida albicans* but not against *B. cereus*. These findings corroborated those of **Dallavecchia *et al.* (2021)**, who reported on the effectiveness of *C. vicina* against *E. coli, Candida albicans* and *Klebsiella. pneumoniae*. Also, **Gordya *et al.*, (2017)** mentioned that *C. vicina* AMPs complex showed a strong killing effect against *S. aureus, E. coli* and *Acinetobacter baumannii* biofilms with No harmful effect on human immunocytes. **Chernysh *et al.*, (2015)** illustrated that against *E. coli, C. vicina* maggot's ES has a high level of inhibition.

Ratcliffe *et al.*, (2015) indicated that flies of the family Calliphoridae have one or more antimicrobial factors assisting in suppression of Gram+ve as well as Gram-ve bacteria in vitro and stated that *Ch. putoria* and *Ch. megacephala* have highly significant antibacterial effects against *S. aureus* and *E. coli*. **Jaklič *et al.*, (2008)** found that *L. sericata* ES stopped growth of *S. aureus*, while it was less effective against *P. aeruginosa* and *E. coli* cultures**.** Also, **Barnes *et al.*, (2010)** mentioned

that *L. sericata* ES can inhibit *E. coli*, *B. cereus*, *P. aeruginosa* and *Proteus miabilis* bacterial growth. **Hassan *et al.*, (2016)** illustrated that ES inhibits the growth of Gram –ve (*E. coli* and *P. aeruginosa*) as well as Gram +ve bacteria (*B. subtilis* and *S. aureus*). **Valachova *et al.*, (2014)** stated that *S. aureus* and *P. aeruginosa* biofilms responded differently to *L. sericata* ES. **Kaihanfar *et al.*, (2018)** mentioned that **t**he growth of both *E. coli* and *B. subtilis* secretions could be inhibited by *L. sericata* larvae and **El-morsy *et al.*, (2020)** proved potent antibacterial activity of *Ch. albiceps* larval ES against both Gram-ve (*P. aeruginosa* and *E. coli*) and Gram+ve (*B. subtilis* and *S. aureus*) bacteria.

More than 1500 proteins, mostly found in insects, have been found to have antimicrobial properties against different taxa, including plants, animals, fungi, and bacteria **(Yi *et al.*, 2014)**. Insects typically produce peptides and polypeptides with a wide range of antibacterial and antifungal activities in response to bacterial and fungal injuries (**Hoffmann 1995; Hoffmann *et al.*, 1996**).

Series of antimicrobial peptides as cecropins, dipterins were found to be synthesized and accumulated in *C. vicina* maggot's hemolymph (**Chernysh *et al.*, 1995**). In the current study, the protein of molecular weight 13 KDa and percentage (7.52%) belongs to antibacterial protein family Gloverins, that is ~ 14 KDa with effect against *E. coli* mutant strains as mentioned by **Axen *et al.*, (1997)** and **Kawaoka *et al.*, (2008)**. The band with a molecular weight 22 KDa and a percentage 3.41% belongs to Attacins family MWt (20-23 KDa) as observed by **Hultmark *et al.*, (1983)**. **Chernysh & Gordija , (2011)** illustrated that diptericins family in *C. vicina* was represented by 4 isomers with molecular weights (9029.1, 8999.7, 8913.9, and 8886.2 Da) and this somehow resemble a band with percentage (1.48%) and molecular weight 9 KDa in this study. Several antibacterial proteins were isolated from different insects with the same molecular weight. **Lung *et al.*, (2001)** detected an antibacterial protein from the male *Drosophila melanogaster* accessory gland having the same molecular weight (28KDa) of the protein in this study. The effectiveness of protein molecules extracted from *C. vicina* maggot's ES during this study was supported by the findings of **Van der plas *et al.*, (2008)** who mentioned that the molecules with beneficial effects may be present in maggots' excretions/ secretions. Also, **Kerridge *et al.*, (2005)** mentioned that exosecretion fractions of insects from 5 to 10 KDa and lower than 1KDa were found to have anti-*staphylococcal* activity.

The most widely used techniques for the separation, quantification, and identification of insect cuticular lipids are gas chromatography and gas chromatography-mass spectrometry **(Nelson *et al.*, 1999)**. Insect cuticular lipids consist of wax esters, hydrocarbons, free fatty acids (FFAs), aldehydes, ketones, alcohols, and acyl glycerols. Analysis of *C. vicina* cuticular lipids illustrated that the majority of the resulted acid chains contain from 16 to 20 carbon atoms with a high percentage of C16 and C18 acid. This finding was in agreement with **Golébiowski *et al.*, (2008)** who studied *C. vicina* cuticular lipids and found that the extracted acids in the alkyl chain containing 5-20 carbon atoms with a high presence of acids containing 16-18 carbon atoms**.** Even-numbered, Saturated and unsaturated FFA ranging from 12 to 20 are identified in the majority of insect species (**Golébiowski *et al.*, 2011, 2012**). Ranges of FFAs were detected in insect cuticular lipids: from 10 to 22 in adults of *Locusta migratoria migratorides* and from 10 to 21in adults of *Schistocerca gregaria* (**Boguś *et al.*, 2010**), from 16 to 36 in *Apis mellifera* (**Blomquist *et al.*, 1980**) and from 14 to 18 in the larvae of *Frankliniella occidentalis* (**Golébiowski *et al.*, 2007**).

During this study the hydrocarbon profile of *C. vicina* illustrated 13 compounds that represent 6 fatty acids, the major acids were palmitic (C16), hexadecanoic (C16) and oleic acid (C18), whereas minor acids were myristic (C14), pentadecanoic (15) and stearic acid (C18). The same observation was illustrated by **Lunas *et al.*, (2019)** who found that the hydrocarbon profile of *Ch. albiceps*, *L. cuprina* and *L. eximia* contain 11 compounds and only 9 compounds for *Ch. megacephala*. These

compounds represent 6 FFAs with 2 major [palmitic and oleic acids] and 4 minor acids [lauric (C12), myristic (C14), palmitoleic (C16) and stearic acids (C18)]. Also**, Golébiowski, (2012)** illustrated that high percentage of C16 and C18 acids were presented in *C. vicina* larval and pupal specimens. The lipid profile of *C. vicina* larvae contains saturated as well as unsaturated FFA with even and odd numbers ranging from C16 to C36, while *C. vomitoria* contain even numbers of carbons ranging from C12, C18 (**Golébiowski *et al*., 2013)**.

Cuticular lipids are considered the initial barrier to bacterial and fungal infections (**Leger, 1991**) and reduction of toxin and chemical penetrations (**Gilby, 1984**). The components of the cuticular lipids especially FFA may play an essential role in the susceptibility and resistance of many insect species to fungal infection (**Golébiowski *et al*., 2008**)**.** Comparing FFA with the same carbon atoms, unsaturated FFA was more active than saturated ones (**Zheng *et al*., 2005; Desbois *et al*., 2008**), and also more effective against Gram + ve bacteria (**Galbraith *et al*., 1971**)**. Kabara *et al*., (1972)** and **Feld Laufer *et al*., (1993)** discussed the effect of orientation of double bonds on FFA activity and found that the Cis orientation double bond possesses a higher antibacterial effect than trans orientation. **Gillespie *et al*., (2000)** mentioned that fatty acids of insect cuticle had fungistatic, toxic, or in some species, stimulatory effects on fungal species. **Dilika *et al*., (2000)** stated that Linoleic and oleic acids inhibit both medically-important fungi and Gram+ve bacteria, but Gram–ve bacteria are resistant to these compounds. Larvae of *C. vicina* were mentioned to have high resistance to soil fungi as, *Conidiobolus coronatus* (**Golébiowski *et al*., 2008**).

Conclusion:

Our findings confirmed molecular and morphological identification of *C. vicinia* and suggest that excretion/ secretions from their maggots show a promising medical property for the inhibition of microbial growth.

References

1. Ames, C.; Turner, B. and Daniel, B. (2006). The use of mitochondrial cytochrome oxidase I gene (COI) to differentiate two UK blowfly species Calliphora vicina and Calliphora vomitoria. Forensic Sci. Int., 164: 179-182.
   DOI: 10.1016/j.forsciint.2006.01.005
2. Arora S.; Baptista, C. and Sing, L.C. (2010). Antibacterial activity of Lucilia cuprina maggot extracts and its extraction techniques. Int. J. Integr. Biol., 9: 43 - 48.
3. Axen, A.; Carlsson, A.; Engstrom, A. and Bennich, H. (1997). Gloverin, an antibacterial protein from the immune hemolymph of Hyalophora pupae. Eur. J. Biochem., 247: 614 – 619.
4. Barnes, K.M.; Dixon, R.A. and Gennard, D.E. (2010). The antibacterial potency of the medicinal maggot, Lucilia sericata (Meigen): Variation in laboratory evaluation. J. Microbiol. Methods, 82:234–237.
5. Benecke, M. (1998).Random amplified polymorphic DNA (RAPD) typing of necrophagous insects (Diptera, Coleoptera) in criminal forensic studies: validation and use in practice. Forensic Sci. Int., 98:157–168.
6. Blomquist, G.J.; Chu, A.J. and Remaley, S.(1980). Biosynthesis of wax in the honeybee, Apis mellifera L. Insect Biochem., 10: 313-321.
7. Boguś, M.I.; Czygier, M.; Golébiowski M. et al. (2010). Effects of insect cuticular fatty acids on in vitro growth and pathogenicity of the entomopathogenic fungus Conidiobolus coronatus. Exp. Parasitol., 125:400–408.
8. Chambers, L.; Woodrow, S.; Brown, A.P. et al. (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.
9. Chernysh, S.; Gordya, N. and Suborova, T. (2015). Insect antimicrobial peptide complexes prevent resistance development in bacteria. PLoS ONE., 10(7): e0130788.

https://doi.org/10.1371/journal.pone.0130788.
10. Chernysh, S.I. and Gordja, N. A. (2011). The Immune System of the Blow Fly (Calliphora vicina) as a Source of Medical Drugs. J. Evo.l Biochem. Physiol. 47: 524-533.
11. Chernysh, S.I.; Simonenko, N.P.; Braun, A. and Meister, M. (1995). Developmental Variability of the Antibacterial Response in Larvae and Pupae of Calliphora vicina (Diptera, Calliphoridae) and Drosophila melanogaster (Diptera, Drosophilidae). Eur. J. Entomol., 93: 203–209.
12. Dallavecchia, D.L.; Ricardo, E.; daSilva, A.S. and Rodrigues, A.G. (2021). Antibacterial and antifungal activity of excretions and secretions of Calliphora vicina. Med. Vet. Entomol., 35:225-229. https://doi.org/10.1111/mve.12486
13. Desbois, A.P.; Lebl, T.; Yan, L. and Smith, V.J. (2008). Isolation and structural characterisation of two antibacterial free fatty acids from the marine diatom, Phaeodactylum tricornutum. Appl. Microbiol. Biotechnol., 81:755–764.
14. Dilika, F.; Bremner, P.D. and Meyer, J.J. (2000). Antibacterial activity of linoleic and oleic acids isolated from Helichrysum pedunculatum: a plant used during circumcision rites. Fitoterapia, 71: 450-2. DOI: 10.1016/s0367-326x(00)00150-7
15. El-morsy, R.H.; Bream, A.S. and Abdel-Samad, M.R.K. (2020). Antibacterial Activities of Chrysomya albiceps Maggots' Extracts (Diptera: Calliphoridae). Egypt. Acad. J. Biol. Sci. (A. Entomology), 13: 99 - 104.
16. El-Tantawy, N.L. (2015). Helminths and insects: maladies or therapies. Parasitol. Res., 114: 359 – 377. DOI:10.1007/s00436-014-4260-7
17. Feld laufer, M.F.; Knox, D.A.; Lusby, W.R. and Shimanuki, H. (1993). Antimicrobial activity of fatty acids against Bacillus larvae, the causative agent of American foulbrood disease. Apidologie, 24:95 – 99.
18. Fleischmann, W.; Grassberger, M. and Sherman, R.A. (2004). Maggot Therapy: A Handbook of Maggot-Assisted Wound Healing. New York, NY: Thieme. j I S B N, 3: 13 - 136.
19. Galbraith, H.; Miller, T.B.; Paton, A.M. and Thompson, J.K. (1971). Antibacterial activity of long chain fatty acids and the reversal with calcium, magnesium, ergocalciferol and cholesterol. J. Appl. Bacteriol., 34: 803–813.
20. Gilby, A.R. (1984). Cuticle and insecticides. In: Bereiter-Hahn J., Matoltsy A.G., Richards K.S., editors. Biology of the integument. Invertebrates, vol 1. Springer-Verlag Berlin, 694–702.
21. Gillespie, J.P.; Bailey, A.M.; Cobb, B. and Vilcinskas, A. (2000). Fungi as elicitors of insect immune responses. Arch. Insect Biochem. Physiol., 44: 49–68.
22. Golébiowski, M. (2012). Comparison of Free Fatty Acids Composition of Cuticular Lipids of Calliphora vicina Larvae and Pupae. Lipids, 47:1001–1009.
23. Golébiowski, M.; Boguś, M.I.; Paszkiewicz, M. and Stepnowski, P. (2011). Cuticular lipids of insects as a potential biofungicides: methods of lipids composition analysis. Anal. Bio. Anal. Chem., 399: 3177–3191.
24. Golébiowski, M.; Cerkowniak, M.; Boguś, M.I. et al. (2013). Free fatty acids in the cuticular and internal lipids of Calliphora vomitoria and their antimicrobial activity. J. Insect Physiol., 59: 416 – 429.
25. Golébiowski, M.; Dawgul, M.; Kamysz, W.; Boguś, M.I. et al. (2012). The antimicrobial activity of the alcohols from Musca domestica. J. Exp. Biol., 215:3419-3428.
26. Golébiowski, M.; Maliński, E.; Boguś, M.I.; Kumirska, J. and Stepnowski, P. (2008). The cuticular fatty acids of Calliphora vicina, Dendrolimus pini and Galleria mellonella larvae and their role in resistance to fungal infection. Insect Biochem. Mol. Biol., 38: 619– 627.
27. Gołębiowski, M.; Maliński, E.; Nawrot, J.; Szafranek, J. and Stepnowski, P. (2007). Identification of the cuticular lipid composition of the Western Flower Thrips Frankliniella occidentalis. Comp. Biochem. Physiol. B, Biochem. Mol. Biol., 147: 288–289.

28. Goodbrod, J.R. and Goff, M. L. (1990). Effects of larval population density on rates or development and interactions between two species of Chrysomya (Diptera: Calliphoridae) in laboratory culture. J. Med. Entomol., 27:338-343.
29. Gordya, N.; Yakovlev, A.; Kruglikova, A.; Tulin, D. et al. (2017). Natural antimicrobial peptide complexes in the fighting of antibiotic resistant biofilms: Calliphora vicina medicinal maggots. PLoS ONE, 12(3):e0173559. DOI: 10.1371/journal.pone.0173559
30. Gunne, H. and Steiner, H. (1993). Efficient secretion of attacin from insect fat body cells requires proper processing of the pro sequence. Eur. J. Biochem., 214:287–293.
31. Hassan, M.I.; Amer, M.S.; Hammad, K.M. and Zidan, M.M. (2016). Antimicrobial activity for excretion and secretion of the greenbottle fly larvae Lucilia sericata (meigen) (Diptera: Calliphoridae). J. Egypt. Soc. Parasitol., 46: 179-184.
32. Hebert, P.D.N.; Cywinska, A.; Ball, S.L. and deWaard, J.R. (2003). Biological identifications through DNA barcodes. — Proc. R. Soc. Lond., 270:313-321. doi: 10.1098/rspb.2002.2218
33. Hoffmann, J.A. (1995). Innate immunity of insects. Curr. Opin. Immunol., 7: 4-10.
34. Hoffmann, J.A.; Reichhart, J.M. and Hertu, C. (1996). Innate immunity in higher insects. — Curr. Opin. Immunol., 8: 8-13.
35. Hultmark, D.; Engstrom, A.; Anderson, K. et al. (1983). Insect immunity. Attacins, a family of antibacterial proteins from Hyalophora cecropia. EMBO J., 2:571–576.
36. Jaklič, D.; Lapanje, A.; Zupančič, K.; Smrke, D. and Cimerman, N.G. (2008). Selective antimicrobial activity of maggots against pathogenic bacteria. J. Med. Microbiol., 57: 617 - 625.
37. Juen, A. and Traugott, M. (2005). Amplification facilitators and multiplex PCR: Tools to overcome PCR-inhibition in DNA-gut-content analysis of soil-living invertebrates. Soil Biol. Biochem., 38:1872–79.
38. Kabara, J.J.; Swieczkowsk,i D.M.; Conley, A.J. and Truant, J.P. (1997). Fatty acids and derivatives as antimicrobial agents. — Antimicrob. Agents Chemother., 2:23–28.
39. Kaihanfar, M.; Moghaddam, M.M.; Moghaddam, M.J.M. et al. (2018). Investigation of antimicrobial effects of treated Lucilia sericata larvae extract on bacteria. Iran. J. Microbiol., 10: 409-16.
40. Kawaoka, S.; Katsuma, S.; Daimon, T.; Isono, R. et al. (2008). Functional analysis of four Gloverin-like genes in the silkworm, Bombyx mori. Arch. Insect Biochem. Physiol., 67:87–96.
41. Kerridge, A.; Lappin-Scott, H. and Stevens, J.R. (2005). Antibacterial properties of larval secretions of the Blow fly, Lucilia sericata. Med. Vet. Entomol., 19:333-37.
42. Leger, R.J. (1991). Integument as a Barrier to Microbial Infections. In: Binnington K. and Retnakaran A., editors. Physiology of the Insect Epidermis. CSIRO, Melbourne, 286-308.
43. Limsopatham, K.; Hall, M.J.R.; Zehner, R.; Zajac, B.K. et al. (2018). A molecular, morphological, and physiological comparison of English and German populations of Calliphora vicina (Diptera: Calliphoridae). — PLoS ONE, 13(12): e0207188. https://doi.org/10.1371/journal.pone.0207188
44. Lunas, B.M.; de Paula, M.C.; Michelutti, K.B. et al. (2019). Hydrocarbon and Fatty Acid Composition from Blowfly Eggs Represents a Potential Complementary Taxonomic Tool of Forensic Importance. J. Forensic Sci., 64:1720-1725.
45. Lung, O.; Kuo, L. and Wolfner, M.F. (2001). Drosophila males transfer antibacterial proteins from their accessory gland and ejaculatory duct to their mates. J. Insect physiol., 47:617-622.
46. Lutz, L.; Williams. K.A.; Villet, M.H.; Ekanem, M. and Szpila, K. (2018). Species identification of adult African blowflies (Diptera: Calliphoridae) of forensic importance. Int. J. Legal Med., 132:831-842.

47. Matsuyama, K. and Natori, S. (1988a). Molecular cloning of cDNA for sapecin and unique expression of the sapecin gene during the development of Sarcophaga peregrina. J. Biol. Chem., 263:17117–17121.
48. Matsuyama, K. and Natori, S. (1988b). Purification of three antibacterial proteins from the culture medium of NIH-Sape-4, an embryonic cell line of Sarcophaga peregrina. J. Biol. Chem., 263:17112–17116.
49. Nelson, D.R.; Fatland, C.L.; Buckner, J.S. and Freeman, T.P. (1999). External lipids of adults of the giant whitefly, Aleurodicus dugesii. Comp. Biochem. Physiol., B 123: 137–145.
50. Nelson, L. A.; Wallman, J. F. and Dowton, M. (2007). Using COI barcodes to identify forensically and medically important blowflies. Med. Vet. Entomol., 21: 44–52.
51. Pavillard, E.R. and Wright, E.A. (1957). An antibiotic from maggots. — Nature, 180: 916-917.
52. Ratcliffe, N.A.; Vieira, C.S.; Mendonc, P.M. et al. (2015). Detection and preliminary physico-chemical properties of antimicrobial components in the native excretions/ secretions of three species of Chrysomya (Diptera, Calliphoridae) in Brazil. Acta Trop., 147: 6–11.
53. Rayner, K. (1999). Larval therapy in wound debridement. Professional nurse, 14 : 329-333.
54. Richardson, M. (2004). The benefits of larval therapy in wound care. Nurs stand, 19: 70
55. Samarakoon, U.; Skoda, S.; Baxendale, F.P. and Foster, J.E. (2013).A molecular key for the identification of blowflies in southeastern Nebraska. J. Forensic Sci., 58:173-178.https://doi.org/10.1111/j.1556-4029.2012.02182.x
56. Sambrook, J.; Fritsch, E.F. and Maniatis, T. (1989). Molecular Cloning: A Laboratory Manual, 2nd ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York, 3:12.
57. Schroeder, H.; Klotzbach, H.; Elias, S.; Augustin, C. and Pueschel, K. (2003). Use of PCR-RGLP for differentiation of calliphorid larvae (Diptera, Calliphoridae) on human corpses. Forensic Sci. Int., 132:76-81.
58. Sherman, R. A. (2005). Age-old therapy gets new approval. Advances in skin & wound care, 18: 12–15.
59. Sherman, R.A. (2014). Mechanisms of maggot-induced wound healing: what do we know, and where do we go from here? Evidence-Based Complementary and Alternative Medicine, Article ID 592419, 13 pages. https://doi.org/10.1155/2014/592419.
60. Simmons, S.W. (1935). A bactericidal principle in excretions of surgical maggots which destroys important etiological agents of pyogenic infections. J. Bacteriol., 30: 253-267.
61. Simon, C.; Frati, F.; Beckenbach, A.; Crespi, B.; Liu, H. and Flook, P. (1994). Evolution, weighting, and phylogenetic utility of mitochondrial gene sequences and a compilation of conserved polymerase chain reaction primers. Ann. Entomol. Soc. Am., 87: 651–701.
62. Telford, G.; Brown, A.P.; Kind, A. English J.S.C., Pritchard D.I. (2011). Maggot chymotrypsin I from Lucilia sericata is resistant to endogenous wound protease inhibitors. British J. Dermatol., 164:192–6.
63. Thomas, S. (2010). Maggot therapy. In: Thomas S (ed). Surgical Dressings and Wound Management. Medetec Publications, P.563-632.
64. Urbanek, A.; Szadziewski, R.; Stepnowski, P.; Gabriel, I. et al. (2012). Composition and antimicrobial activity of fatty acids detected in the hygroscopic secretion collected from the secretory setae of larvae of the biting midge Forcipomyia nigra (Diptera: Ceratopogonidae). J. Insect Physiol., 58: 1265–1276.
65. Valachova, I.; Bohová, J.; Kozanek, M.; Takac, P. and 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, 1745-1753.

66. Van der plas, M.J.; Jukema, G.N.; Wai, S.W.; Dogterom-Ballering, H.C. et al. (2008). Maggot excretions/secretions are differentially effective against biofilms of Staphylococcus aureus and Pseudomonas aeruginosa. J. Antimicrob. Chemother., 61: 117-122.
67. Wainwright, M. (1990). Miracle Cure: The Story of Penicillin and the Golden Age of Antibiotics. Oxford, England: Basil Blackwell
68. Wells, J.D. and Williams, D.W. (2007). Validation of a DNA-based method for identifying Chrysomyinae (Diptera: Calliphoridae) used in a death investigation. Int. J. Legal Med., 121:1–8.
69. Wollina, U.; Liebold, K.; Schmidt, W.D.; Hartmann, M. and Fassler, D. (2002). Biosurgery supports granulation and debridement in chronic wounds—clinical data and remittance spectroscopy measurement. Int. J. Dermatol., 41: 635–639.
70. Yi, H.Y.; Chowdhury, M.; Huang, Y.D.; Yu, X.Q. (2014). Insect antimicrobial peptides and their applications. Appl. Microbiol. Biotechnol., 98: 5807–5822. DOI: 10.1007/s00253-014-5792-6
71. Zasloff, M. (2002). Antimicrobial peptides of multicellular organisms. Nat., 415:389–395.
72. Zheng, C.J.; Yoo, J.S.; Lee, T.G.; Cho, H.Y.; Kim, Y.H. and Kim, W.G. (2005). Fatty acid synthesis is a target for antibacterial activity of unsaturated fatty acids. FEBS Lett., 579:5157–5162.

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DA, EZ conducted experiments—investigations and statistical analyses. ER conducted statistical analyses, contributed material and writing—original draft. SR, AA helped in conceptualization, methodology, contributed material, secured study supervision, writing—original draft, writing—review and editing. All authors read and approved the final manuscript.

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