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Antimicrobial, antioxidant, wound healing, and cytotoxic effects of maggot extract from molecularly identified *Chrysomya albiceps* (Diptera: Calliphoridae)

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ABSTRACT

The medical industry is currently experiencing a deficit of effective therapies to prevent infections, as the evolution of multidrug resistance has outpaced the research and development of new effective medications. Blowfly maggots have attracted high interest for their medicinal and therapeutic potential. This study was conducted to assess the antibacterial efficacy of *Chrysomya albiceps* larval extract against four multidrug-resistant pathogenic bacteria, including Gram-positive (*Listeria monocytogenes* ATCC 19155 and *Staphylococcus aureus* ATCC 43300) and Gram-negative bacteria (*Escherichia coli* ATCC 8739 and *Salmonella* sp. ATCC 14028). The extract strongly inhibited all tested bacterial species with inhibition zones ranging from 18 to 25 mm, showing the highest effect on *S. aureus*. Additionally, the extract exhibited potent antioxidant activity with an IC_{50} of 44.89 μg/mL and was non-toxic to human fibroblast (HFB4) cells up to 250 μg/mL. In vitro wound healing assays demonstrated that the extract significantly accelerated wound closure within 48 h. GC-MS analysis identified 17 bioactive compounds, most with known antimicrobial properties. Molecular docking studies confirmed stable interactions between these compounds and key bacterial protein targets, supporting the observed bioactivity. As part of the genetic diversity, larval samples were identified by nucleotide sequencing of the mitochondrial cytochrome *c* oxidase subunit I (COXI gene). These results were then compared with similar sequences that had already been put in GenBank. Moreover, a molecular docking study was performed to confirm and rationalize the observed biological activity. Overall, these results suggest that *C. albiceps* larval extract possesses antibacterial, antioxidant, and wound-healing activities and represents a safe, natural candidate for developing novel pharmaceutical and therapeutic agents.

1. Introduction

New strains of drug-resistant pathogens are emerging and spreading faster than the discovery of antimicrobials [1]. The antimicrobial-resistant pathogens have resulted in a concerning mortality rate exceeding 50 % in several places [2,3]. The global burden of chronic wounds and oxidative stress has intensified the search for novel bioactive products from natural sources such as plants, microorganisms, and insects [4,5]. In this context, insects, particularly carrion-feeding flies of Calliphoridae, have attracted considerable interest for their medicinal and therapeutic potential. The antibacterial effects against various Gram-positive and Gram-negative bacteria were examined by the excretions and secretions of maggots of many blue flies, such as *Lucilia sericata* [6], *Lucilia cuprina* [7], *Calliphora vicina* [8], and *Chrysomya* species [9].

Chrysomya albiceps, a blowfly species widely distributed in Egypt [10] and across many other countries, has shown promise as a source of

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bioactive compounds with multiple pharmacological activities. It is widely distributed across tropical and subtropical regions, including North Africa and the Middle East, with Egypt being one of its most common habitats. Its ecological abundance and ease of collection in rural and peri-urban environments make it a highly accessible biological resource. Unlike exotic species that require laboratory rearing or importation, *C. albiceps* is readily available year-round in the local environment, offering a cost-effective and sustainable option for bioactive compound extraction. These characteristics position it as a valuable native candidate for the development of affordable therapeutic products, particularly in low- and middle-income settings where access to conventional pharmaceuticals may be limited.

The larvae of *C. albiceps* secrete a rich mixture of biologically active molecules during feeding, which includes antimicrobial peptides (AMPs), proteolytic enzymes, and immune-modulating compounds. These secretions have demonstrated potent antibacterial activity against a broad range of Gram-positive and Gram-negative bacteria, including drug-resistant strains [11–13].

In addition to their antimicrobial effects, maggot extracts have been found to exhibit significant antioxidant activity, attributed to the presence of free radical scavenging enzymes and small-molecule antioxidants. These compounds can mitigate oxidative stress, a major factor in impaired wound healing and chronic inflammation [14]. Extracts from larval secretions have been reported to enhance fibroblast proliferation, angiogenesis, and extracellular matrix remodeling—key processes in tissue repair and regeneration [15].

Furthermore, the cytotoxic potential of insect-derived extracts, including those from *C. albiceps*, is under investigation for possible applications in anticancer therapy. Certain fractions have demonstrated selective toxicity against tumor cell lines while sparing normal cells, indicating a possible therapeutic window for drug development [16].

The numerous advantages of maggots have significantly heightened interest in these little larvae [17]. It possesses a broad range of antibacterial, antioxidant, wound healing, and cytotoxicity properties. The maggot extract of *C. albiceps* represents a valuable candidate for pharmacological exploration. Although maggot-based therapy has gained increasing attention, there aren't many detailed studies that look at all four important biological activities in *C. albiceps* at the same time. Most existing reports focus on individual biological effects or other blowfly species. So, this study aims to address this lack of research by looking at the combined effects of the antimicrobial, antioxidant, wound-healing, and cytotoxic properties of *C. albiceps* larval extract.

While maggot-based therapies have garnered increasing scientific interest, the majority of published studies have focused on isolated biological effects or other blowfly species, such as *L. sericata*, which is the most commonly studied. In contrast, *C. albiceps*, despite its wide geographic distribution and evident pharmacological potential, remains significantly underexplored. To date, there is a notable lack of comprehensive research evaluating the full spectrum of its biological activities—namely antimicrobial, antioxidant, wound-healing, and cytotoxic effects—in an integrated framework. Given the urgent global need for multi-functional bioactive agents, particularly from sustainable and readily available sources, a holistic investigation of *C. albiceps* larval extract is both timely and scientifically justified [3,15].

As previously mentioned, carrion flies serve as an excellent source of evidence for forensic entomologists, are relevant to issues in public health and medicine, and are useful in the production of many therapeutic medicinal compounds. However, the need to correctly identify these flies 'limits access to useful information about them [18]. The majority of insect species important for forensics in all geographic areas still lack a taxonomy key that includes all larval stages. Forensic entomologists find identifying larval species challenging due to the many morphological similarities that make it difficult to differentiate between closely related species. Currently, mitochondrial DNA is chosen for use in forensics because it is more common in tissues than nuclear DNA and is therefore simpler to extract from even tiny amounts of sample [19,20]. The CO1 gene in mitochondrial DNA is thought to be the best one to use as a molecular marker [21,22]. Using the nucleotide sequences of cytochrome oxidase I (COXI) to make a phylogenetic tree gives a useful picture of where insects came from biologically and how their genes have moved around.

This study aims to answer whether *C. albiceps* larval extract exhibits antimicrobial, antioxidant, wound-healing, and non-toxic cytotoxic activities. We hypothesize that the extract contains bioactive compounds with effective therapeutic properties and minimal toxicity. The objectives are to evaluate its antibacterial effects against multidrug-resistant bacteria, assess antioxidant capacity, test cytotoxicity on human fibroblasts, examine wound healing promotion in vitro, and identify active compounds via chemical and molecular analyses. The flowchart of the article summarizes the steps for our study as shown in Fig. 1.

2. Materials and methods

2.1. Stock colony

The study was conducted in the summer season (June–July 2024) on the campus of the Faculty of Science, Benha University, Qalyubiya, Egypt (30°27′34"N 31°11′8"E). Maggots of *C. albiceps* were obtained from a lab colony collected from a fresh rabbit carcass in Benha city and were identified by morphological keys as well as molecular identification and phylogenetic analysis. A laboratory colony of *Chrysomya albiceps* was established and maintained for several successive generations under controlled conditions. Larvae were initially collected from a freshly decomposed rabbit carcass and reared to adulthood. The emerged adults were housed in well-ventilated rearing cages measuring 38 × 38 × 56 cm, maintained at 31.2 ± 1.5 °C, 47.6 ± 5 % relative humidity, and a 14-h light:10-h dark photoperiod. Each cage had a wooden base and wire mesh on three sides, while the fourth side was made of wood with a circular access port fitted with a muslin sleeve to facilitate feeding, cleaning, and egg collection. Adults were provided daily with a diet consisting of granulated sucrose, clean water, and minced beef. Water was supplied using a cotton wick placed in a bottle, while 50 g of ground beef were offered in a 400 ml plastic container. Egg batches were collected daily and transferred to rearing bowls (35 cm in diameter) containing fresh beef as a larval medium. The bowls were covered with muslin cloth secured by rubber bands to prevent contamination while ensuring adequate aeration. At the pre-pupal stage, autoclaved dry sawdust was added to serve as a pupation substrate. The resulting pupae were carefully sieved from the sawdust and transferred into clean adult emergence cages of identical design to maintain colony continuity.

2.2. Chrysomya albiceps identification

2.2.1. Sample preparation

The sample was stored in a single sterile microcentrifuge tube and kept at −70 °C until used. The larvae that were collected were washed twice with clean water to get rid of any extra dirt, rinsed once with 70 % ethanol, cut up, and ground with phosphate buffered saline (PBS) using a clean mortar and pestle. At 4 °C, each tissue homogenate was centrifuged for 10 min at 3000 rpm (1008×*g*), and the clear supernatant was frozen at −70 °C for molecular identification.

2.2.2. Molecular identification

DNA extraction, gene amplification, and sequencing of the mtCOXI gene. First, the GeneJET Genomic DNA Purification Kit (Thermo Scientific, Cat. No. K0721) was used to extract DNA according to the manufacturer's instructions. The NanoDrop Qubit 3.0 Fluorometer was used to assess the quantity and quality of DNA in the extracted sample. Molecular identification relies on the sequence characterization of the mito-

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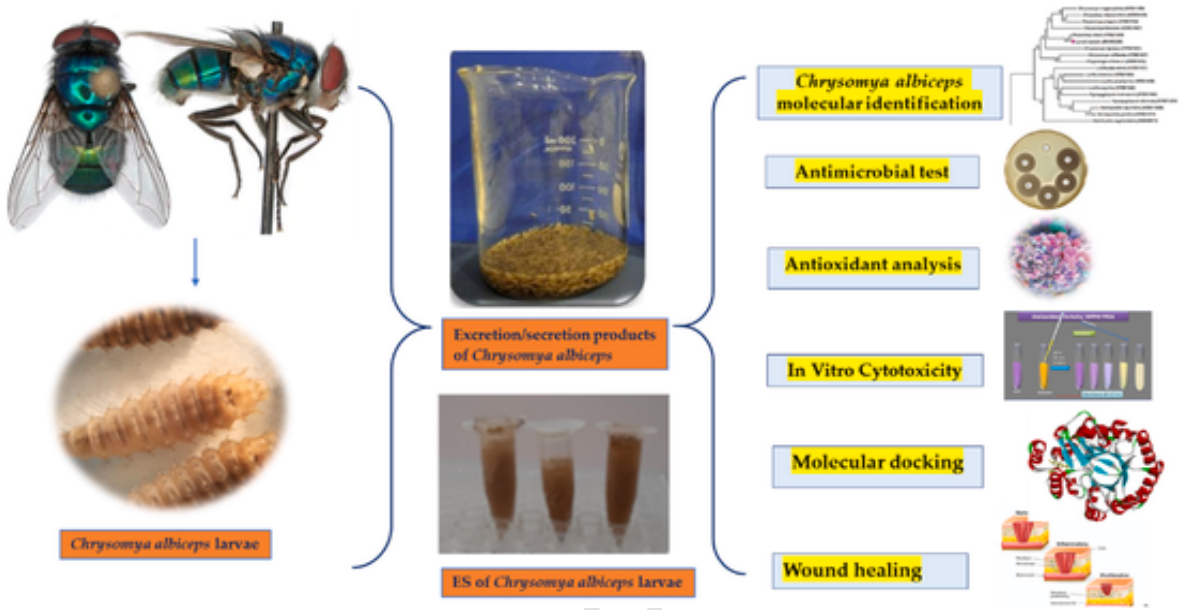

Fig. 1. Flowchart of research structure.

chondrial cytochrome *c* oxidase subunit I (COXI) gene. PCR amplification was initiated using a pair of universal primers, LCO1490 forward primer 5′-GGT CAA CAA ATC ATA AAG ATA TTG G-3′ and HCO2198 reverse primer 5′-TAA ACT TCA GGG TGA CCA AAA AAT CA-3′, as described by Folmer et al. [23], to amplify a specific COXI gene fragment ranging from 600 to 700 bp. Follow the steps in the kit's manual to make the PCR reaction mixture. It should have 25 μL, 5 μL of DNA, 12.5 μL of AmpliTaq Gold® 360 Master Mix, 3 μL of each primer, and 1.5 μL of nuclease-free water. Amplification of the target genes was carried out in a BIO-RAD® PCR system T100 thermocycler (BioRad, Hercules, California, USA) as previously described. For larvae identification, we used a temperature profile of 95 °C for 5 min (predenaturation), followed by 35 repeated cycles, each lasting 1 min at 95 °C (denaturation), 52 °C (annealing), and 72 °C (extension). The final extension was carried out for an additional 10 min at 72 °C and scheduled for a final hold at 4 °C. The amplified fragment was analyzed on a 1.5 % agarose gel stained with ethidium bromide by using the Molecular Imager Gel DocTM XR + Imaging System (BIO-RAD) and Image LabTM software for gel image analysis. Identification of molecular weight was guided by a 1 kb DNA ladder (Thermo Scientific™, Waltham, MA, USA). According to the manufacturer's instructions, PCR products were purified using the QIAquick® Gel Extraction Kit (QIAGEN, USA, Cat. No. 28704) and then sequenced with the same previous primers using the 3500 genetic analyzer sequencer and the BigDye® Terminator v3 kit (Thermo Fisher, Waltham, MA, USA) and cycle sequencing kit.

2.2.3. Sequence analysis and phylogenetic tree

The resulting sequence was edited using BioEdit 7.1.10 software [24] and was compared to those in the GenBank database (http://www.ncbi.nlm.nih.gov/blast, accessed on December 10, 2022). A set of 20 COX1 genes from the GenBank database of related genera was utilized to construct a phylogenetic tree, in addition to the 2 COX1 genes of *Musca domestica* as an outgroup. Thompson et al. [25] was utilized for nucleotide sequence alignment. A phylogenetic tree was constructed using a maximum likelihood method based on the Tamura model [26] by MEGAX inferred from 1000 bootstrap replicates [27]. The resulting sequence was deposited in the GenBank database.

2.3. Extraction of larval excretion/secretion (ES)

A modified method from Kerridge [28] was used for extraction. Briefly, approximately 250 larvae (~40 g) were used in each assay. Larvae were washed with ethyl alcohol for 5 min, replaced with 0.5 % formaldehyde for another 5 min, and finally rinsed twice times with sterile phosphate buffer saline (PBS) (pH 7.2). Larvae were incubated in 2 ml of PBS for 60 min at 27 °C and 50 % RH in darkness. The resultant liquid was then extracted using a pipette and centrifuged at 8000 g for 10 min at 4 °C. For antibacterial testing, the supernatant was filtered through a 0.22 μm membrane (Xi'an Zenlab), put into clean Eppendorf vials, and kept at −20 °C. The extract was turned into a powder using a freeze-dryer, Alpha 1–4 LSCplus. Gas chromatography-mass studies were done on this powder to find its active compounds, cell toxicity, and antioxidant levels.

2.4. Antimicrobial test

Antimicrobial activity of *C. albiceps* extract was tested against Gram-positive bacteria (*Listeria monocytogenes* ATCC 19155, *Staphylococcus aureus* ATCC 43300) and Gram-negative bacteria (*Escherichia coli* ATCC 8739, *Salmonella* sp. ATCC 14028) via the agar diffusion approach, according to Al-Shemy et al. [29]. The bacteria were grown in a nutrient-liquid medium on a shaker bed at 200 rpm for 24 h at 37 °C. The bacteria (1.5×10^8 CFU) were swabbed on Mueller-Hinton agar plates. Then, 200 μL of the extracts were put into wells that had been cut into agar plates and then incubated at 37 °C for 24 h. All the conducted studies were carried out in triplicate for all tested strains, where the inhibition zones surrounding the discs were quantified in millimeters.

2.5. Antioxidant activity (DPPH assay)

The antioxidant activity of *C. albiceps* maggot extract at various concentrations was carried out using the DPPH (2,2-diphenyl-1-picrylhydrazyl) method by Dawwam et al. [30] with minor modifications. Different concentrations of crude extracts (1000, 500, 250, 125, 62.5, 31.25, 15.625, 7.81, and 3.9 μg/mL) were used to determine the scavenging of DPPH radicals. The antioxidant activity of the standard and extracts was measured by checking how much they lowered DPPH

levels, using the formula [(control absorbance – extract absorbance)/(control absorbance) × 100] [31].

2.6. In vitro cytotoxicity assay

The cytotoxicity of *C. albiceps* extract at concentrations of 1000–32.25 μg/l was determined using the MTT protocol [32] with minor modification against normal human fibroblast cell lines (HFB4 cell lines) that were collected from ATCC. The viability and inhibition percentages were calculated as shown in Eqs. 1 and 2 as follows:

$$\text{Viability\%} = \text{Test OD/Control OD X100} \quad (1)$$

$$\text{Inhibition\%} = 100 - \text{Viability\%} \quad (2)$$

2.7. In vitro wound healing assay

Normal human skin cells (3 x 10^5 cells per well) were grown in 24-well plates and kept in a humid environment with 5 % CO_2 and 95 % air at 37 °C, using RPMI that had 1 % penicillin-streptomycin, 1 % nonessential amino acids, and 10 % fetal bovine serum (FBS). After incubation, the cell's monolayers were washed twice with PBS. A vertical scratch was applied using a 10–100 μL pipette tip across each well. The washing process through PBS helped to remove the detached cells; this phase was followed by adding low-serum fresh medium to each well, and then the image for the scratch was taken at zero time. 20 mg of maggot extract was used after sterilization for 30 min under UV. After 48 h, we imaged the scratch closure using ZOE Fluorescent Cell Imager fluorescence microscopy (BIO-RAD, USA). The analysis of the scratch width was calculated using the Image J public domain software [33].

2.8. Phytochemical analysis of larval extract

2.8.1. Gas chromatography-mass spectrometry (GC-MS) analysis

The extract was taken from the common blue botfly larvae (*C. albiceps*) and was analyzed by GC-TSQ mass spectrometer (Thermo Scientific, Austin, TX, USA) with a direct capillary column TG–5MS (30 m × 0.25 mm x 0.25 μm film thickness). The column oven temperature was initially held at 60 °C and then increased by 5 °C/min to 250 °C with a hold of 2 min, then increased to 300 °C at 30 °C/min. The injector temperature was kept at 270 °C. Helium was used as a carrier gas at a constant 1 ml/min flow rate. The solvent delay was 4 min, and diluted samples of 1 μl were injected automatically using the autosampler AS3000 coupled with GC in the split mode. EI mass spectra were collected at 70 eV ionization voltages over the range of *m/z* 50–650 in full scan mode. The ion source and transfer line were set at 200 °C and 280 °C, respectively. The components were identified by comparison of their mass spectra with those of the WILEY 09 and NIST14 mass spectral databases [34].

2.8.2. Molecular docking studies

A molecular docking study was performed using Accelrys's Discovery Studio 2.5.5 software (Accelrys Inc., San Diego, CA, USA) at the Faculty of Pharmacy, Ain Shams University, Egypt. However, the results were visualized using BIOVIA Discovery Studio Visualizer (Dassault Systems, 2025). The 3D structure of the different targets used in the docking study in the current study (*E. coli* pdb code: 4YFX, *Salmonella* sp. pdb code: 3NA5, *S. aureus* pdb code: 8CF3, and *L. monocytogenes* code: 4CDB) were obtained from the Protein Data Bank at the Research Collaboration for Structural Bioinformatics (RCSB) website (www.rcsb.org). Afterwards, hydrogen atoms were added to the downloaded 3D structures, followed by applying a force field for energy calculation and then energy minimization with fixed atom constraints to avoid the distortion of the original 3D structure. The minimized proteins were then used in the docking of the major bioactive compounds in larvae extract using the CDOCKER protocol. After the docking run was completed, CDOCKER interaction energies of the docked compounds were recorded and compared to those of the original lead compound of the specified target. Also, the binding interactions of the docked compounds were observed and compared to the reported interactions with essential amino acids at the target active site. Moreover, the validation of our molecular docking studies was performed through the calculation of root mean square deviation (RMSD) value. This value determines the degree of superimposition between the original X-ray pose of a certain ligand versus its docked pose. For a reliable and successful docking process, the value should not exceed 2.0 A°. In the presented study, the RMSD values of the docking studies in the forementioned targets ranged between (1.19–1.82 A°) which confirms the reliability of the docking results recorded (Fig. 2.)

2.9. Statistical analysis

Data were analyzed using IBM SPSS software version 23 (USA). The assumptions of normality and homogeneity of variances were assessed using the Shapiro-Wilk test and Levene's test, respectively. Upon confirmation that these assumptions were met, one-way analysis of variance (ANOVA) was performed to evaluate differences among group means. The post hoc Tukey's Honestly Significant Difference (HSD) test for pairwise comparisons to identify statistically significant differences between specific groups. The significant levels were set at $P < 0.05$.

3. Results

3.1. Antimicrobial assay

The results of the antibacterial activity of *C. albiceps* maggot ES using disc diffusion are presented in Fig. 3. Results showed that *C. albiceps* larval extract highly inhibited the growth of MDR bacteria. The inhibition efficiency ranged from 18 to 25 mm. The highest inhibition zone (25 mm) diameter was observed for *S. aureus*, whereas the least inhibition zone (18 mm) was observed for *E. coli* and *Salmonella* sp.

3.2. Antioxidant activity

The antioxidant compounds are used to reduce the effect of free radicals. In this study, the antioxidant activity of ascorbic acid (positive control) and extract from *C. albiceps* maggots was evaluated at different concentrations from 1000 to 1.95 μg/mL, as shown in Fig. 4. Results revealed that *C. albiceps* extract exhibited strong antioxidant activity compared to ascorbic acid. Antioxidant activity of *C. albiceps* extract at concentrations of 62.5–1000 μg/mL was above 50 % for all, while at concentrations of 31.25 and 1.95 μg/mL, it was 47.3 % and 12.8 %, respectively. Moreover, the IC_{50} of *C. albiceps* extract was 44.89 μg/ml using the DPPH assay.

3.3. In vitro cytotoxicity assay

Evaluation of the cytotoxicity of natural metabolic products is the first step to determining their safety in noncancerous human cells [35]. Cytotoxicity of *C. albiceps* maggot extract was determined toward the HFB cell line, as illustrated in Fig. 5. The results showed that the HFB4 cell line was not affected by the *C. albiceps* extract at a concentration of 250 μg/ml. Also, IC_{50} was 350.58 ± 4.23 μg/mL, where if the IC_{50} is ≥ 90 μg/mL, the compound is classified as not cytotoxic. Eventually, *C. albiceps* extract is non-toxic and safe for use.

3.4. In vitro wound healing assessment

The effectiveness of *C. albiceps* maggot extract in helping normal skin cells heal a scratch was tested in a lab using normal human skin fibroblast cells (HFB4 cells). Results showed that the *C. albiceps* extract

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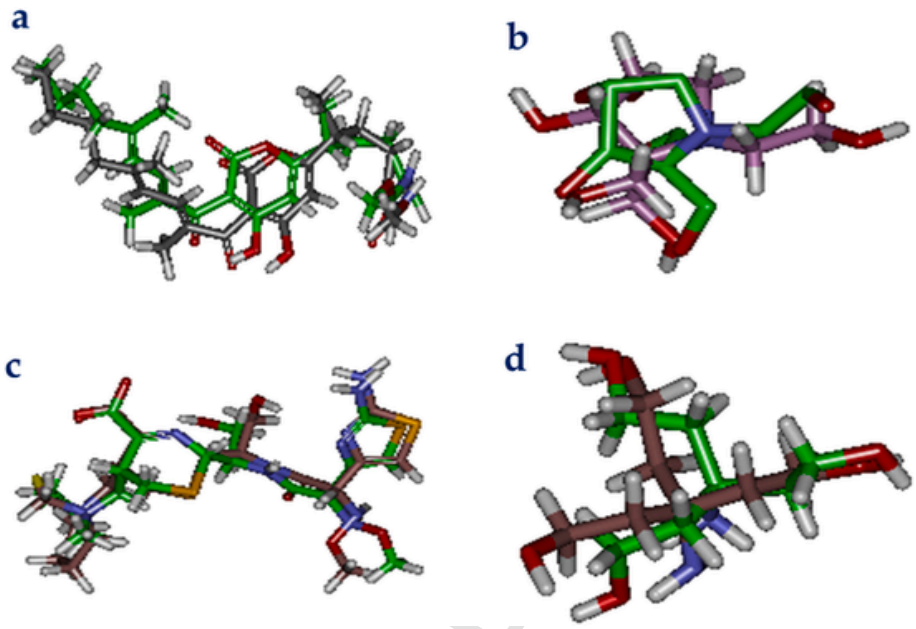

Fig. 2. Superimposition of the original X-ray pose of the ligand (green) and its docked pose of different targets: a) *Escherichia coli* ligand (1.19 A˚), b) *Salmonella* sp. ligand (1.82 A˚), c) *Staphylococcus aureus* ligand (1.30 A˚), d) *Listeria monocytogenes* ligand (1.50 A˚).

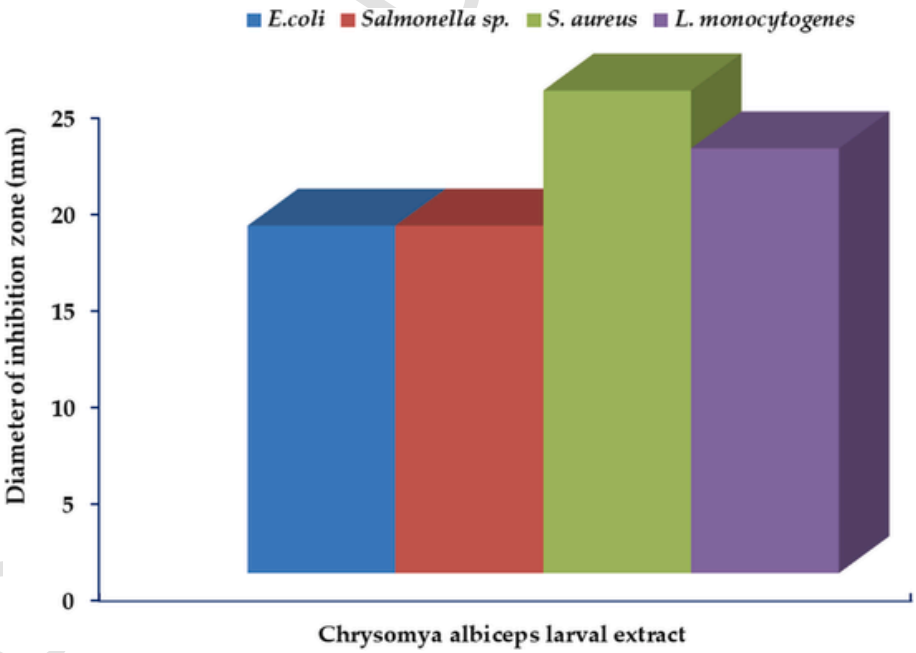

Fig. 3. Antibacterial activity of *Chrysomya albiceps* maggot ES against several pathogenic microorganisms.

worked to heal the scratch (Fig. 6). The scratch examination after treatment revealed that *C. albiceps* extract accelerated the wound healing process by decreasing the scratch gap from both sides. The results reflected a remarkable impact of *C. albiceps* extract in inducing the scratch closure within 48 h.

3.5. Molecular identification of Chrysomya albiceps and construction of phylogenetic tree

PCR amplification resulted in a ~680 bp fragment (Fig. 7A). These fragments corresponded to the COXI gene and were subsequently sequenced. All the mtCOXI sequences obtained were aligned with those deposited in GenBank, and they displayed 98–100 % similarities to the mtCOXI of *C. albiceps* using the BLASTn algorithm retrieved from the GenBank database. These sequences were then deposited in GenBank and assigned accession number PV210134.1. Employing the MEGA X

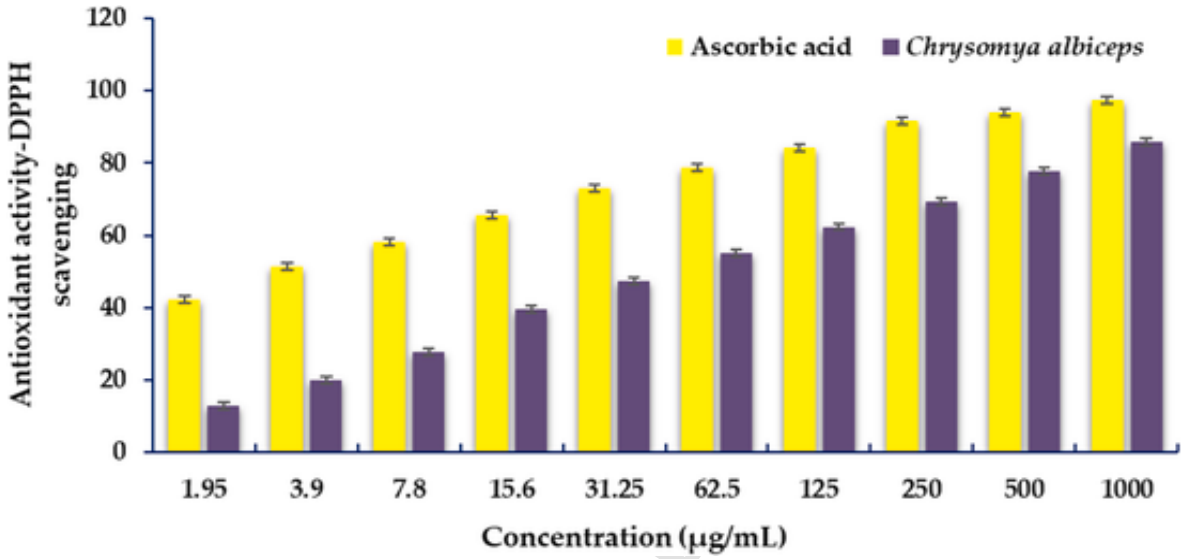

Fig. 4. Antioxidant activity of *Chrysomya albiceps* maggot extract using DPPH assay.

program and the maximum likelihood method analysis with 1000 replicates for bootstrap support, an original tree with well-supported nodes was generated (Fig. 7B). The species identified and collected in this study could thus be specified based on their COI gene, resulting in 100 % compatibility between molecular and taxonomic identification, indicating that the COI barcode is a useful tool to supplement taxonomy for blowfly species identification.

3.6. Larval extract identification

3.6.1. Gas chromatography-mass spectrometry (GC-MS) analysis

The GC-MS technique is still considered one of the best methods for identifying the constituents of volatile matter, long- and branched-chain hydrocarbons, alcoholic acids, esters, and other organic compounds [36]. The results of GC-MS analysis led to the identification of several compounds. These compounds were identified through mass spectrometry attached to GC. The mass spectrometer analyzes the compounds eluted at different times to determine their nature and structure. Interpretation of the mass spectrum GC–MS was conducted using the database of the National Institute of Standards and Technology (NIST). The retention time (RT), relative amounts (peak area %), names of the compounds, molecular formulas, and molecular weights (MW) of the test materials were determined; the components found by the GC-MS analysis are shown in Table 1. All of the tested fractions had a high concentration of bioactive components, according to the gas chromatography-mass detector results. This could give a clue to a wide medicinal activity they may possess. A preliminary study found acids, alcohols, aldehydes, aromatic compounds, esters, and ketones in the extract of *C. albiceps* larvae (Table 1). The *Chrysomya albiceps* extract's GC-MS analysis revealed 17 bioactive compounds, as shown in Fig. 8 and Table 1. These compounds possess different properties. Most of these compounds can fight germs or bacteria, including 2-piperidinone, palmitleic acid, hexadecanoic acid, 9,12-octadecadienoic acid (z,z)-, oleic acid, hexadecadienoic acid methyl ester, 9-octadecenamide, and ethyl iso-allocholate.

3.6.2. Molecular docking

The results of the molecular docking studies performed using the CDOCKER protocol (Accelrys's Discovery Studio 2.5.5 software (Accelrys Inc., San Diego, CA, USA) for the highly available bioactive compounds present in the larvae extract at the active sites of DNA-directed RNA polymerase beta subunit in *E. coli*, phosphoglucomutase enzyme in *Salmonella* sp., regulatory protein BlaR sensor domain in *S. aureus*, and the essential virulence factor Listeriolysin O in *L. monocytogenes*, respectively, are shown in Table 2 as CDOCKER interaction energy relative to the co-crystallized ligands in the 3D structures obtained from the protein data bank. Also, the interaction of the docked compounds with the essential amino acids at the active sites of different previously mentioned targets was observed and compared to the reported interactions by the co-crystallized ligand of each target and shown as 2D interaction diagrams (Figs. 9–12). The results reflected that most of the docked compounds had comparable CDOCKER interaction energies relative to the value of the docked co-crystallized lead compound in each specified target. Moreover, the 2D interaction diagrams revealed the well-fitting and binding of the represented docked compounds with at least one amino acid at the active site of the specified target. In conclusion, these observations could rationalize the recorded antimicrobial activities for the larvae extract.

4. Discussion

Natural extracts exhibit various biological activities, including anticancer, antiparasitic, and antimicrobial effects. Insects, particularly Calliphoridae larvae, are emerging as valuable sources of bioactive compounds with therapeutic potential [55]. Despite their richness in proteins, lipids, and peptides, their biomedical utility remains underexplored. With the alarming rise of antibiotic resistance, these larvae represent a promising natural alternative due to their ability to produce antimicrobial peptides that may help combat resistant bacterial infections [56,57].

This study looks at how the compounds from *C. albiceps* larvae affect the growth of specific Gram-negative and Gram-positive bacteria. Despite *C. albiceps* excretion/secretion (ES) being derived from uninfected larvae cultivated on raw beef, the findings of this investigation demonstrated that the extract of *C. albiceps* larvae possesses strong antibacterial activity against multiple drug-resistant bacterial strains, with inhibition zones ranging from 18 to 25 mm, showing the highest efficacy against *S. aureus*. The inhibition may be due to antimicrobial peptides secreted by secretory cells in maggots [28]. This study confirms that secretory cells continuously synthesize numerous insect antimicrobial peptides without the need for bacterial stimulation. No noticeable differences in how well larval extracellular secretions fight bacteria have

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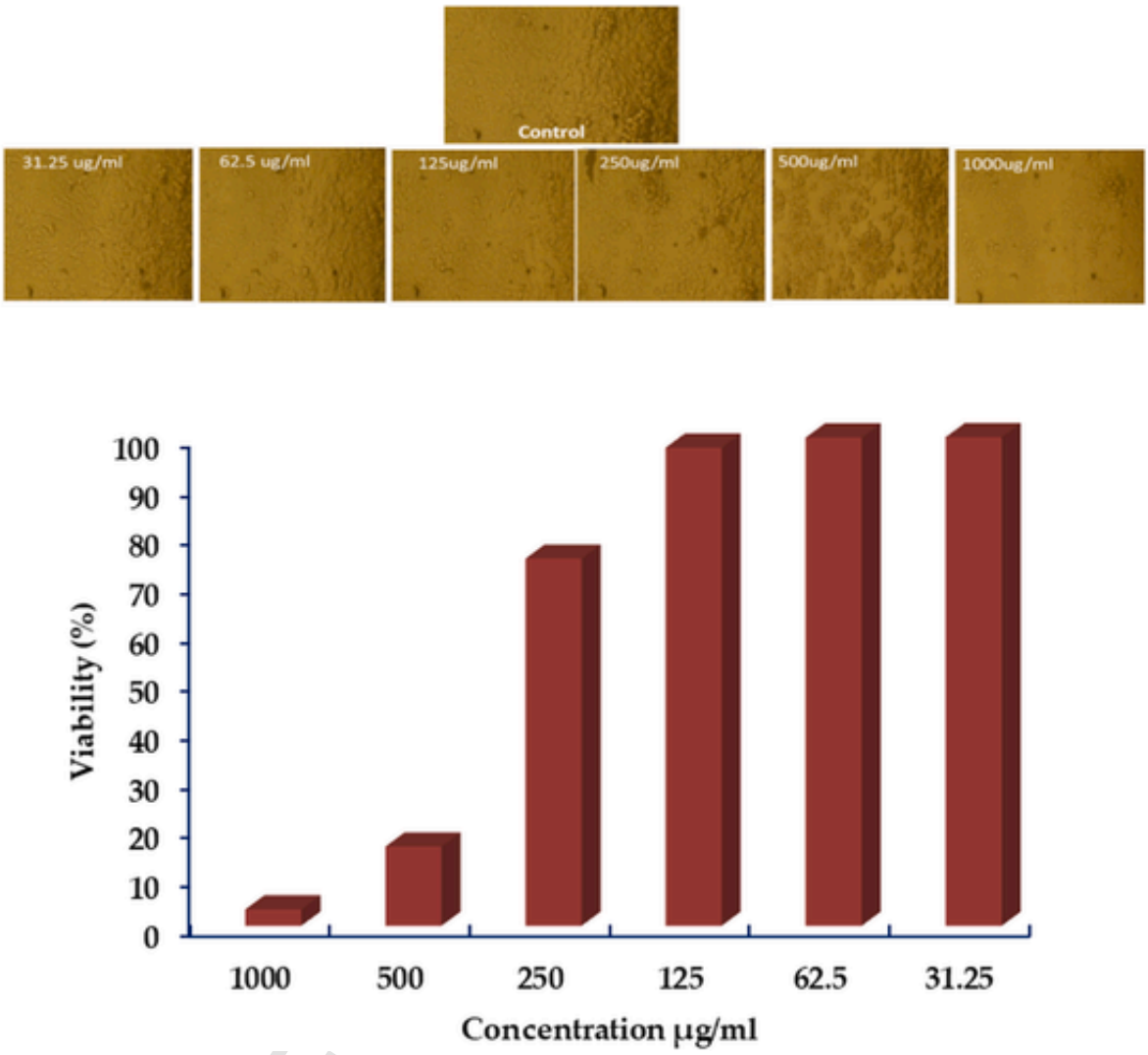

Fig. 5. Cytotoxicity of *Chrysomya albiceps* extract against HFB4 cell line.

been found between infected and uninfected larvae, indicating that their effectiveness remains constant.

These findings are consistent with previous studies. For instance, Ref. [13] found that *C. albiceps* extracts were very effective at stopping the growth of both Gram-positive and Gram-negative bacteria, such as *S. aureus* and *E. coli*. Similarly [58], who demonstrated the antimicrobial potential of extracts from *L. sericata* and *M. domestica*. Furthermore, Kerridge [28] confirmed that larval excretions from blowflies contain antimicrobial peptides that remain effective regardless of bacterial stimulation. These findings together back up our results, indicating that *C. albiceps* larval secretions have natural and wide-ranging antimicrobial properties. Also, Hou [59] discovered that high concentrations of housefly larvae extract exhibited enhanced antibacterial activity against both Gram-positive and Gram-negative bacteria. Furthermore, they determined that the housefly extract exhibits extensive antibacterial activity against both Gram-positive and Gram-negative bacteria, a conclusion consistent with the current investigation and may indicate a wide range of antibacterial activity of maggots of many or all species of Diptera.

The extract also exhibited potent antioxidant activity, with an IC_{50} value of 44.89 µg/ml, highlighting its role in reducing oxidative stress that impedes wound healing. Moreover, experiments showed that the extract is non-toxic to human fibroblast cells at a concentration of 250 µg/ml, confirming its safety for potential therapeutic applications. Additionally, the extract enhanced wound healing in skin cells within 48 h, demonstrating its effectiveness in promoting tissue regeneration. Some studies have demonstrated that maggot therapy is effective in treating infected wounds [60]. A study looked at how the extracellular parts of the *Wohlfahrtia nuba* maggot stopped gram-negative and gram-positive bacteria from growing. Even though the waste products from *W. nuba* maggots were collected from those that ate raw beef without any bacteria, all the tests in this study showed that these waste products can kill all the bacteria that were tested in the lab [13].

We confirmed what Kerridge [28] found, which was that many of the antibacterial chemicals that maggots make are always present and don't need to be activated [61], especially those that are made in secretory cells [62]. In a different study [63], found that the fluid from *Lucilia sericata* maggots that had been exposed to bacteria killed bacteria better than fluid from maggots that had not been exposed to bacteria.

The maggot's waste products are important for more than just killing different types of bacteria; they also contain substances that make cleaning up injuries that are infected easier. New studies have examined how effective sterile *L. sericata* ES is at preventing the growth and breaking apart bacterial biofilms of *S. aureus* and *P. aeruginosa* [64]. ES stopped and broke up *S. aureus* biofilms right away, while it encouraged the growth of *P. aeruginosa* biofilms for 10 h after incubation.

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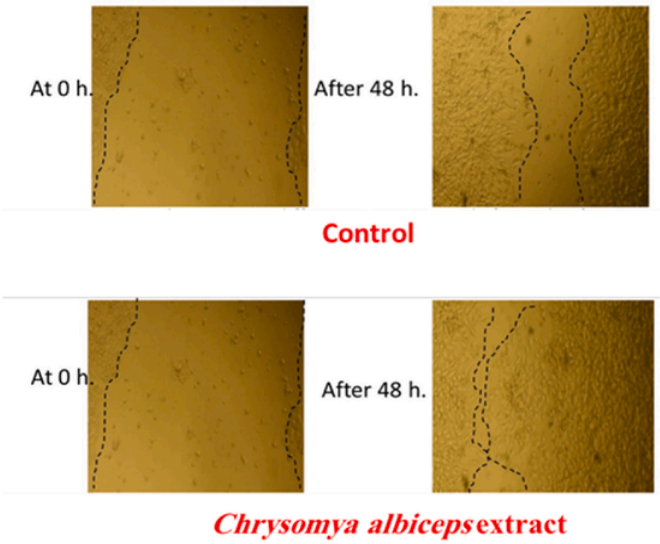

Fig. 6. Microscope images from the in vitro wound healing assay of *Chrysomya albiceps* extract. The photos were taken at 0, and 48 h.

After that, it started breaking down *P. aeruginosa* biofilms [65]. The integration of essential oils and conventional antibiotics may guarantee the thorough eradication of biofilms [55]. According to Pöppel [66], most insects make a wide range of antimicrobial peptides (AMPs) as part of their innate immune responses. The complex interactions between these AMPs make antimicrobial defense work well.

The research on the antibacterial effects of *C. albiceps* fits into maggot debridement therapy, where larvae are placed on wounds to remove dead tissue, reduce bacteria, and help healing. Researchers have discovered that the waste and secretions from *C. albiceps* larvae raised in a clean environment can effectively fight various types of bacteria. The data indicate that exposure to ambient microorganisms does not elicit antibacterial activity. However, they provide no evidence of bacterial influence on the efficacy of the ES. Furthermore, previous studies have not examined the impact of exposure to harmful bacteria on the survival rate to adulthood (wound healing) of these insects.

The oxidative response is crucial for human health. An antioxidant is a chemical that can inhibit or prevent the oxidation of other molecules, hence averting pathogenic alterations. Certain physiological processes in the body produce free radicals to eradicate invading pathogenic germs. An overload of oxidative stress and weak antioxidant defenses inside cells can lead to several diseases, including cancer [67], neurological disorders, autoimmune skin diseases, heart diseases, and chronic renal insufficiency [68]. As a result, antioxidants can help reduce the harmful effects of unstable and reactive free radicals, protecting cells from their damage.

This study demonstrates the potential of whole-body extracts from maggots as antioxidant and anticancer agents. Notably, these extracts exhibited therapeutic effects, particularly the *C. albiceps* extract. It was more than 50 % effective as an antioxidant when the concentration was between 62.5 and 1000 μg/mL. These results are consistent with previous research on insect-derived antioxidants. For instance, El-Garawani [55] demonstrated that *M. domestica* larvae injected with lipopolysaccharides produced potent antioxidant responses. Similarly [36,44],

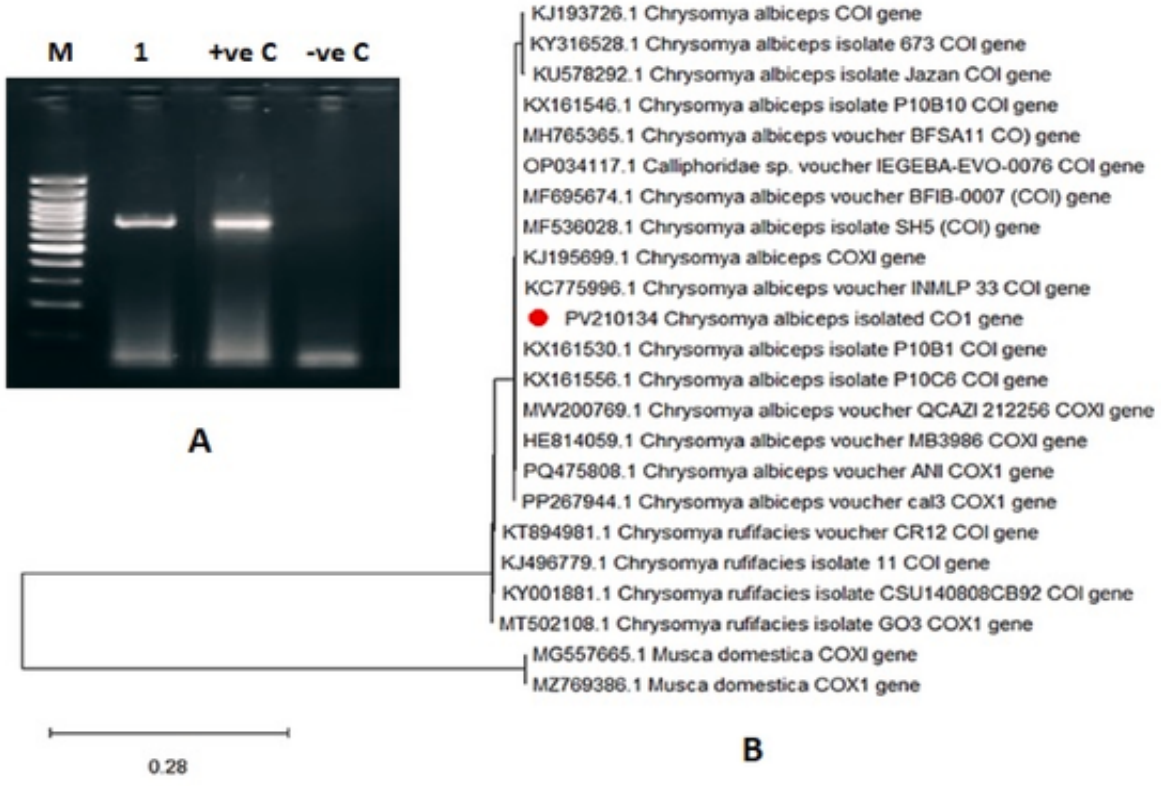

Fig. 7. A. Amplification of COI gene produces 680bp of PCR products from *Chrysomya* sp. Lane M: 100bp DNA ladder; Lane 1: DNA of *Chrysomya albiceps*, Lane + ve C: Positive control and Lane -ve C: Negative control, B. Evolutionary relationships among 20 *C. albecips* isolate utilizing cytochrome oxidase subunit 1 (COXI) sequences; 1000 bootstrap replicates were used to assess robustness using MEGA X. Sequence from the present study is denoted by a red circle on the tree. The red circles represent the insect specimens under study. The *Musca domestica* COXI sequence was used as an out-group.

Table 1
The major chemical constituents of *Chrysomya albiceps* larvae extracts using GC-MS chromatogram.

No	RT	Compound Name	Area %	M. F.	M.W	Biological Activity
1	9.05	2-piperidinone	3.53	C_5H_9NO	99	Antimicrobial activity [37]
2	15.18	Benzenepropanoic acid, trimethylsilyl ester	2.69	$C_{12}H_{18}O_2Si$	222	Antioxidant, Antibacterial, Antiplasmodial and Anticancer Activity [38]
3	27.16	Palmitoleic acid	3.27	$C_{16}H_{30}O_2$	254	Antibacterial activity [39]
4	27.78	Hexadecanoic acid	19.62	$C_{16}H_{32}O_2$	256	Antibacterial Activity [40]
5	29.10	Palmitic acid, tms derivative	5.58	$C_{19}H_{40}O_2Si$	328	Antioxidant and antimicrobial activity [41]
6	30.78	Ethyl (9z,12z)-9,12-octadecadienoate #	13.55	$C_{20}H_{36}O_2$	308	Antioxidant and antidiabetic activity [42]
7	30.90	9,12-Octadecadienoic acid (z,z)-	10.44	$C_{18}H_{32}O_2$	280	Antibacterial activity [43]
8	30.97	Ethyl oleate	8.98	$C_{20}H_{38}O_2$	310	Antibacterial, and antioxidant activity [44]. Antibacterial activity [45]
9	31.06	Cis-vaccenic acid	7.77	$C_{18}H_{34}O_2$	282	Cytotoxic and antimicrobial activity [46]
10	31.38	Oleic acid	1.56	$C_{18}H_{34}O_2$	282	antifungal and antibacterial activities [47]
11	33.45	5,8,11,14-Eicosatetraenoic acid, methyl ester, (all-z)-	0.97	$C_{21}H_{34}O_2$	318	Antimicrobial activity and cytotoxicity [48]
12	33.83	Hexadecadienoic acid, methyl ester	1.06	$C_{17}H_{30}O_2$	266	Cytotoxicity and antibacterial activity [48,49]
13	33.95	9-Octadecenamide	1.59	$C_{18}H_{35}NO$	281	Antifungal activity [50]
14	40.59	1-Heptatriacotanol	2.71	$C_{37}H_{76}O$	536	Antioxidant and Wound Healing Properties [51]
15	40.98	Ethyl iso-allocholate	2.43	$C_{26}H_{44}O_5$	436	Antimicrobial activity [52]
16	41.30	1,25-Dihydroxyvitamin d3, tms derivative	3.19	$C_{30}H_{52}O_3Si$	488	Antioxidant and antibacterial activities [53]
17	43.76	Cholest-5-en-3-ol (3á)-	7.27	$C_{27}H_{46}O$	386	Antioxidant compound [54]

identified fatty acids such as oleic and palmitoleic acid as key contributors to antioxidant activity in insect extracts. Importantly, we found these same compounds in our GC-MS profile of *C. albiceps*, which supports their role in reducing oxidative stress and confirms the extract's potential for medical use.

The active compounds in the extract were analyzed by GC-MS, identifying 17 bioactive compounds with diverse properties, including fatty acids and peptides with antibacterial activity. Computational molecular docking studies supported the antibacterial efficacy of these active compounds by showing interactions with active sites on vital bacterial proteins. Despite these promising findings, the study is limited to in vitro experiments without in vivo or clinical evaluations, and the molecular interaction data do not allow precise quantitative assessment of binding strengths between the compounds and proteins. Therefore, the study recommends further research involving comprehensive in vivo experiments, isolation of effective compounds, and investigation of their synergistic effects with conventional antibiotics to develop natural and effective therapeutic strategies against infections and chronic wounds. The molecular docking results demonstrated high compatibility between the bioactive compounds present in the *C. albiceps* larval extract and the active sites of key proteins responsible for the activity and virulence of the tested pathogenic bacteria (*E. coli, Salmonella* sp., *S. aureus*, and *L. monocytogenes*). Compounds such as hexadecanoic acid, ethyl (9z,12z)-9,12-octadecadienoate, 9,12-octadecadienoic acid, ethyl oleate, and cis-vaccenic acid exhibited the ability to interact with essential amino acids at the active sites of these proteins. These interactions likely contribute to inhibiting the proteins' functions and consequently impair the bacteria's pathogenic capabilities. Such interactions support the molecular rationale for the antimicrobial activity observed in the laboratory experiments. Although this study did not include a quantitative assessment of the binding energies between the compounds and proteins, the findings provide significant evidence of the potential use of these compounds as sources for antibacterial drug development, demonstrating the importance of conducting further studies to determine the relative efficacy and optimal compound concentrations for pharmaceutical advancement.

To fight off pathogens, insects have a complex immune system that includes hemocytes in the hemolymph and physical barriers like the gut and skin [69]. The epithelial and midgut tissues also make antimicrobial agents, specifically peptides, which act as effector molecules [70]. Researchers have found polar chemicals with antibacterial properties on the epicuticular layer of arthropods, including social insects [58]. The self-defense mechanism operates by scavenging free radicals and repairing damaged biomolecules essential for survival [71]. The substantial protein content of insects enhances their nutritional value [72]. Bioactive peptides can be a good source of nutrition because they have good biological properties like reducing inflammation, fighting free radicals, lowering blood pressure, and lowering cholesterol. They do this by activating GST and CAT antioxidant enzymes and DPPH radical scavenging activities. In insects, special anti-pathogen peptides can be produced in their fat bodies, but the skin can also create antibacterial and antifungal peptides when there are local infections.

The cytotoxicity results show that *C. albiceps* extract is safe for normal human fibroblast cells (IC_{50} = 350.58 μg/mL), which means it can be used safely in treatments. This matches what [73] said, which is that substances with IC_{50} values over 90 μg/mL are considered non-cytotoxic. Moreover, Amer [58] reported similar safety profiles for whole-body insect extracts, highlighting their potential as safe alternatives for drug development. The lack of harmful effects strengthens the extract's promise for use on the skin or in the body, especially when looking at other synthetic antimicrobials that have known side effects. The ability of the maggot extract to help wounds heal faster, shown by quicker closure of scratches in fibroblast cells within 48 h as shown in our data, adds to the increasing research on how blowfly larvae can help with healing. Van der Plas [65] showed that the waste products from Lucilia sericata maggots helped wounds heal faster by reducing inflammation and breaking down biofilms. Similarly, Pöppel [66] identified proteolytic enzymes in maggot secretions that promote tissue regeneration. The results of this study affirm that *C. albiceps* may offer similar therapeutic benefits, reinforcing its use in wound management strategies.

Several investigations have been published that used DNA-based identification to identify some forensically significant blowfly specimens [20]. This molecular method may help resolve many issues related to identifying tiny pieces of insect matter or very early larvae, particularly those associated with morphological defects [74]. As previously indicated, mtDNA has various advantages over nuclear DNA. The COI sequencing of the blow flies used in this study is being published for the first time in this country; these flies were collected from decomposing rabbit carcasses in the Benha area, Egypt. The sequencing information of the 680 bp COI gene may be utilized to differentiate between blowfly species. The chosen COI sequence demonstrated sufficient specificity to identify the collected blowfly specimens. This conclusion

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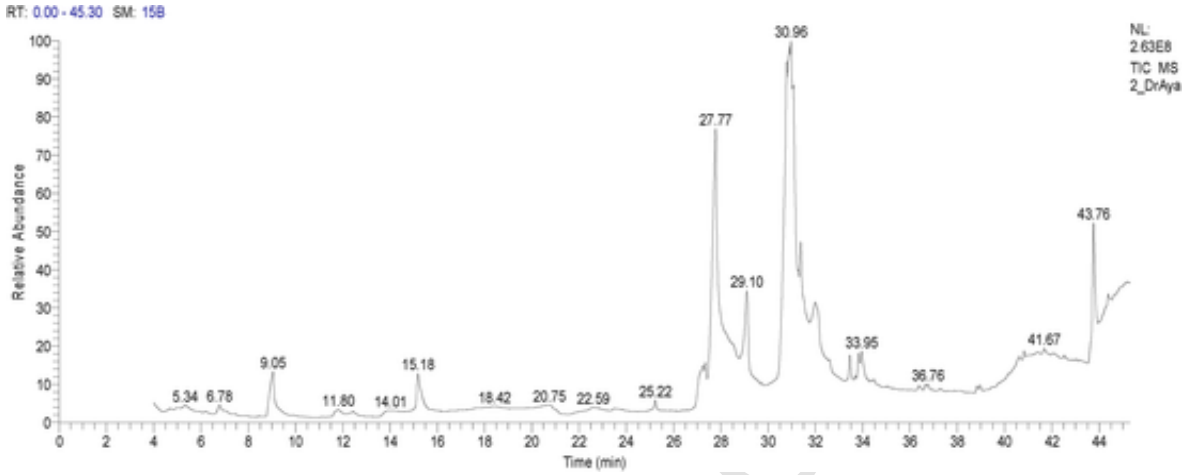

Fig. 8. GC-MS chromatogram of compounds secreted by *Chrysomya albiceps*.

is consistent with the findings of Mona [75], who found that in Western Australia, forensically significant fly species were distinguished using a brief COI sequence, amplified the CO1 region of 658 bp, and identified *C. megacephala* in 142 samples and *Chrysomya rufifacies* in 34 samples. In the northwest of South America, Amat [76] evaluated a brief COI fragment sequence (~342 bp) for the identification of 28 species of blow fly.

The genetic analysis data, which agreed with the conventional morphological classification, demonstrated its utility for detecting the type of fly. Researchers have discovered that COI serves as a descriptive tool for Chrysomyiinae identification. Researchers have extensively used the mitochondrial genome for species-level identifications [77]. Ames [78] looked at several gene regions and found that the COI "barcode" region was the best at telling the difference between Australian Chrysomya (Diptera: Calliphoridae). The COI region was enough for Ames [78] to tell the difference between *Calliphora vomitoria* and *C. vicina* in the UK. Wallman and Donnellan [79] made a similar discovery recently. They found that forensic entomology in Belgium and France can use the 304-bp fragment of the COI gene to help identify Chrysomyiinae species.

This study's results indicate that DNA analysis, in conjunction with other methods, can accurately identify various types of blowflies. The accessibility of such a DNA database will aid forensic cases by making the identification of immature phases possible. Examining preserved larvae or damaged specimens could enhance the accuracy of identification.

Molecular docking guided the understanding of compound safety and pharmacokinetics. In vivo analyses confirmed immunomodulatory effects, supported by biomarker assays showing changes in cytokines, tissue remodeling, and reduced oxidative stress [80,81].

In the current study, molecular docking studies were conducted to give a better understanding of the antimicrobial activities recorded against the 4 tested microorganisms: *E. coli*, *Salmonella* sp., *S. aureus*, and *L. monocytogenes*. The targets used in the molecular docking study were essential in the activity and the virulence of the microorganism; thus, the binding of the docked compounds at the active site of the target would negatively affect the microorganism and inhibit its activity. 4YFX3NA58CF34CDB

The bacterial RNA polymerase in *E. coli* (pdb code: 4YFX) is responsible for gene expression as well as bacterial cell growth and viability. Accordingly, binding of an inhibitor to the RNA polymerase binding site inhibits RNA elongation, which negatively affects bacterial survival [82].

Karim et al. [83] addresses the crisis of bacterial resistance to traditional antibiotics by evaluating 33 new quinolone derivatives, where the molecular studies highlighted the distinction between specific compounds (28, 29, 32, and 33) in interacting with bacterial targets, with compound 29 confirming its stability and potential efficacy as an anti-MRSA agent.

Interestingly, the phosphoglucomutase enzyme (pdb code: 3NA5) is a well-known bacterial protein present in *Salmonella* sp. It plays an essential role in metabolism as well as bacterial virulence and infectivity [84]. However, the extracellular domain of the BlaR protein is the BLAR sensor domain (pdb code: 8CF3), which is responsible for resistance to β-lactam antibiotics, especially in *S. aureus*. Thus, binding an inhibitor at this site is supposed to inhibit this response and restore bacterial sensitivity toward antibiotics [85].

Moreover, Listeriolysin O (LLO) is the critical essential virulence factor of *L. monocytogenes* and has been isolated by X-ray crystallography (pdb code: 4CDB) [86]. Listeriolysin O is responsible for the incidence of listeriosis. It is a member of the cholesterol-dependent cytolysins (CDC) family, the largest family among bacterial pore-forming toxins (PFTs), the most common bacterial virulence factors [87]. Therefore, in our presented research, our molecular modeling studies were performed using the aforementioned targets due to their importance in bacterial survival or virulence.

The observation of the active site of the DNA-directed RNA polymerase beta subunit in *E. coli* (PDB code: 4YFX) showed hydrogen bonding with essential amino acids Gly344, Lys1348, and Ile1352. The 2D interaction diagram of bacterial phosphoglucomutase in *Salmonella* sp. responsible for its virulence (pdb code: 3NA5) reflected hydrogen bonding with Pro 164 and Asp 166 along with interactions via unconventional carbon-hydrogen bonding with Asn 168 and Asp 378. The binding of cefepime to the regulatory protein BlaR sensor domain in *S. aureus* (PDB code: 8CF3) showed the interaction with Ser 389, Lys 526, Thr 529, and Ile 531. Finally, the investigation of the active site of the essential virulence factor in *L. monocytogenes*, Listeriolysin O (pdb code: 4CDB), showed an essential hydrogen bonding with Tyr 520. Also, the observation of the 2D interaction diagrams of the highly abundant bioactive compounds in the larvae extract (hexadecanoic acid, ethyl (9z,12z)-9,12-octadecadienoate, 9,12-octadecadienoic acid (z,z), ethyl oleate, and cis-vaccenic acid) revealed the well-fitting and interaction of these compounds in the previously mentioned targets with at least one amino acid at each active site. The docking results in *E. coli* showed that all represented docked compounds exhibit hydrogen bonding with Lys 1348 except hexadecenoic acid, which demonstrated an alternatively unreported interaction with Ala 1323. Also, the docking re-

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Table 2
CDOCKER interaction energies of the docked bioactive compounds.

Escherichia coli (pdb code: 4YFX)		*Salmonella* sp. (pdb code: 3NA5)	
Compound	CDOCKER interaction energy	Compound	CDOCKER interaction energy
Ligand (Myxopyronin B)	40.90	Ligand (2-[BIS-(2-hydroxyethyl)-amino]-2-hydroxymethyl-propane-1,3-diol)	27.02
Hexadecanoic acid	39.07	Hexadecanoic acid	25.39
Palmitic acid, tms derivative	31.45	Palmitoleic_acid	21.04
Ethyl (9z,12z)-9,12-octadecadienoate	37.30	Ethyl (9z,12z)-9,12-octadecadienoate	22.89
9,12-Octadecadienoic acid (z,z)	34.97	9,12-Octadecadienoic acid (z,z)	23.54
Ethyl oleate	35.5	Ethyl oleate	25.06
Cis-vaccenic acid	36.01	Cis-vaccenic acid	26.28
1,25-Dihydroxyvitamin d3, tms derivative	33.14	1,25-Dihydroxyvitamin d3, tms derivative	26.22
		Benzenepropanoic acid trimethylsilyl ester	26.08
		2-piperidinone	22.99
		Cholest-5-en-3-ol (3a)	21.58
		Palmitic acid trimethylsilyl ester	21.98
Staphylococcus aureus (pdb code: 8CF3)		*Listeria monocytogenes* (pdb code: 4CDB)	
Compounds	CDOCKER interaction energy	Compounds	CDOCKER interaction energy
Ligand (**cefepime**)	48.06	Ligand (Tris (Hydroxyethyl) Aminomethane)	23.76
Hexadecanoic acid	36.49	Hexadecanoic acid	26.03
Palmitoleic acid	32.72	Palmitoleic acid	20.38
Ethyl (9z,12z)-9,12-octadecadienoate	39.44	2-piperidinone	21.77
9,12-Octadecadienoic acid (z,z)	38.10	9,12-Octadecadienoic acid (z,z)	27.16
Ethyl oleate	37.79	Ethyl oleate	23.20
Cis-vaccenic acid	35.79	Cis-vaccenic acid	26.57
1,25-Dihydroxyvitamin d3, tms derivative	33.44	1,25-Dihydroxyvitamin d3, tms derivative	20.01
Benzenepropanoic acid trimethylsilyl ester	34.65		
2-piperidinone	35.87		

sults at the *Salmonella* sp. target reflected the interaction of all previously mentioned docked compounds with one of the reported amino acids except for cis-vaccenic acid, which showed unreported hydrogen bonds with Asn 488 and Trp 375.

Regarding interaction diagrams for docked compounds at the sensor domain in *S. aureus,* the docked compounds showed at least one interaction with reported amino acids except for ethyl (9z,12z)-9,12-octadecadienoate and ethyl oleate, which revealed unreported interactions with Thr 527 and Thr 536, respectively. Finally, the docked compounds at the Listeriolysin O active site in *L. monocytogenes* showed a different pattern of interactions than reported (hexadecanoic acid with Thr 494, ethyl (9z,12z)-9,12-octadecadienoate Pi-alkyl interaction with Tyr 520, 9,12-octadecadienoic acid (z,z) with Arg 499, ethyl oleate with Asp 498 and Arg 499 and Pi-alkyl interaction with Tyr 520, and cis-vaccenic acid with Pro 518), but all of them were able to interact with at least one amino acid present at the active site. These findings suggest that all tested compounds can bind to active sites in bacterial virulence proteins, although some displayed unique or unreported interaction profiles. This structural evidence supports the observed antimicrobial activity *in vitro*. However, quantitative docking scores or binding affinities were not reported, limiting precise evaluation of the compounds' relative binding strengths. Including binding energy values in future analyses would enhance the molecular interpretation and help prioritize the most effective compounds for drug development.

5. Conclusion

Insects have long been a valuable source of natural products, including silk, honey, beeswax, propolis, and royal jelly, and traditional medicine across many cultures has utilized insects and their derivatives to treat various ailments. Among these, dipteran fly larvae like *Chrysomya albiceps* are of particular interest due to their rich content of proteins, polyunsaturated fats, vitamins, and minerals. The extract of *C. albiceps* larvae demonstrates clear potential as a natural source of antibacterial and antioxidant agents with a stimulatory effect on wound healing. This study adds to growing evidence that *C. albiceps* larval extract is a safe and effective option for antimicrobial and antioxidant treatments. GC-MS profiling and molecular docking analyses revealed key bioactive compounds interacting with vital microbial proteins, providing a molecular explanation for the extract's observed antimicrobial efficacy. Furthermore, the extract's safety on non-cancerous human cells enhances its prospects for development as a natural therapeutic agent. Overall, this study supports the use of insect-derived products in medicine and calls for further *in vivo* studies to evaluate clinical efficacy and optimize formulations for practical medical applications.

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[49].

CRediT authorship contribution statement

Ghada E. Dawwam: Writing – review & editing, Writing – original draft, Validation, Supervision, Methodology, Investigation, Formal analysis. **Abdelwahab A. Ibrahim:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Investigation, Formal analysis, Conceptualization. **Ramy E. El-Ansary:** Writing – original draft, Project administration, Methodology, Investigation, Formal analysis, Conceptualization. **Abeer H.A. Abdelhafiz:** Writing – review & editing, Writing – original draft, Supervision, Formal analysis, Data curation. **Aya S. Omara:** Writing – original draft, Methodology, Funding acquisition, Data curation. **Abla D. Abdel-Meguid:** Writing – review & editing, Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Data curation. **Mohamed M. Baz:** Writing – review & editing, Writing – original draft, Supervision, Methodology, Investigation, Funding acquisition.

Informed consent statement

Not applicable.

Institutional review board statement

The study was conducted according to the guidelines of the Declaration of Benha University, and approved by Ethics Committee of Faculty of Science, Benha University (Code: BUFS 2025-26Ent).

Consent for publication

Not applicable.

Funding

The study did not receive fund

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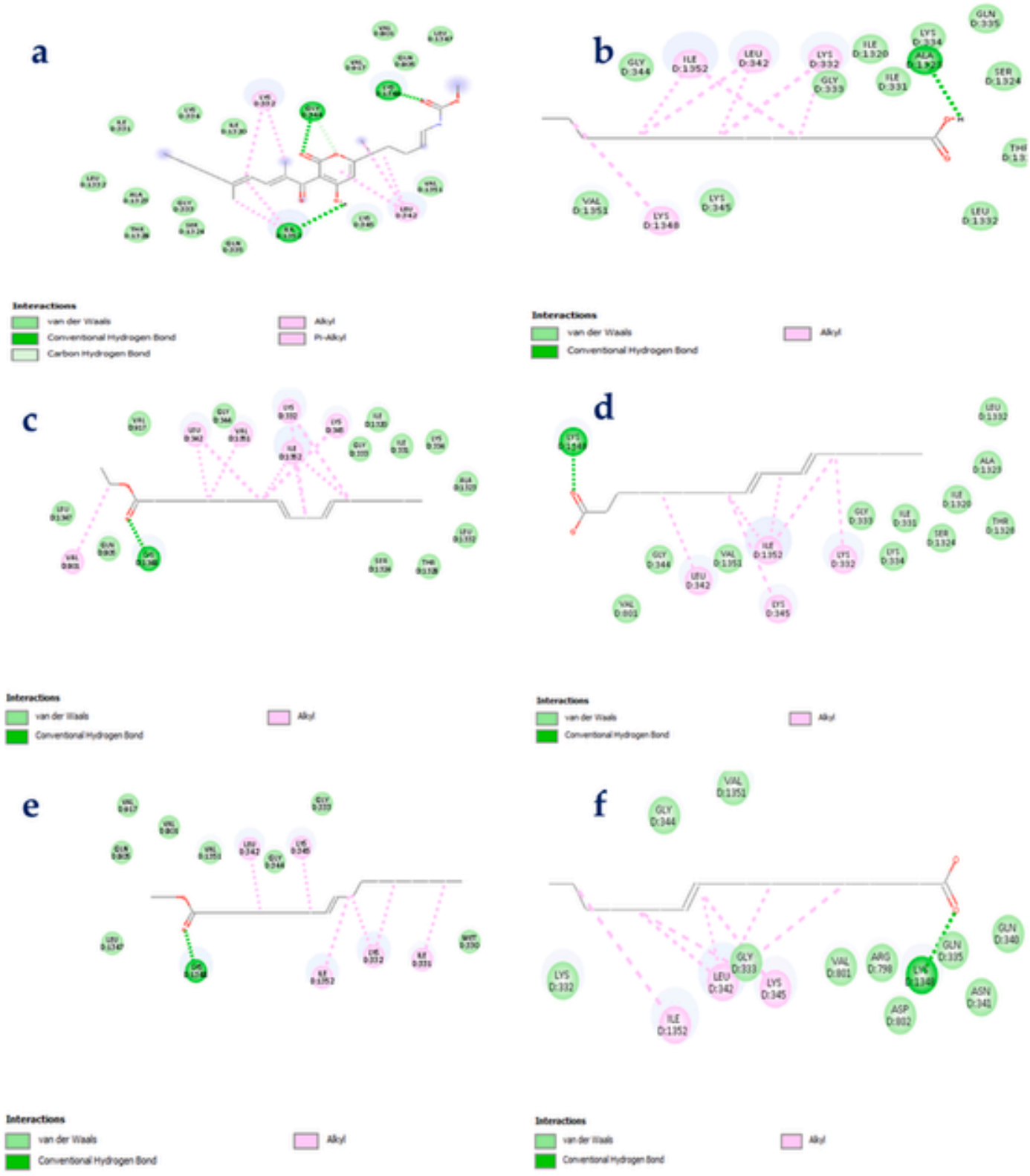

Fig. 9. 2D interaction diagrams of different compounds bound to DNA-directed RNA polymerase beta subunit of *Escherichia coli.* (a) ligand, (b) Hexadecanoic acid, (c) Ethyl (9z,12z)-9,12-octadecadienoate, (d) 9,12-Octadecadienoic acid (z,z), (e) Ethyl oleate, (f) Cis-vaccenic acid.

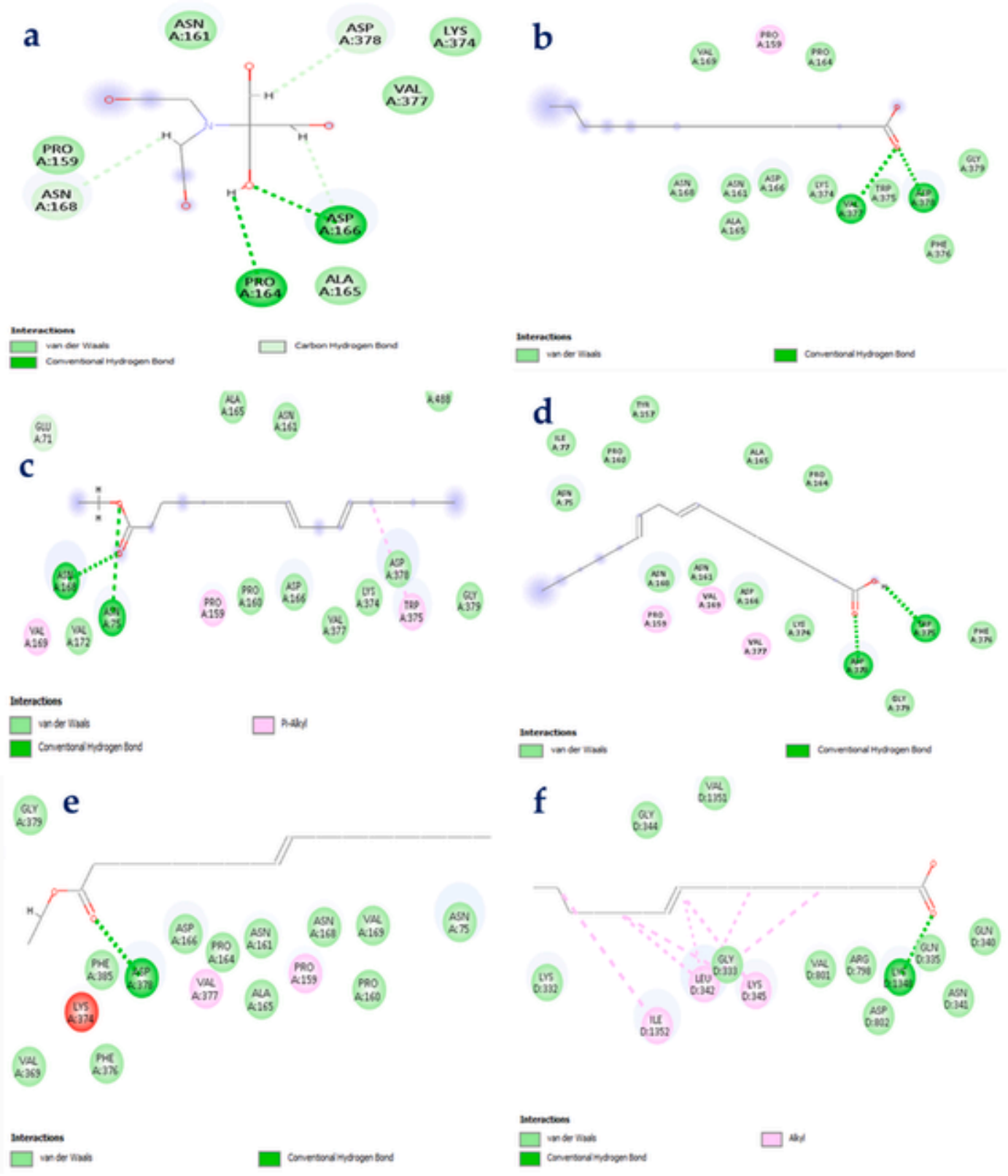

Fig. 10. 2D interaction diagrams of different compounds bound to phosphoglucomutase enzyme in *Salmonella* sp. (a) ligand, (b) Hexadecanoic acid, (c) Ethyl (9z,12z)-9,12-octadecadienoate, (d) 9,12-Octadecadienoic acid (z,z), (e) Ethyl oleate, (f) Cis-vaccenic acid.

Fig. 11. 2D interaction diagrams of different compounds bound to BlaR sensor domain in *Staphylococcus aureus*. (a) ligand, (b) Hexadecanoic acid, (c) Ethyl (9z,12z)-9,12-octadecadienoate, (d) 9,12-Octadecadienoic acid (z,z), (e) Ethyl oleate, (f) Cis-vaccenic acid.

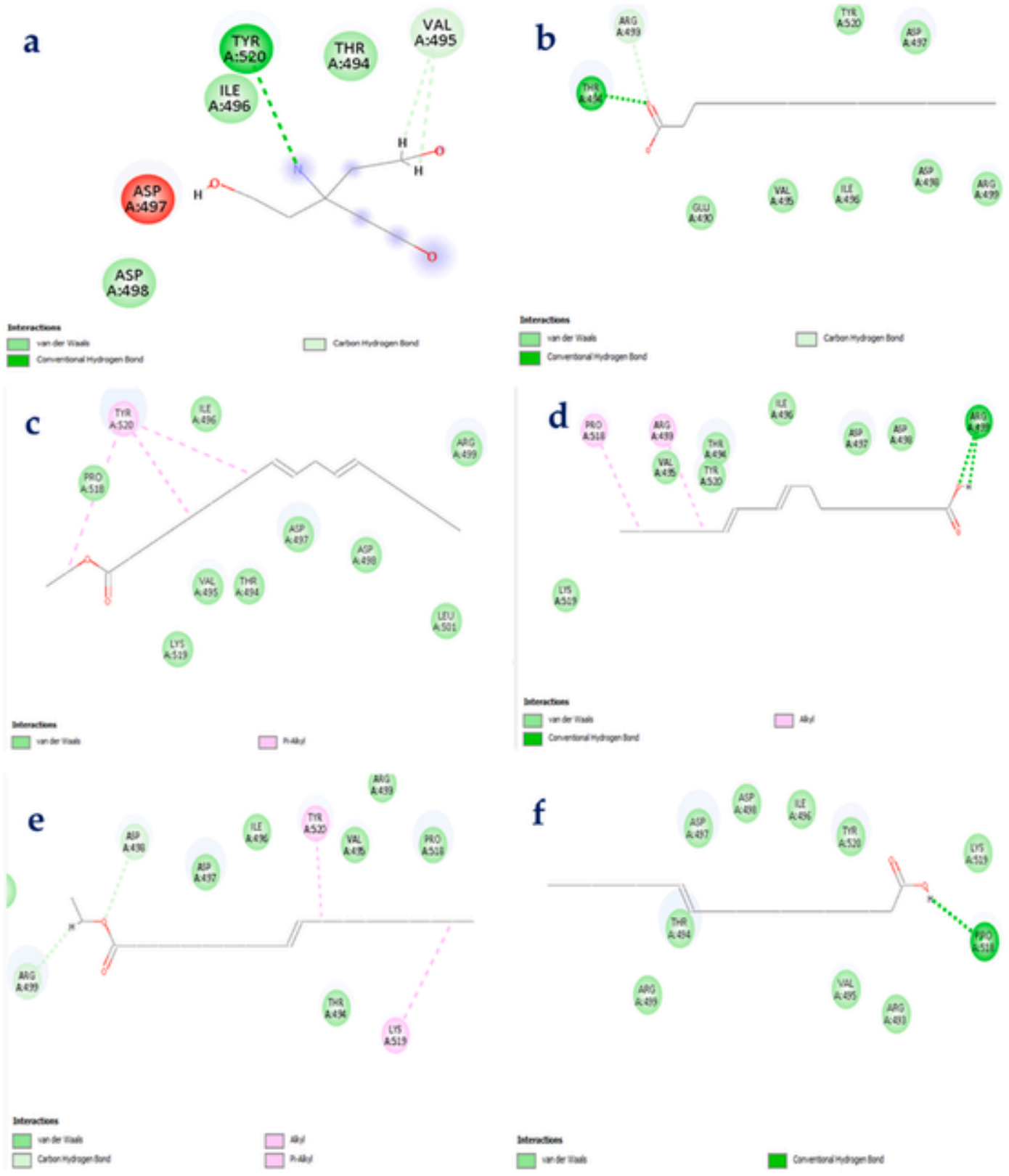

Fig. 12. 2D interaction diagrams of different compounds bound to Listeriolysin O enzyme in *Listeria monocytogenes* (a) ligand, (b) Hexadecanoic acid, (c) Ethyl (9z,12z)-9,12-octadecadienoate, (d) 9,12-Octadecadienoic acid (z,z), (e) Ethyl oleate, (f) Cis-vaccenic acid.

Declaration of competing interest

The authors declare that they have no competing interests.

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Data availability

Data will be made available on request.

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