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Acta Parasitologica (2025) 70:129
https://doi.org/10.1007/s11686-025-01066-3

RESEARCH

Efficacy of Hot *Capsicum annuum* Extracts Against the Biological Activity of *Culex pipiens* and *Musca domestica* Larvae with their Phytochemical Profiles

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Received: 4 March 2025 / Accepted: 20 May 2025

Abstract

The extensive use of chemical pesticides poses risks to the environment and human health due to the toxicity and poor biodegradability. Alternative natural practices, including the use of natural molecules, are needed to achieve more sustainable production methods to meet consumer and societal expectations. Plants contain a wide range of potential phytochemicals that target a specific target, are rapidly biodegradable, are environmentally friendly, and have a variety of therapeutic effects, making them a treasure trove of biological materials.

Methods Toxicity of hot *Capsicum annuum* extracts was tested against 3rd instar larvae of *Culex pipiens* and *Musca domestica*. LC_{50} values were determined using serial concentrations, and phytochemical profiling was performed to identify active compounds with molecular docking studies.

Results In this study, different exposure periods of various *Capsicum annuum* extracts showed high insecticidal activity against mosquito and housefly larvae. The petroleum ether (CAPe) extract from *C. annuum* was the most effective (100 MO%) against *Culex pipiens* (LC_{50} = 150.46 ppm) and *Musca domestica* larvae (LC_{50} = 0.18 mg/ml) 24 h after treatment. The LC_{50} dose of the CAPe extract led to a negative effect on the insect metabolism process represented by a significant decrease in the activity level of protease, lipase, α-amylase, and invertase enzymes in both mosquito and fly larvae. Antimicrobial activity tests showed that the CAPe extract killed all of the microbes that were tested, except for *Penicillium glabrum*. The UPLC/MS comparison of the four *Capsicum* extracts led to the possible identification of eighty metabolites. The large amounts of flavonoids, phenolic acids, and capsaicinoids were in line with what has been written about the genus *Capsicum*. Moreover, the multivariate data analysis showed that capsaicinoids, sophorolipids, triterpenoids, and phenolic acids were abundant in the methanol extract compared to flavonoids, triterpenoids, and fatty acids for the petroleum ether extract. Simultaneously, the docking results showed that all of the docked compounds could fit into the digestive lysosome active site of *M. domestica* (2H5Z).

Conclusions The major compounds in petroleum ether extract were able to interact with essential amino acids at the target sites of both *Cx. pipiens* and *M. domestica*, and therefore the insects' life-supporting functions were negatively affected. Overall, CAPe extract from *Capsicum annuum* could be a promising ecofriendly bioinsecticide.

Keywords *Capsicum annuum* · Bioinsecticides. · Biological activity · Phytochemical profiles · *Culex pipiens* · *Musca domestica*

Introduction

Vector-borne illnesses represent a significant global public health issue, especially in tropical and subtropical areas. More than three billion individuals reside in polluted environments, which poses additional risks to public health [1]. Arthropod vectors are capable of disseminating a diverse array of harmful pathogens, resulting in the transmission of various infectious diseases to both humans and animals [2].

Mosquitoes serve an important function as vectors for disease transmission [3]. They are capable of transmitting various diseases, including dengue, malaria, filariasis, yellow fever, and Japanese encephalitis. *Culex pipiens* is a vector

Extended author information available on the last page of the article

Published online: 09 June 2025

for the West Nile virus, which has been enzootic in southern and central Europe, Asia, and Africa, with significant prevalence in Egypt. The house fly serves as a significant mechanical vector for numerous diseases, with the potential to transmit nearly 100 diseases to humans and animals. This includes various bacteria, such as *Escherichia coli, Shigella* species, and *Salmonella*, as well as viruses. Human food, animal dung, garbage, and decaying animal waste attract adult houseflies [4].

Various vector control measures and monitoring techniques routinely address the threats posed by mosquitoes and flies. Medical insect control programs have progressively integrated synthetic insecticides alongside other pest management strategies. Frequent and excessive use of insecticides can have detrimental effects, disrupt the food chain, and lead to environmental pollution; moreover, vectors develop resistance to these chemicals.

To eliminate these pests, it was necessary to find an effective alternative to synthetic pesticides. These natural chemicals have been found to have many useful properties, such as responding to environmental stresses, signaling defense responses, and offering protection against pests and diseases [5]. These chemicals have been found to have a significant effect on insects, including mosquitoes and houseflies, by eliminating eggs, larvae, and adults; inhibiting egg laying, growth, and feeding; repelling mosquitoes; preventing hatching; and hindering mosquito emergence [6, 7].

Moreover, phytochemicals exert a variety of effects on insect physiology, hindering their ability to eat and restricting their growth by altering the activity of digestive enzymes that aid in food digestion. Without the activity or role of digestive enzymes, insects die because they are unable to digest their food [8–10].

In addition to the impact of plant extracts on digestive enzymes, insects (such as mosquitoes and houseflies) possess various metabolic enzymes, particularly "detoxification enzymes." These enzymes play a crucial role in the biodegradation of pesticides or foreign materials and the elimination of their insecticidal effects, which primarily consist of amino acids [6, 11]. Therefore, the impact on the amino acids or proteins of the insect is considered a key crucial factor in the elimination of insect pests. Since protein constitutes the majority of the macronutrients in edible insects, some researchers have carefully considered the role of plant extracts in disrupting the amino acids of these insects [12, 13].

Phytochemists, pharmacists, and ethnobotanists have been practicing ethnomedicine worldwide for a long time, using knowledge from traditional healers to select and test new medicinal plants [14]. Communities have traditionally used plants as mosquito repellents due to their affordability, availability, and renewable nature. Plant-derived chemicals are generally considered safer for the environment than their synthetic counterparts because they are part of the ecosystem [15]. Commercial production of many other medicines from other plants is currently underway [14, 16]. Every day, researchers discover chemicals with broad efficacy against many disease vectors and parasites, both in killing larvae and repelling adults [17, 18]. The global increase in malaria incidence and drug failure rates necessitates the search for alternative antimalarial drugs. One important strategy to reduce outdoor mosquito bites is to control the malaria vector through the development of plant-based mosquito repellents [19].

Capsicum annuum L., commonly referred to as chili pepper, is a member of the Solanaceae family and is native to tropical America. Chili peppers represent a significant category of vegetable and spice crops grown globally. Thailand, India, China, Pakistan, and Peru represent the foremost producers of dried chili worldwide [20]. Food additives frequently use chili peppers to impart a hot and pungent flavor, enjoying global popularity. Capsaicinoids, unique to the *Capsicum* genus, are responsible for the intense heat or burning sensation associated with chili peppers. The fruits' placenta biosynthesizes these compounds through the condensation of vanillylamine and medium-chain-length fatty acids [21]. Capsaicin, dihydrocapsaicin, nordihydrocapsaicin, homocapsaicin, and homodihydrocapsaicin are the main chemicals that make up capsaicinoids [22].

Capsaicinoids are responsible for the pungency, which is proportional to the total concentrations of the several vanillyl amides that are referred to as capsaicinoids. Likewise, dihydrocapsaicin (8-methyl-N-vanillyl-nonanamide) and capsaicin (trans-8-methyl-N-vanillyl-6-nonenamide) are found in most foods. They are responsible for about 90% of the heat sensation. There are other smaller capsaicinoids found in peppers besides these two main ones. These include nordihydrocapsaicin, norcapsaicin, homocapsaicin I and II, homodihydrocapsaicin I and II, nornorcapsaicin, nornornorcapsaicin, and nonivamide [23]. The relative concentrations of these analogues vary with taxa and genotype. The interest in these compounds extends far beyond their roles as flavor ingredients in food; they also have medical, toxicological, and forensic implications [24]. Capsaicinoids are well-known for their pharmacological properties, such as their ability to stop cells from migrating and multiplying, cause organisms to die, and protect against mutations and tumors. Aside from that, they're known for killing germs and protecting cells from damage, relieving pain, and changing the way nerve cells respond to pain and inflammation. These substances are also talked about as a means of controlling obesity, and capsaicin is currently used to treat psoriasis, diabetic neuropathy, osteoarthritis, and post-herpetic neuralgia. The food industry, medicine, and defensive sprays have extensively researched capsaicinoid compounds due to their characteristics and current use [25]. Researchers have

documented various methods for the isolation and analysis of these secondary metabolites [26]. The main parts of capsaicinoids are capsaicin and dihydrocapsaicin. They are lipophilic and are very good at activating TRPV1 (transient receptor potential cation channel subfamily V member 1). One difference is that they have two bonds on the side carbonic chain. Commercial creams and lotions used to treat peripheral neuropathic pain contain these substances. However, these medications have serious side effects, like pungency and a local burning sensation, when taken in such formulations, which lowers treatment efficacy and adherence [27].

The present study investigated the effect of different solvent plant extracts of *C. annuum* L. on toxicity, digestive enzymatic activities, and the midgut cells of *Musca domestica* 3rd larval instar. Furthermore, molecular docking studies were performed on an essential fatty acid binding protein (FABP) present in insect muscle (pdb code: 2FLJ) as well as a digestive lysozyme present in *Musca domestica* (pdb code: 2H5Z). Interestingly, the results were able to expect the binding modes of the docked active ingredients at the active sites and deduce the possible mechanism for the insecticidal activity observed practically.

Material and Methods

Plant Collection and Extracts

Fruits of hot Sudanese peppers or *Capsicum annuum,* was purchased from the Nefertari Company (Cairo, Egypt) for natural plant oils, medicinal herbs and cosmetics. The collected plant was then identified and authenticated by Dr. Trease Labib, a plant taxonomy consultant at the Egyptian Ministry of Agriculture, and a voucher specimen was deposited at the herbarium of the Pharmacognosy Department, Faculty of Pharmacy, Ain Shams University, under the code (PHG-P-CA-526). About 50 g from the dried hot peppers were soaked separately in 70% methanol (CAM), 50% acetone (CAA), and in 100% petroleum ether (CAPe) then filtered and the excess solvent was evaporated using Rotavap®. The resulting extracts were weighed and recorded as: methanol extract (CAM, 18 g), acetone extract (CAA, 15 g), petroleum ether extract (CAPe, 22 g). The prepared extracts were dried till no residual solvent and kept in freezer for further use.

Insect Larvicidal Assay

Mosquito Colony

Culex pipiens mosquito larvae were obtained from the Medical Entomology Division, Entomology Department, Faculty of Science, Benha University, Egypt, under laboratory conditions (27 ± 2 °C, 75–80% RH, and light period 12:12 h (L/day). Larvae were reared in enamel dishes measuring 25 × 20 × 10 cm with 2 L of dechlorinated water and fed with fish food (Tetramin®) and ground dog biscuits every other day, with observation of water content and growth of larvae and avoiding the formation of a gelatinous layer on the surface that hinders larval respiration and growth. Adults were provided with 8–10% sucrose solution as a food source. For current and future scientific experiments, adults and larvae were kept under the same laboratory conditions [28].

Housefly Colony

Adult houseflies were collected from the vegetable market in Benha, Qalyubiya, Egypt. They were then placed in 40 × 30 × 30 cm^3 wooden cages with wire tops and kept at room temperature (28–30 °C) in the insect rearing laboratory, Medical Entomology Division, Department of Entomology, Faculty of Science, Benha University. Cotton wool absorbed a mixture of 10% syrup and 10% milk, which formed their diet. Besides, 300 g of mackerels were carefully cooked in a tray measuring 18 × 25 × 9 cm^3 with a mixture of dry bread and mashed potatoes, which created an ideal environment for houseflies to feed and lay their eggs [29].

Larvicidal Bioassays

Culex pipiens: Plant extracts were tested according to Larvicides [30] to see if they might effectively control *Cx. pipiens* third larval instar. A glass beaker containing 250 ml of different concentrations (62.5, 125, 250, 500, 1000, and 1500 ppm) with twenty-five mosquito larvae were added (dipping technique). The control groups were treated with water alone, without the addition of any plant extracts. The experiments were conducted three times. The mortality rates of *Cx. pipiens* larvae were recorded after 24 and 48 h after treatment (PT) at 27 ± 2 °C and 70–80% RH. The group without treatment received only distilled water as a control.

Musca domestica: Bioassays were performed to determine the effects of plant extracts on fly larvae using the contact method, placing the larvae in a treated culture medium [31]. We placed 25 early third-instar larvae in small paper cups (5 cm in diameter and 7 cm high) filled with 5 g of rearing medium. The cups were then treated with 0.05, 0.1, 0.2, 0.5, 1.0, and 2.5 mg/mL plant extracts. Untreated groups were treated with dechlorinated water only. The treated and untreated cups were covered with a cotton cloth tied with a rubber band to prevent larvae from escaping. The experiment was repeated three times. Dead larvae were counted after 24, and 48 h, and then 3 g of sawdust was added to each Petri dish for pupation.

Digestive Enzyme Assays

Protease

In this assay, β-casein was used as a substrate. If protease digests casein, the amino acid tyrosine is liberated along with other peptide fragments. Folin's reagent reacts with free tyrosine to generate a blue-colored product, which is quantifiable and measured as an absorbance value on the spectrophotometer at 660 nm. A tyrosine standard calibration curve is constructed to determine the amount of tyrosine released after the proteolytic activity. A series of tyrosine standard solutions at different concentrations (5–50 µg/mL) were prepared from the 0.18 mg/mL L-tyrosine stock solution with deionized water.

Lipase Activity

The lipase activities were assayed as described by [32]. The standard reaction mixture (10 mM 2,3-dimercapto-1-propanol tributyrate (DMPTB), 40 mM 5,5-dithiobis(2-nitrobenzoic acid) (DTNB), 0.5 Ethylenediaminetetraacetic acid, 10% TritonX-100, and1MTris-Cl, pH 7.5) was prepared in a microcentrifuge tube. Microplate wells were filled with 180 L of this mixture, and 20 L (10 g) of the enzyme samples were added to each well. The reaction was incubated at 37 °C for 30 min and the absorbance was measured at 405 nm using a Flex Station III microplate reader (Molecular Devices, Sunnyvale, CA, USA). We used a blank that contained no DMPTB. In the DMPTB–DTNB method, free thiol groups generated by the lipase hydrolysis of DMPTB reduce DTNB to create a yellow color. Three samples were analyzed for each experimental point. The assay was read to an endpoint and the molar extinction coefficient of DTNB 13.6 M1 cm 1 was used for calculations.

Hydrolyzing Enzymes

The method used to determine the digestion of trehalose, starch, and sucrose by amylase and invertase enzymes, respectively, was similar to that described by [33]. The free aldehydic group of glucose formed after trehalose, starch, and sucrose digestion was determined using 3, 5-dinitrosalicylic acid reagent. 0.2 ml of 4% sucrose (substrate), 0.1 ml of phosphate buffer (pH 5.4), and 0.2 ml of larval homogenate made up the invertase reaction mixture. 0.2 ml of 2% starch (substrate), 0.160 ml of phosphate buffer (pH 5.4), and 0.2 ml of larval homogenate. The dinitrosalicylic acid reagent was made by dissolving 1 g of 3,5-dinitrosalicylic acid in 20 ml of 2N NaOH and 50 ml of distilled water. Potassium sodium tartrate (30 g) was added, and magnetic stirring continued until a clear solution was obtained. Distilled water was then added to bring the final volume to 100 mL. All test tubes were incubated at 37 °C for exactly 60 min. 0.8 ml of 3,5-dinitrosalicylic acid reagent was then added. The reaction mixture was heated for 5 min. at 100 °C in a boiling water bath followed by immediate cooling in an ice bath. The optical density (O.D.) of the produced color is measured at 550 nm using a spectrophotometer. The enzymatic activity was expressed as mg glucose released/g body weight/min.

Antimicrobial Assay

The agar-well diffusion method: The agar well diffusion method is widely used to evaluate the antimicrobial activity of plants or microbial extracts. In the same way that the disk-diffusion method is used, a volume of the microbial inoculum is spread over the whole surface of the agar plate to add bacteria. Then, a sterile cork borer or tip is used to make a 6 mm hole in the well. The well is then filled with 100 mL of the antimicrobial agent or extract solution at the concentration that was chosen. Next, the test microorganism determines the suitable conditions for incubating the agar plates. The antimicrobial agent diffuses in the agar medium and inhibits the growth of the microbial strain tested [34]. After incubation times of 16 to 24 h for bacteria and 48 h for fungi, the resulting inhibition zone diameters (in mm) surrounding the wells should be measured to the nearest whole millimeter at the point at which there is a prominent reduction in growth. The microbial strains tested in this study are obtained from Thermo Fisher Specialty Diagnostics Ltd, Hampshire, UK.

Phytochemical Identification

UPLC/MS Analysis

UPLC-ESI–MS (+ve & –ve ion modes) were performed using a XEVO TQD triple quadrupole instrument, Waters Corporation, Milford, MA01757 U.S.A., mass spectrometer using the method adopted from Elhawary et al. [35] and Yagi et al.[36]. UPLC-ESI–MS for both positive and negative ions. The acquisition modes were performed using a Waters Corporation, Milford, XEVO TQD triple quadruple instrument. The instrument used for the acquisition was a Waters Corporation, Milford, XEVO TQD triple quadruple, model MA01757, located in the USA. Ten microliters of the material were injected into a UPLC apparatus equipped with a C-18 reverse phase column (BEH's Accuracy UPLC, 2.1 × 50 mm column; 1.7 µm particle size) in order to separate it. The sample solution (100 µg/mL) was prepared. Prior to injection, the gas was released by sonication using a membrane-filtered HPLC-grade methanol disc filter (0.2 µm) disc, and the results were then examined using LC–ESI–MS/MS. Eluents A and B are acidified with 0.1% formic acid and 0.1% formic acid, respectively, in H_2O and

MeOH. There are two eluents in the gradient mobile phase. Elution occurred at a flow rate of 0.2 mL/min; 10% B was produced between 0 and 5 min, 30% between 5 and 15 min, 70% between 15 and 22 min, 90% between 22 and 25 min, and 100% between 25 and 29 min. The negative ion mode study was conducted using the following parameters: source temperature 150 °C, cone voltage 30 eV, capillary voltage 3 kV, desolvation temperature 440 °C, cone gas flow 50 L/h, and desolvation gas flow 900 L/h [37]. Mass spectra were recorded in Electrospray ionization (ESI) (+ ve and -ve ion modes) (*m/z* 100–1000) using Masslynx 4.1 software, and tentative identification was done by comparing their retention times (R_t), mass spectra, and fragmentation patterns with reported data [38].

GC/MS Analysis

The biochemical studies of the plant fruits acetone and aqueous extracts using Thermo Scientific Trace GC Ultra/ISQ Single Quadrupole MS and TG-5MS fused silica capillary columns, measuring 0.1 mm, 0.251 mm, and 30 m in length. The process was conducted utilizing an electronic ionizer with an ionization energy of 70 eV. Helium gas was employed as the carrier gas at a flow rate of 1 ml/min. The MS transmission line and injector were both calibrated to 280 °C. The oven was preheated to 35 °C, subsequently raised to 150 °C at a rate of 7 °C per minute, then to 270 °C at a pace of 5 °C per minute (with a 2-min pause), and finally to 310 °C at a rate of 3.5 °C per minute (maintained for 10 min). A relative peak area was utilized to quantify all identified components. The identification of the substances was achieved by comparing their retention periods and mass spectra with data from the NIST and Willy libraries on the GC/MS equipment. Identification was conducted utilizing the composite spectrum of user-generated reference libraries. Single-ion chromatographic reconstructions were conducted to assess peak uniformity. Co-chromatographic analysis of reference chemicals was employed whenever feasible to validate GC retention durations.

Multivariate Data Analysis Through Clustered Heatmap

A clustered heat map was built using NCSS. 12® software with Euclidean distance and the unweighted pair group method [35].

Molecular Docking Studies

All the docking studies in the current study were performed using Accelrys® Discovery Studio (2.5.5) software (Accelrys Inc., San Diego, CA, USA), at the Faculty of Pharmacy, Ain Shams University, Cairo, Egypt. Noteworthy, the crystallized 3D structure of the fatty acid binding protein (FABP) of *Cx. pipiens* does not exist in the protein data bank (http://www.rcsb.org). However, the crystal structure of the fatty acid binding protein (FABP) present in locust flight muscle was used instead. This structure has been used as an alternative structure for *Cx. pipiens* [39–43]. On the other hand, the crystal structure of a digestive lysosomal enzyme present in *Musca domestica* was available on the protein data bank (pdb code: 2H5Z) and was used in the presented docking studies. The crystal structure of the locust muscle fatty acid binding protein bound to oleate (pdb code: 2FLJ) and the digestive lysosomal enzyme in *Musca domestica* bound to chitotetraose (pdb code: 2H5Z) were downloaded from the protein data bank website. Afterwards, hydrogen atoms were added to the downloaded structures, followed by the creation of fixed atom constraints and then energy minimization using the energy minimization protocol. The active compounds present in the petroleum ether extract as major components were subjected to docking at the active site of both targets mentioned above using the C-DOCKER protocol, and all binding interactions were compared to those observed in the original ligands bound to the downloaded crystal structures. Also, C-DOCKER interaction energies of the docked compounds were compared relative to those of original ligands co-crystallized with their targets.

Statistical Analysis

The data were analyzed using SPSS V23 software from IBM in the USA. It was used to do probit analyses to find the lethal concentration (LC) values and the one-way analysis of variance (ANOVA) (Post Hoc/Turkey's HSD test). The significant levels were set at $P < 0.05$. Mass spectra were recorded in Electrospray ionization (ESI) (+ ve and -–ve ion modes) (m/z 100–1000) using Masslynx 4.1 software, and tentative identification was done by comparing their retention times (R_t), mass spectra, and fragmentation patterns with reported data [38].

Results

Insect Larvicidal Activity

Mosquito Larvicidal Activity

This research assessed *Capsicum annuum* extracts on 3rd instar larvae of *Culex pipiens*. All plant extracts evaluated in this study showed significant insecticidal efficacy against mosquito larvae, *Cx. pipiens*, following various exposure times. The mortality rate (MO%) of *Cx. pipiens* 24 h post-treatments with 1500 ppm plant extracts of *C. annuum* (CAM, CAA, and CAPe) and *C. annuum* aqueous extracts was 98.67, 100, 100, and 85.33%, respectively (Table 1).

Table 1 Efficacy of *Capsicum annuum* extracts on *Culex pipiens* larval mortality, 24 and 48 h post-treatment

Time (h)	Solvent	Concentration (ppm)						
		0	62.5	125	250	500	1000	1500
24	Methanol	0.0 ± 0^{aG}	14.6 ± 1.33^{cF}	26.6 ± 1.33^{cE}	36.0 ± 2.31^{cD}	62.6 ± 2.67^{cC}	82.6 ± 3.53^{bB}	98.6 ± 1.33^{aA}
	Acetone	0.0 ± 0^{aF}	18.6 ± 1.33^{bE}	32.0 ± 2.31^{bD}	56.0 ± 2.31^{bC}	85.3 ± 1.33^{bB}	100.0 ± 0.00^{aA}	100.0 ± 0.00^{aA}
	Petroleum	0.0 ± 0^{aE}	21.3 ± 1.33^{aD}	36.02.31^{aC}	60.0 ± 2.31^{aB}	92.0 ± 2.51^{aA}	100.0 ± 0.00^{aA}	100.0 ± 0.00^{aA}
	Aqueous	0.0 ± 0^{aG}	10.6 ± 1.33^{dF}	18.6 ± 1.33^{dE}	30.6 ± 2.67^{dD}	50.6 ± 2.67^{dC}	68.0 ± 2.31^{cB}	85.3 ± 3.53^{bA}
48	Methanol	1.3 ± 1.33^{aF}	29.3 ± 1.33^{cE}	42.6 ± 1.33^{bD}	65.3 ± 1.33^{cC}	92.0 ± 2.31^{bB}	100.0 ± 0.00^{aA}	100.0 ± 0.00^{aA}
	Acetone	1.3 ± 1.33^{aE}	33.3 ± 1.33^{bD}	58.6 ± 1.33^{aC}	88.0 ± 2.31^{bB}	100.0 ± 0.00^{aA}	100.0 ± 0.00^{aA}	100.0 ± 0.00^{aA}
	Petroleum	1.3 ± 1.33^{aE}	36.0 ± 2.31^{aD}	60.0 ± 0.00^{aC}	94.6 ± 1.33^{aB}	100.0 ± 0.00^{aA}	100.0 ± 0.00^{aA}	100.0 ± 0.00^{aA}
	Aqueous	1.3 ± 1.33^{aG}	20.0 ± 2.31^{dF}	34.6 ± 3.53^{cE}	50.6 ± 2.67^{dD}	72.0 ± 2.31^{cC}	85.3 ± 3.53^{bB}	97.3 ± 1.33^{aA}

a, b & c: There is no significant difference ($P > 0.05$) between any two means for each time, within the same column have the same superscript letter; A, B & C: There is no significant difference ($P > 0.05$) between any two means, within the same row have the same superscript letter

The LC_{50} (50%, median lethal concentration) values were 288.42, 183.62, 150.46, and 450.47 ppm, respectively. The highest larval mortality was recorded after 48 h of post-treatments, where the mortality reached 100% in CAM, CAA, and CAPe and 97.3% in aqueous extracts (CAAq), respectively, at 1500 ppm (Table 1). Based on lethal concentrations, CAPe (50% of the median lethal concentration) seemed to be the most effective against *Cx. pipiens* larvae ($LC_{50} = 86.33$ ppm), followed by CAA ($LC_{50} = 96.40$), CAM ($LC_{50} = 136.32$) and CAAa ($LC_{50} = 226.43$ ppm), 48 h post-treatment (Table 2).

Housefly Larvicidal Activity

All tested plant extracts had significantly higher mortality rates than the controls. The percentage of dead larvae in plant extract-treated contact medium at a high concentration of 2.5 mg/ml was 92, 97.3, 100, and 80% for CAM, CAA, CAPe, and CAAa, respectively, compared to 0.0% in control groups 24 h post-treatments (Table 3). After 48 h post-treatment, the CAM, CAA, and CAPe extracts had the highest larval mortality, reaching 100%, while the CAAa extract mortality reached 80%. The LC_{50} values for CAM, CAA, CAPe, and CAAa were 0.41, 0.24, 0.18, and 0.72 mg/ml for 24 h and 0.19, 0.14, 0.09, and 0.46 mg/ml, 48 h post-treatments, respectively. These values showed that CAPe was more toxic to house fly larvae than other plant extracts (Table 4).

Digestive Enzyme Assays

The secondary metabolites in the *C. annuum* extract significantly inhibited the activity of all four digestive enzymes (protease, lipase, α-amylase, and invertase) in both *M. domestica* and *Cx. pipiens* larvae (Table 5). In *M. domestica* 3rd larval instar, the inferences of the present study elucidated that the LC_{50} dose of the tested plant extract induced a marked reduction in the level of protease, lipase, α-amylase, and invertase activity (272.20 ± 27.91, 10.92 ± 1.0 U/gm, 7.71 ± 0.78, and 6.59 ± 1.34 µg/min/gm/larva, respectively) in accordance with control (678.84 ± 128.67, 17.72 ± 0.31, 11.57 ± 0.50 U/gm, and 11.136 ± 0.84 µg/min/gm/larva, respectively). The same results are achieved in *Cx. pipiens* larvae, where the level of protease, lipase, α-amylase, and invertase activity (368.24 ± 3.86, 18.77 ± 0.68 U/gm, 10.37 ± 0.07, and 2.52 ± 0.03 µg/min/gm/larva, respectively) are also inhibited compared to control (517.56 ± 2.61, 27.91 ± 0.36 U/gm, 16.35 ± 0.19, and 3.28 ± 0.02 µg/min/

Table 2 Lethal concentrations (ppm) of *Capsicum annuum* extracts on *Culex pipiens* larval mortality, 24 and 48 h post-treatment

Time (h)	Solvent	LC_{50} (Low-Up.)	LC_{90} (Low-Up.)	LC_{95} (Low-Up.)	Slope ± SE	Chi (Sig.)
24	Methanol	288.42 (189.48–424.06)	1353.16 (1013.39–2870.73)	2097.26 (1572.75–5116.56)	1.901 ± 0.136	14.128 (0.006)
	Acetone	183.62 (160.12–209.67)	658.31 (536.40–858.83)	945.40 (739.47–1308.86)	2.311 ± 0.186	5.532 (0.236)
	Petroleum	150.46 (116.72–198.75)	501.09 (388.59–791.73)	711.60 (549.89–1210.41)	2.525 ± 0.182	9.848 (0.043)
	Aqueous	450.47 (383.94–532.52)	2810.93 (2067.73–4221.93)	4723.49 (3262.75–7755.30)	1.611 ± 0.128	3.672 (0.452)
48	Methanol	136.32 (95.44–179.86)	480.67 (369.56–790.75)	687.06 (522.60–1248.87)	2.341 ± 0.178	9.961 (0.041)
	Acetone	96.40 (83.63–109.01)	264.55 (226.50–323.77)	352.20 (291.99–453.55)	2.923 ± 0.265	3.537 (0.472)
	Petroleum	86.33 (72.93–103.42)	236.01 (212.47–300.73)	319.20 (272.99–414.80)	2.953 ± 0.276	4.113 (0.390)
	Aqueous	226.43 (188.92–261.31)	1246.21 (972.44–1718.55)	2028.59 (1498.33–3027.01)	1.716 ± 0.135	2.642 (0.619)

Table 3 Efficacy of *Capsicum annuum* extracts on *Musca domestica* larval mortality, 24 and 48 h post-treatment

Time (h)	Treatment	Concentration						
		0	0.05	0.1	0.2	0.5	1.0	2.5
24	Methanol	0.0 ± 00^{aG}	8.0 ± 2.31^{cF}	17.3 ± 1.33^{cE}	26.6 ± 1.33^{cD}	52.0 ± 2.31^{cC}	77.3 ± 1.33^{cB}	92.0 ± 4.00^{bA}
	Acetone	0.0 ± 00^{aG}	12.0 ± 2.31^{bF}	24.0 ± 2.31^{bE}	45.3 ± 4.81^{bD}	69.3 ± 3.53^{bC}	88.0 ± 4.00^{bB}	97.3 ± 2.67^{aA}
	Petroleum	0.0 ± 00^{aG}	16.0 ± 2.31^{aF}	28.0 ± 4.00^{aE}	52.0 ± 6.11^{aD}	74.6 ± 2.67^{aC}	90.6 ± 1.33^{aB}	100.0 ± 0.00^{aA}
	Aqueous	0.0 ± 00^{aF}	6.6 ± 1.33^{cE}	8.0 ± 2.31^{dE}	20.0 ± 0.00^{dD}	34.6 ± 2.67^{dC}	62.6 ± 4.81^{dB}	80.0 ± 0.00^{cA}
48	Methanol	0.0 ± 00^{aG}	13.3 ± 3.53^{cF}	28.0 ± 0.00^{cE}	45.3 ± 3.53^{cD}	84.0 ± 4.62^{cC}	92.0 ± 2.31^{bB}	100.0 ± 0.00^{aA}
	Acetone	1.3 ± 1.33^{aF}	18.6 ± 3.53^{bE}	32.0 ± 4.62^{bD}	60.0 ± 2.31^{bC}	92.0 ± 2.31^{bB}	100.0 ± 0.00^{aA}	100.0 ± 0.00^{aA}
	Petroleum	1.3 ± 1.33^{aE}	29.3 ± 2.67^{aD}	45.3 ± 3.53^{aC}	82.6 ± 3.53^{aB}	100.0 ± 0.00^{aA}	100.0 ± 0.00^{aA}	100.0 ± 0.00^{aA}
	Aqueous	1.3 ± 1.33^{aG}	9.3 ± 1.33^{dF}	13.3 ± 1.33^{dE}	32.0 ± 4.00^{dD}	48.0 ± 4.62^{dC}	70.6 ± 1.33^{cB}	90.6 ± 1.33^{bA}

a, b & c: There is no significant difference ($P > 0.05$) between any two means for each time, within the same column have the same superscript letter; A, B & C: There is no significant difference ($P > 0.05$) between any two means, within the same row have the same superscript letter

Table 4 Lethal concentrations (ppm) of *Capsicum annuum* extracts on *Musca domestica* larval mortality, 24 and 48 h post-treatment

Time (h)	Treatment	LC_{50} (Low-Up.)	LC_{90} (Low-Up.)	LC_{95} (Low-Up.)	Slope ± SE	Chi (Sig.)
24	Methanol	0.41 (0.35–0.48)	2.35 (1.79–3.32)	3.86 (2.79–5.85)	1.686 ± 0.118	3.566 (0.467)
	Acetone	0.24 (0.20–0.28)	1.21 (0.96–1.63)	1.93 (1.46–2.75)	1.809 ± 0.126	0.697 (0.951)
	Petroleum	0.18 (0.16–0.22)	0.93 (0.74–1.23)	1.45 (1.11–2.04)	1.874 ± 0.133	3.062 (0.547)
	Aqueous	0.72 (0.60–0.88)	5.13 (3.58–8.24)	8.96 (5.86–15.79)	1.500 ± 0.116	4.330 (0.363)
48	Methanol	0.19 (0.17–0.22)	0.78 (0.64–1.01)	1.17 (0.92–1.59)	2.095 ± 0.144	3.758 (0.439)
	Acetone	0.14 (0.13–0.16)	0.46 (0.39–0.59)	0.65 (0.53–0.86)	2.487 ± 0184	5.357 (0.252)
	Petroleum	0.09 (0.08–0.10)	0.26 (0.22–0.33)	0.35 (0.29–0.47)	2.829 ± 0.250	7.192 (0.126)
	Aqueous	0.46 (0.39–0.54)	2.86 (2.14–4.22)	4.87 (3.42–7.70)	1.601 ± 0.115	3.134 (0.535)

Table 5 The effect of *Capsicum annuum* extracts on digestive enzymes activity of *Culex pipiens* and *Musca domestica* larvae at 24 h post-treatment

Sr. no	Analysis	*Cx. pipiens*		*M. domestica*	
		Control	Tested	Control	Tested
1	Protease (U/gm)	517.56 ± 2.61	368.24* ± 3.86	678.84 ± 128.67	272.20* ± 27.91
2	Lipase (U/gm)	27.91 ± 0.36	18.77* ± 0.68	17.72 ± 0.31	10.92* ± 1.09
3	α-amylase (µg/min/gm	16.35 ± 0.19	10.37* ± 0.07	11.57 ± 0.50	7.71* ± 0.78
4	Invertase (µg/min/gm	3.28 ± 0.02	2.52* ± 0.03	11.13 ± 0.84	6.59* ± 1.34

Values are mean ± SE (standard error). Tukey's test and significance analyzed data at $*p < 0.05$ compared with control

gm/larva, respectively). The lower activity of enzymes suggests that the extract may mess up these larvae's digestive systems, which could make it harder for them to absorb nutrients and grow (Table 5).

Antimicrobial Activity

To investigate the antimicrobial activity of the *Capsicum annuum* petroleum ether extracts, six different pathogenic microbes were used in the antimicrobial activity analysis. The diameters of the inhibition zones served to express the results of antimicrobial activity. The information in Table 6 shows that the petroleum ether extract from *C. annuum*

Table 6 Antimicrobial activities of *Capsicum annuum* petroleum ether extract against several pathogenic microorganisms

Microorganism	Inhibition zone (mm)	
	Treated	Control
Bacillus subtilis (ATCC 6633)	21 ± 10	28 ± 10
Staphylococcus aureus (ATCC 6538)	23 ± 10	23 ± 10
Klebsiella pneumoniae (ATCC 13883)	21 ± 10	21 ± 10
Salmonella typhi (ATCC 6539)	19 ± 10	19 ± 10
Candida albicans (ATCC 10221)	23 ± 20	23 ± 10
Penicillium glabrum (Op 694,171)	NA	30 ± 10

killed all of the tested microbes except for the *Penicillium glabrum* microbe. Divergence in the extent of the inhibition area among the diverse groups of microbes, either bacteria or fungi, can be relatively disclosed. The highest inhibition zone was observed for *Staphylococcus aureus* (23), *Candida albicans* (23), *Klebsiella pneumoniae* (21), and *Salmonella typhi* (19). *Bacillus subtilis*, on the other hand, showed the lowest inhibition zone, measuring 21 mm. No antibacterial activity was recorded for *Penicillium glabrum* (Fig. 1).

Phytochemical Analysis of Plant Extracts

UPLC/MS Tentative Identification of Metabolites from Different Solvent Extracts of *Capsicum annum* Fruits

UPLC/MS in ESI positive and negative ionization modes was selected for the analysis and profiling of four extracts prepared from the fruits of *Capsicum annum*. The solvents utilized in the extraction were petroleum ether (Pet. ether), acetone (Acet.), methanol (Meth.), and water (Aq.). The metabolic content of the four extracts was revealed and compared through UPLC/MS, where their spatial component profiles were recorded in Table S1 and the different classes of identified secondary metabolites were illustrated in Fig. 2. The comparative UPLC/MS analysis of the four extracts led to the tentative identification of eighty metabolites. The abundant presence of flavonoids, phenolic acids, and capsaicinoids aligned with the reported literature on the genus *Capsicum.* Moreover, the negative ion mode was abundant in flavonoids and phenolic acids, while the positive ion mode primarily identified capsaicinoids. Capsaicinoids are a group of compounds responsible for the spicy, pungent taste of hot chili peppers (*C. annuum* and *C. frutescens*). Chemically, we classify them as a unique type of alkaloid, possessing one or more nitrogen atoms in their skeleton.

Among the main identified metabolites, certain components were denoted as major, and they will be discussed in detail as follows. Four peaks were detected in both ESI modes, and they belonged to capsaicinoids (Table S1). These peaks were found at *m/z* 205 (278), 304 (306), 306 (308), and 295. Based on this, they were likely identified as nornorcapsaicin [16], capsaicin [7], dihydrocapsaicin, dihydro [7, 17], and nornordihydrocapsaicin [16]. Similarly, three flavonoid peaks were assigned to luteolin-hexuronide

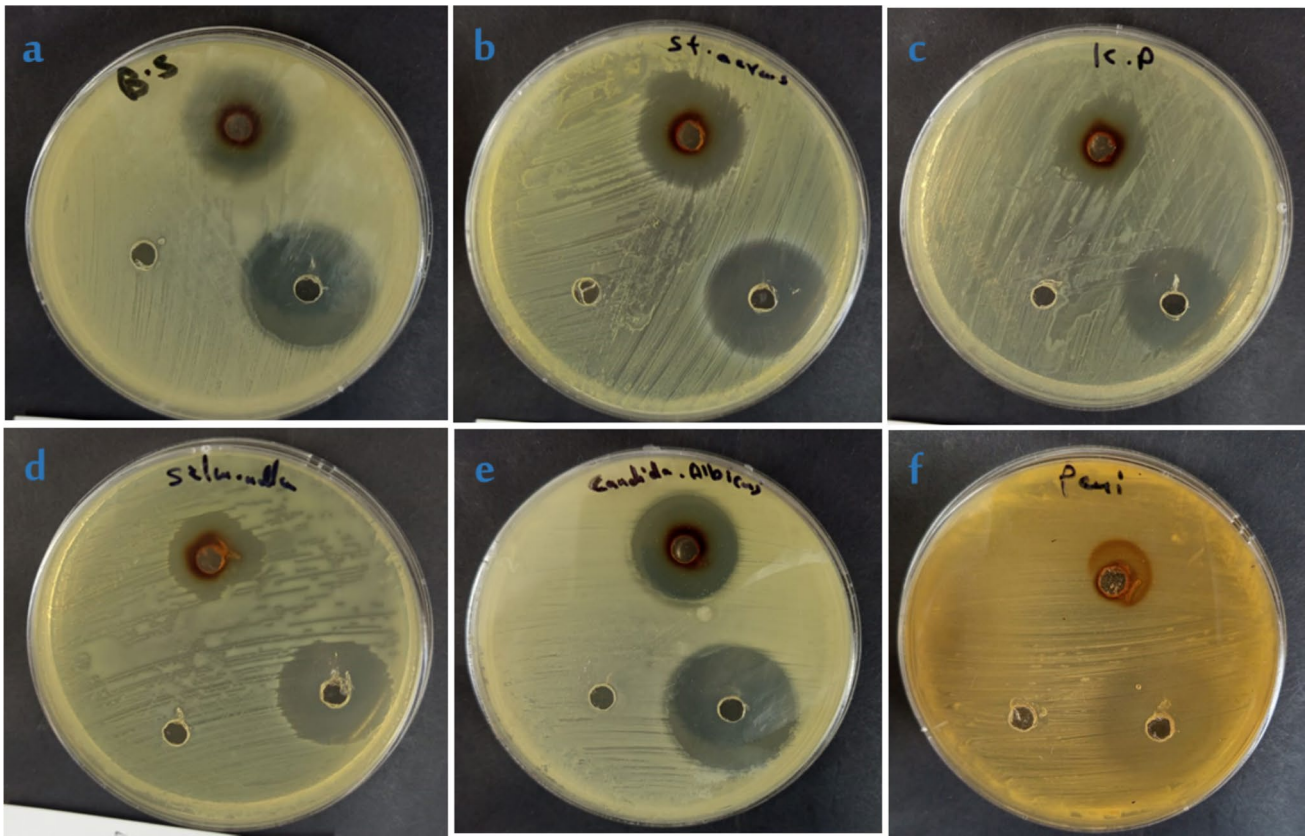

Fig. 1 Antimicrobial activities of petroleum ether extract of *Capsicum annuum* against **(a)** *Bacillus subtilis* (ATCC 6633), **(b)** *Staphylococcus aureus* (ATCC 6538), **(c)** *Klebsiella pneumoniae* (ATCC 13883), **(d)** *Salmonella typhi* (ATCC 6539), **(e)** *Candida albicans* (ATCC 10221), and **(f)** *Penicillium glabrum* (Op 694,171) microorganisms

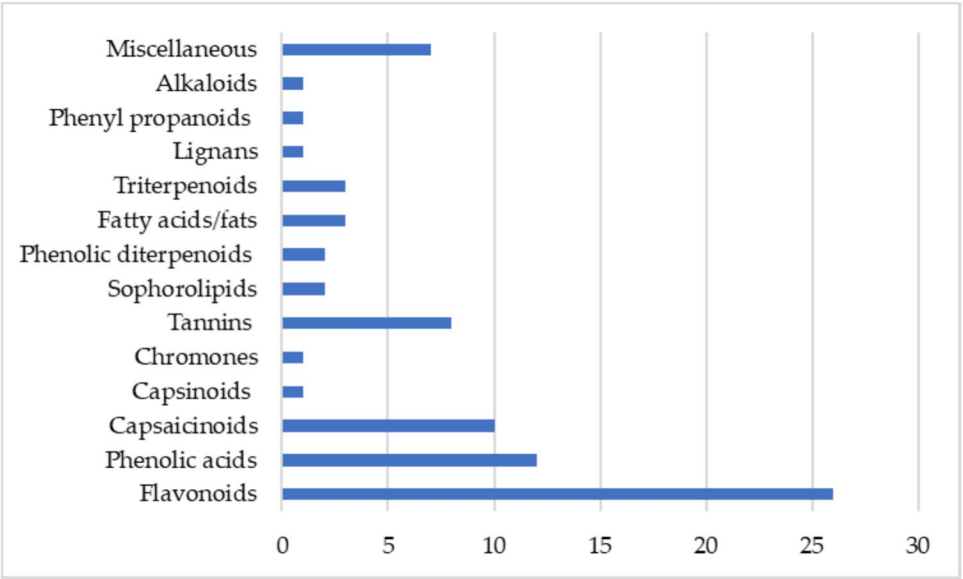

Fig. 2 Bar chart showing the classes of tentatively identified components from different extracts of *Capsicum annum* fruit

derivative [*m/z* 513 (521), abundant in the methanol and water extracts] [13, 18], puerarin [*m/z* 415 (417), only detected in the methanol extract] [9], and quercetin-galloyl-pentoside [*m/z* 587, acetone and methanol extracts] [13, 19, 20]. In addition to that, compounds 2, 16, and 24 presented deprotonated peaks at *m/z* 294, 311, and 353 in ESI negative mode, and they were assigned to the phenolic acids: benzoic acid derivative (8.16% methanol extract only) [21], caftaric acid (3.87% acetone extract) [9], and chlorogenic acid (11.62% aqueous extract) [8, 11, 13, 17, 22, 23], respectively. Capsaicinoids primarily identify the genus *Capsicum*, but certain other phytochemical classes also contribute to its metabolic profile. The metabolic profile of chili is made up of different parts, such as sophorolipids, methoxyphenols, lignans, lignins, triterpenoids, and more. Table S1 shows the names and amounts of lignan, methoxyphenol, medioresinol, enterolactone, and sophorolipids I and IV (*m/z* 659 and 643, respectively) [17], nordnordihydrocapsiate (methoxyphenol, *m/z* 293, 10.65% acetone extract) [17], and medioresinol (lignan, *m/z* 387(343) [21].

Multivariate Data Analysis Using Clustered Heat Maps

A clustered heat map was constructed for the predominant metabolites in the positive mode and another one for the abundant components in the negative mode, where the components with % composition ≥ 3 were selected. The resulting heat maps were presented in Figs. 3 and 4 for components from the positive and negative ion modes, respectively. As shown in Fig. 3, the most abundant components were in red while the least abundant were in green. In the methanol extract, the main substances were capsaicinoids, flavonoids, and lignin. In the petroleum ether extract, the main substances were flavonoids, tannins, and phenolic acids. Moreover, capsaicinoids and triterpenoids predominated in the aqueous extract, and one phenolic acid appeared as the main component of the acetone extract. On the other hand, the major classes and metabolite distribution changed dramatically when the negative ion mode components were selected for the heat map in Fig. 4. Capsaicinoids, sophorolipids, triterpenoids, and phenolic acids were abundant in the methanol extract compared to flavonoids, triterpenoids, and fatty acids for the petroleum ether extract. In addition to capsaicinoids, flavonoids, and phenolic acids for the aqueous extract and triterpenoids, flavonoids, phenolic acids, and vitamin E derivatives for the acetone extract (Fig. 4).

GC/MS Analysis

Metabolomic analysis of the four *Capsicum annum* extracts, petroleum ether, acetone, methanol, and aqueous, was conducted using GC–MS analysis. The analysis allowed the identification of large numbers of compounds, including terpenes, esters, fatty acids, alkanes, and phenols, in the fruit of *Capsicum annuum* extracts with a high probability (Table 7). The main components in CAPe are 9-octadecenoic acid (*Z*)-, 2,3-dihydroxypropyl ester (32.95%), 2-hydroxy-3-[(9*E*)-9-octadecenoyloxy]propyl (9*E*)-9-octadecenoate (24.39%), hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester ($C_{19}H_{38}O_4$) (12.9%), 9-octadecenoic acid, (e) (12.51), and hexadecanoic acid (7.07%). The most abundant phytocompounds identified in CAA were 9-octadecenoic acid (*Z*)-, 2,3-dihydroxypropyl ester (22.85%), 11-octadecenoic acid, methyl ester ($C_{19}H_{36}O_2$) (12.46%), 2-hydroxy-3-[(9*E*)-9-octadecenoyl oxy]propyl (9*E*)-9-octadecen oate (11.07), hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester

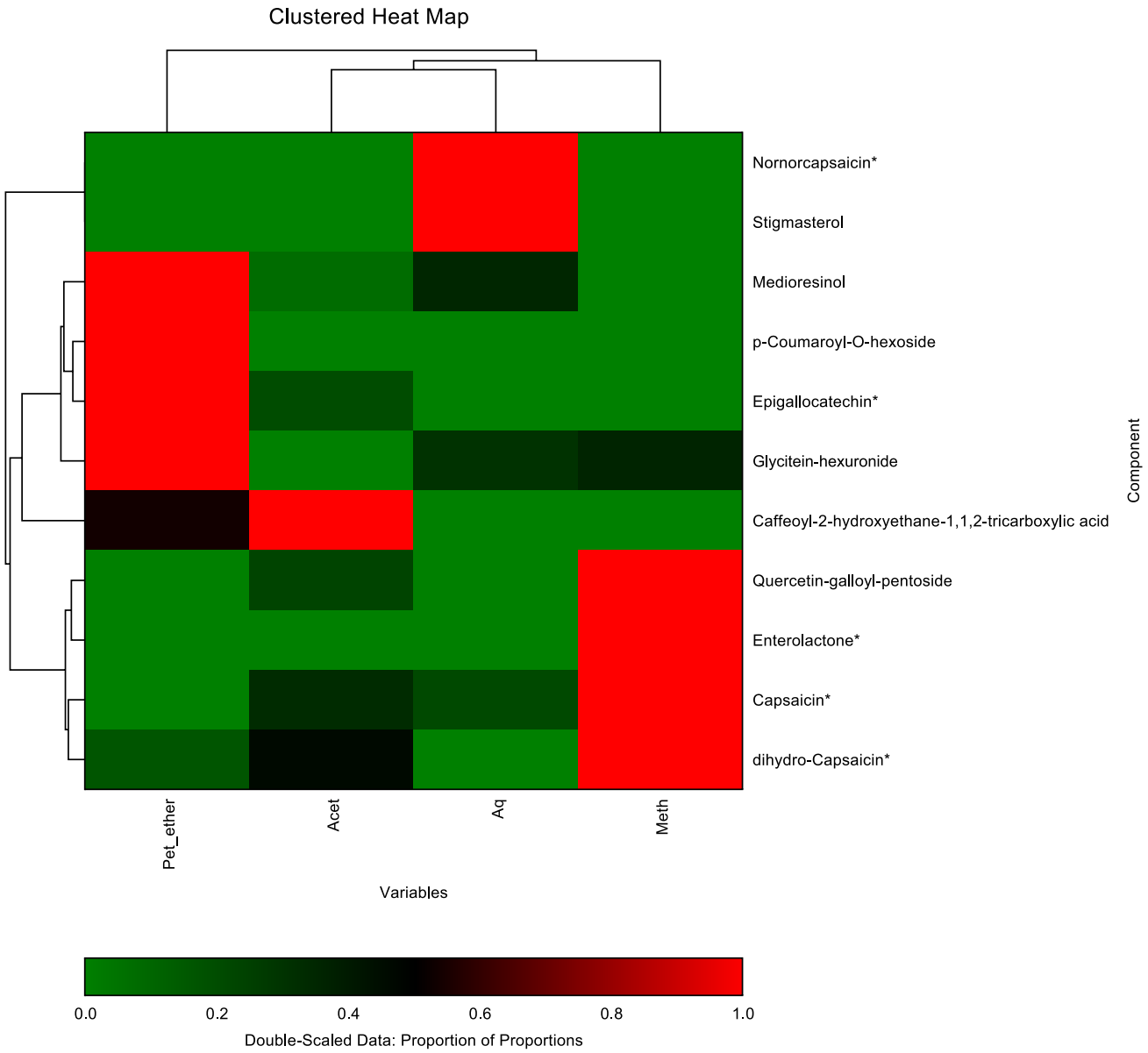

Fig. 3 Clustered heat map for the main identified components from *Capsicum annum* fruit extracts in positive ESI mode [heat map was constructed using Euclidean distance and the unweighted group method, components with % composition ≥ 3 were included, Pet. ether; petroleum ether extract, Acet.; acetone extract, Meth.; methanol extract and Aq.; aqueous extract]

(8.71%), 9-octadecenoic acid, (2-phenyl-1,3-dio xolan-4-yl) methyl ester, *cis* (8.59%) Dihydrocapsaicin (8.13%), and finally capsaicin (7.13%). Our results showed that 9-octadecenoic acid (z)-, 2,3-dihydroxypropyl ester was the common component among the four extracts.

Molecular Docking Studies

In the current research, initial investigation of the binding interactions of oleate and chitotetraose at their binding sites were initially investigated (Figs. 5, 6). It was observed that oleate at FABP active site shows two essential interactions with two amino acids (Arg 108 and Arg 128) (Fig. 5). On the other hand, chitotetraose showed hydrogen bond interactions with Asn 57, Trp 61, Gln 100 and Ala 104 in addition to sigma-pi interaction with Tyr 60 (Fig. 6). Binding interactions of the best docked active ingredients in petroleum ether extract at FABP active site (Figs. 7, 8) and digestive lysosomal active site (Figs. 9, 10, 11, 12) were investigated. Also, C-DOCKER interaction energies of all docked compounds were recorded and compared to the values of reference compounds oleate and chitotetraose against their specified targets (Table 8).

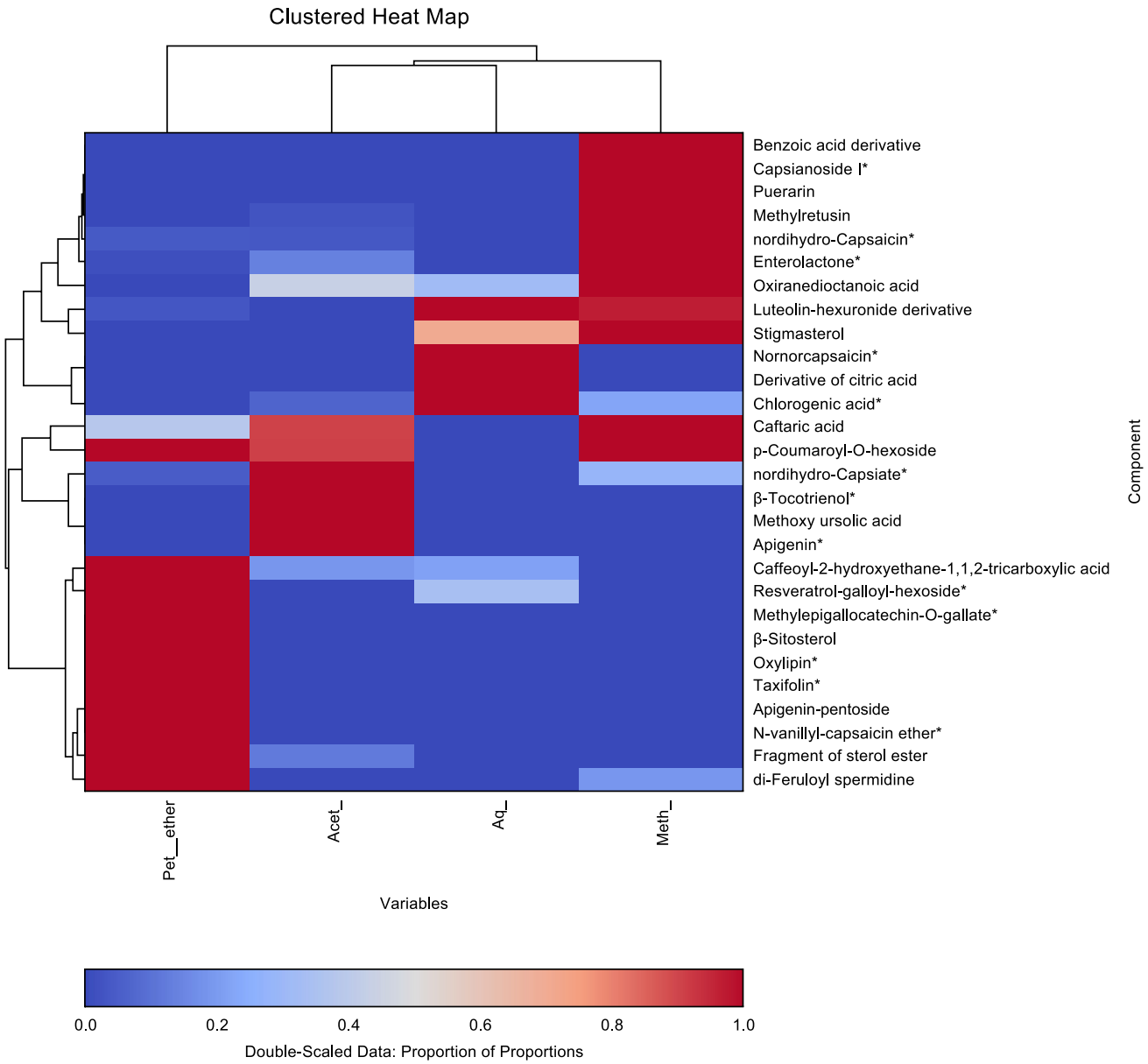

Fig. 4 Clustered heat map for the main identified components from *Capsicum annum* extracts in negative ESI mode [heat map was constructed using Euclidean distance and the unweighted group method, components with % composition ≥ 3 were included, Pet. ether; petroleum ether extract, Acet.; acetone extract, Meth.; methanol extract and Aq.; aqueous extract]

Discussion

Naturally occurring compounds found in plant extracts and essential oils are very important. These compounds can be used safely to control pests and diseases because they are simple to get and don't harm the environment when broken down [44, 45]. Notwithstanding their advantages as pesticides, approximately 5% of global pesticides are biopesticides [46, 47]. Biopesticides are experiencing significant expansion and are projected to surpass chemical pesticides in the near future, with an average annual growth rate of 9–20% owing to their distinctive qualities that promote usage, including their environmental nontoxicity [48]. It is estimated that around 90% of pesticides are lost in their effectiveness during or post-application, necessitating the development of ecologically sustainable pesticides that are both cost-efficient and effective in achieving the desired outcomes [49].

The current study investigated the efficacy of chili pepper extract against two highly medically important disease vectors, mosquitoes and flies. It is well known that mosquitoes, along with flies, are vectors of many diseases and

Table 7 The major GC/MS identified chemical constituents of *Capsicum annum* petroleum ether, acetone, methanol and aqueous extracts

No	Compound Name	M.F	M.W	Area %			
				PE	AC	ME	AQ
1	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	270	–	5.01	–	–
2	*n*-Hexadecanoic acid	$C_{16}H_{32}O_2$	256	–	4.21	–	–
3	11-Octadecenoic acid, methyl ester	$C_{19}H_{36}O_2$	296	–	12.46	–	–
4	Heptadecanoic acid, 16-methyl-, methyl ester	$C_{19}H_{38}O_2$	298	–	1.04	–	–
5	Hexadecanoic acid, 2,3-dihydroxypropyl ester	$C_{19}H_{38}O_4$	330	–	3.37	-	-
6	*cis*-13-Octadecenoic acid	$C_{18}H_{34}O_2$	282	–	5.73	-	-
7	2-Hydroxy-3-[(9*E*)-9-octadecenoyl oxy]propyl (9*E*)-9-octadecen oate	$C_{39}H_{72}O_5$	620	–	11.07	28.21	3.73
9	Hexadecanoic acid, 2-hydroxy-1-(hy droxymethyl)et hyl ester	$C_{19}H_{38}O_4$	386	12.91	8.71	11.20	-
10	Capsaicin	$C_{18}H_{27}NO_3$	305	–	7.13		-
11	Dihydrocapsaicin	$C_{18}H_{29}NO_3$	307	–	8.13	3.74	-
12	9-octadecenoic acid, (*Z*)-, 2,3-dihydroxypropyl ester	$C_{21}H_{40}O_4$	356	32.95	22.85	32.26	28.0
13	Hexadecanoic acid, 1-(hydroxymethy l)-1,2-ethanediylester	$C_{35}H_{68}O_5$	568	–	1.70	–	3.49
14	9-Octadecenoic acid, (2-phenyl-1,3-dio xolan-4-yl)methyl ester, *cis*	$C_{28}H_{44}O_4$	444	–	8.59	–	14.32
15	2,3-dihydro-3,5-*di* hydroxy-6-methy l-4 H-pyran-4-one	$C_6H_8O_4$	144	–	–	1.28	
16	Benzothieno[2,3-*C*]quinolin-6(5 H)-o ne, 2-methoxy	$C_{16}H_{11}NO_2S$	281	–	–	0.44	
17	Oxiraneundeca noic acid, 3-pentyl-, methyl ester, *cis*	$C_{19}H_{36}O_3$	312	–	–	0.63	
18	Hexadecanoic acid	$C_{16}H_{32}O_2$	256	7.07	–	7.49	7.59
19	9(*Z*)-Octadecenoic acid	$C_{18}H_{34}O_2$	282	–	–	14.75	12.7
20	Cholestan-3-ol, 2-methylene-, (3á,5à)	C28H48O	400	0.57	–	–	–
21	pentadecanoic acid, 14-methyl-, methyl ester	$C_{17}H_{34}O_2$	270	1.60	–	–	1.96
22	*trans*-13-Octadecenoic acid, methyl ester	C16H32O2	256	3.58	–	–	–
23	9(*E*)-Octadecenoic acid,	$C_{19}H_{36}O_2$	296	12.51	–	–	–
24	9-Octadecenoic acid (*Z*)-, oxiranylmethyl ester	$C_{21}H_{38}O_3$	338	1.29	–	–	4.5
25	(6*E*)-*n*-(4-hydrox y-3-methoxybenzyl)-8-methyl-6 nonenamide	$C_{18}H_{27}NO_3$	305	3.13	–	–	–
26	2-Hydroxy-3-[(9*E*)-9-octadecenoyloxy]propyl (9*E*)-9-octadecenoate	$C_{39}H_{72}O_5$	620	24.39	–	–	–
27	10-Octadecenoic acid, methyl ester	$C_{19}H_{36}O_2$	296	–	–	–	4.48
28	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl) ethyl ester	$C_{19}H_{38}O_4$	330	–	–	–	13.41
29	9-Octadecenoic acid (*Z*)-, 2-[(trimethylsily l)oxy]-1-[[(trime thylsilyl) oxy]m ethyl]ethyl ester	$C_{27}H_{56}O_4Si_2$		–	–	–	2.33
30	Hexadecanoic acid, 1-(hydroxymethy l)-1,2-ethanediyl ester	$C_{35}H_{68}O_5$		–	–	–	3.49

PE petroleum ether, *AC* acetone, *ME* methanol, *AQ* aqueous

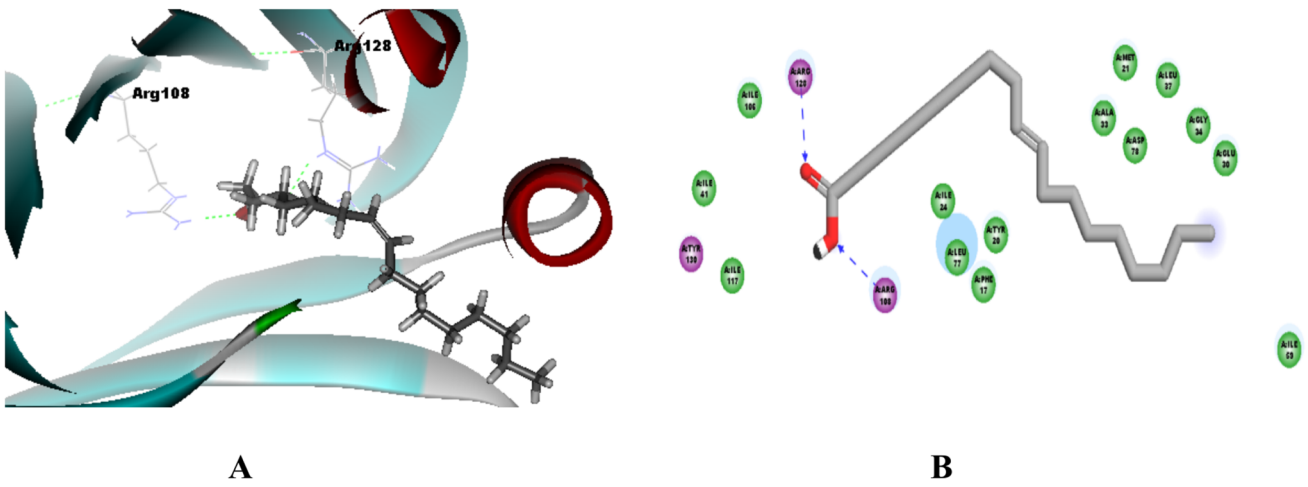

Fig. 5 Binding interactions of oleate at FABP active site. **A** 3-D representation, **B** 2-D representation

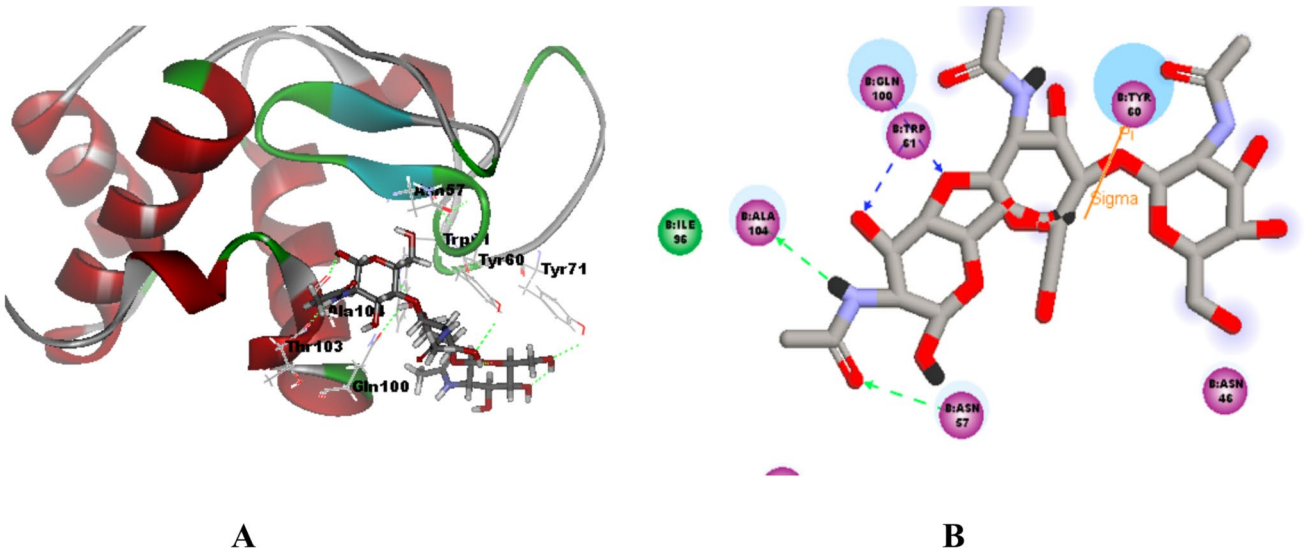

Fig. 6 Binding interactions of chitotetraose at digestive lysosome active site. **A** 3-D representation, **B** 2-D representation

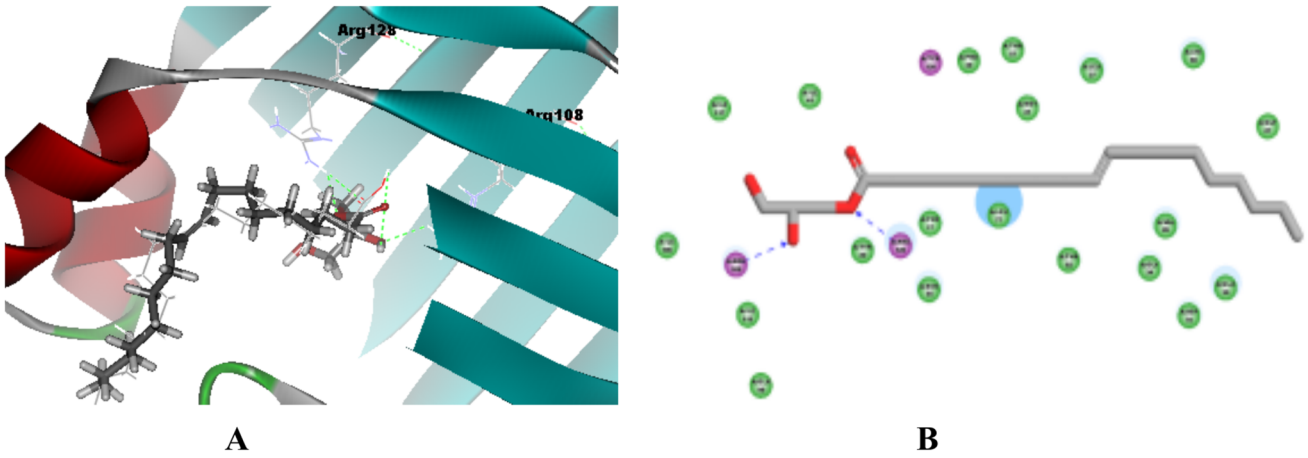

Fig. 7 Binding interactions of 9-octadecenoic acid (*Z*)-, 2,3-dihydroxypropyl ester at FABP active site. **A** 3-D representation, **B** 2-D representation

spread globally, making their control a complex matter [50, 51]. This has led many researchers to explore new natural pesticides using plants available in nearby or distant environments [39]. Our data revealed that all *C. annuum* extracts tested showed strong insecticidal effectiveness against mosquito and housefly larvae. The *C. annuum* plant extracts killed 98.7% to 100% of the mosquito larvae and 92% to 100% of the housefly larvae 24 h after treatment. *C. annuum* petroleum ether extracts (CAPe) were the most effective plant extracts against mosquito and housefly larvae. Similar to our findings, several researchers have demonstrated the apparent efficacy of *C. annuum* against the larvae and adults mosquitoes [52], which found that the ethanol extract of *C. annuum* worked well enough against *Culex quinquefasciatus* and *Anopheles stephensi*. According to the LC_{50} values, *Cx. quinquefasciatus* (LC_{50} = 0.0097) was more susceptible than *An. stephensi* (LC_{50} = 0.011).

The results of our study also confirm that the larval lethality of both species obtained after 24 and 48 h of exposure increases with increasing concentrations. These results are agreed upon by many authors [53, 54], who revealed that the larvicidal efficacy increases with increasing concentration.

The petroleum ether extract is thought to kill more larvae because it contains extra compounds, as shown by Farahat et al. [55], who discovered that tannic acids slowed the growth of *Cx. pipiens* larvae, suggesting that these chemicals can effectively get rid of pests. In addition to other secondary chemicals, the method used to extract secondary metabolic compounds from chili pepper fruits and plant parts has an effect on physiological aspects that lead to insect-killing. In

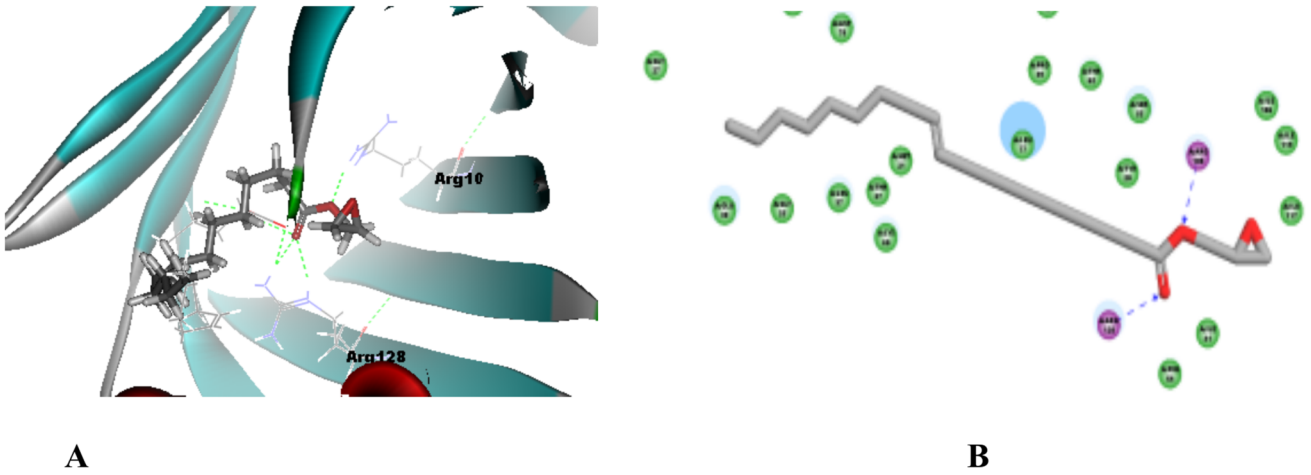

Fig. 8 Binding interactions of 9-octadecenoic acid (*Z*)-, oxiranylmethyl ester at FABP active site. **A** 3-D representation, **B** 2-D representation

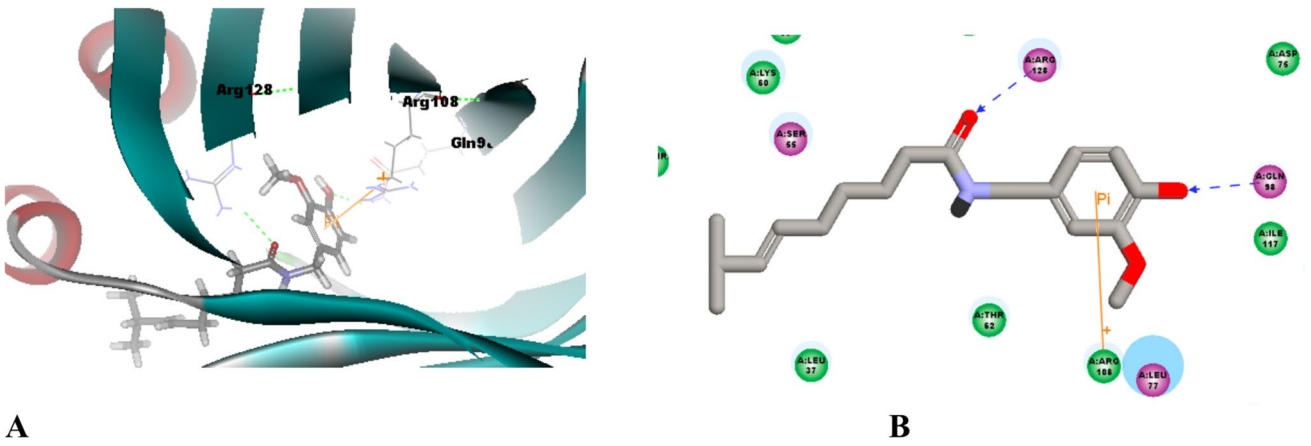

Fig. 9 Binding interactions of (6*E*)-*n*-(4-hydrox y-3-methoxybenzyl)-8-methyl-6 nonenamide at FABP active site. **A** 3-D representation, **B** 2-D representation

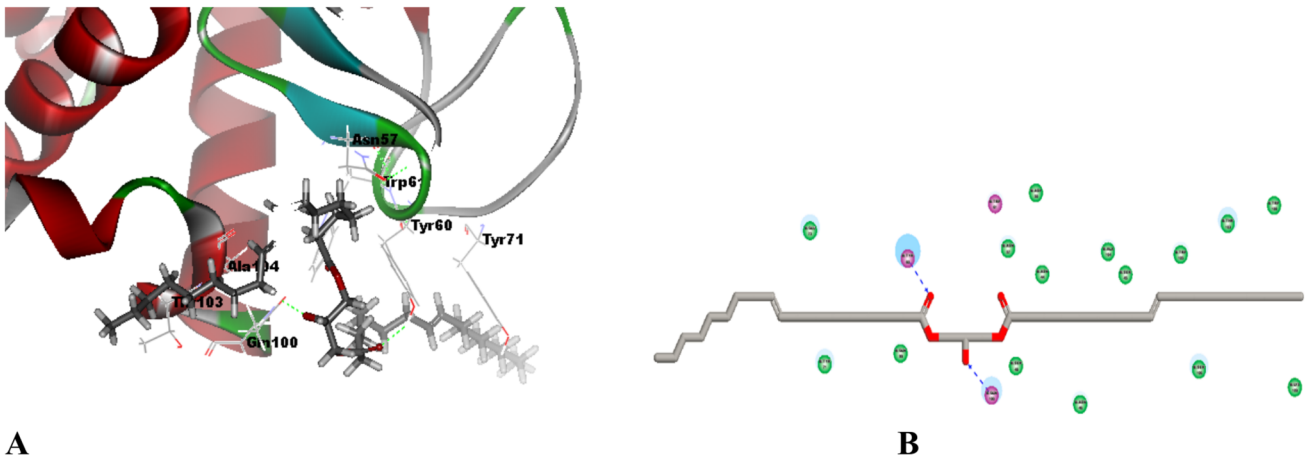

Fig. 10 Binding interactions of 2-hydroxy-3-[(9*E*)-9-octadecenoyl oxy]propyl (9*E*)-9-octadecenoate at 2H5Z active site. **A** 3-D representation, **B** 2-D representation

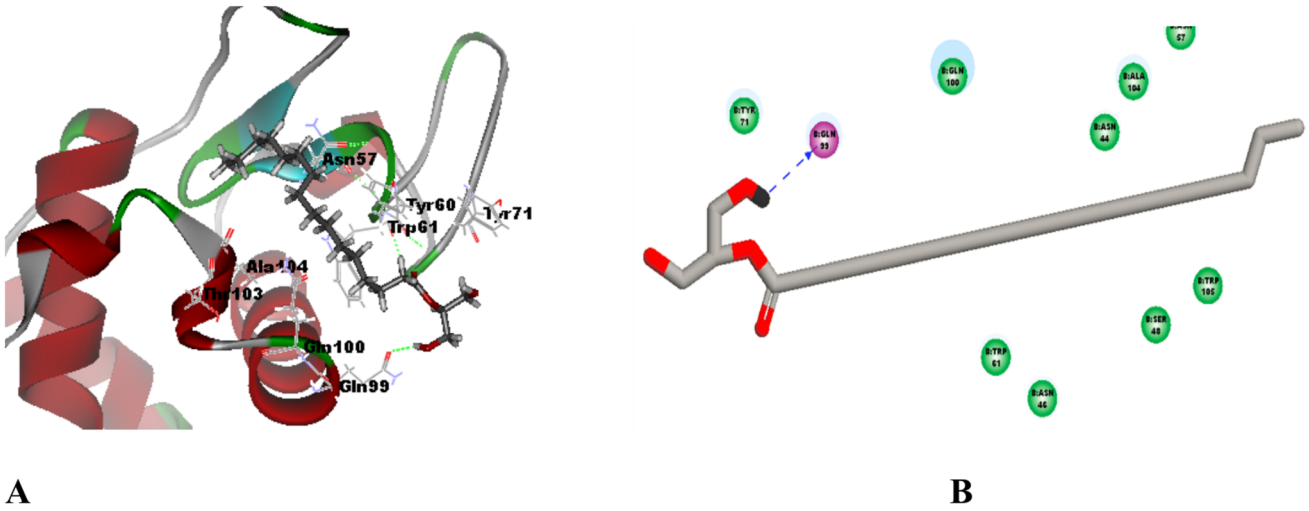

Fig. 11 Binding interactions of hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester at 2H5Z active site. **A** 3-D representation, **B** 2-D representation

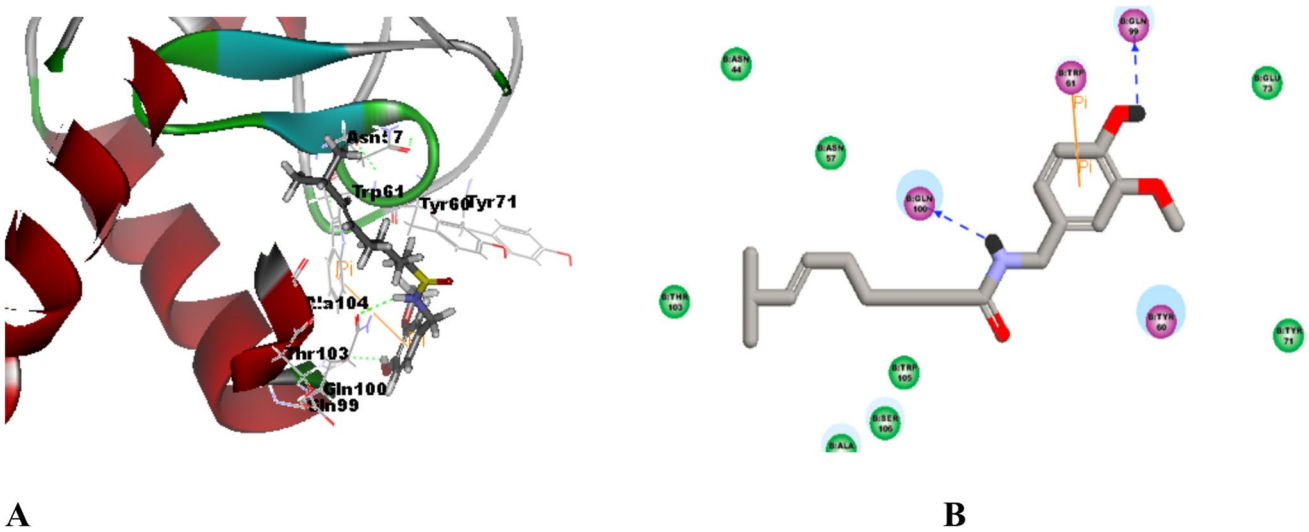

Fig. 12 Binding interactions of (6*E*)-*n*-(4-hydroxy-3-methoxybenzyl)-8-methyl-6 nonenamide at 2H5Z active site. **A** 3-D representation, **B** 2-D representation

Table 8 Docking results of all docked compounds

	CDOCKER interaction energy (-Kcal/mol)	
	2FLJ	2H5Z
9-Octadecenoic acid (*Z*)-, 2,3-dihydroxypropyl ester	47.39	38.17
2-Hydroxy-3-[(9*E*)-9-octadecenoyl oxy]propyl (9*E*)-9-octadecenoate	ND	41.73
Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester	44.48	38.36
9-Octadecenoic acid, (*E*)	38.90	35.67
(6*E*)-*n*-(4-hydroxy-3-methoxybenzyl)-8-methyl-6 nonenamide	40.23	39.37
Chitotetraose (Reference compound 2H5Z)	–	43.44
Oleate (Reference compound 2FLJ)	38.58	–

ND not determined

their study, Izah et al. [56] examined how the crude, ketone, and ethanol extracts of *Capsicum frutescens* impacted the larvae of *Anopheles gambiae*. They found that the ethanolic extract killed more larvae, indicating that this extraction method might help obtain more secondary chemicals from pepper fruits. Similarly, 3rd larval instar of *An. stephensi* and *Cx. pipiens* died when the essential oil from the leaves of *T. orientalis* was used to test it [57]. Moreover, Samuel, Oliver, Coetzee and Brooke [58] studied how piperine and ground *P. nigrum* killed *Anopheles* larvae (3rd and 4th stages) and discovered that *P. nigrum* worked much better than piperine.

It was reviewed that most of the plant medicine extracts were highly effective as mosquito larvicides and insecticides, where Pam et al. [59] looked at how well *Millettia aboensis* leaf powder killed *An. gambiae* and *Cx. quinquefasciatus* larvae, and Kholhring Lalchhandama [60] looked at how well *Millettia pachycarpa* killed *Aedes aegypti* larvae and eggs, finding that the mosquitoes died the most at the highest concentration after being exposed for the longest time. Chukwujekwu et al. [61], found similar results when they examined anti-plasmodial diterpenoids from the leaves of *H. suaveolens*.

It is noteworthy that researchers found *C. annuum* extracts to be effective against a variety of pests, including stored grain pests. Erdoğan [62] study indicated that *C. annuum* extract exhibited the most significant F1 progeny effect on wheat weevil, *Sitophilus granarius* and was the best plant among other plants in repelling this pest [63]. Olotuah [64] found when he tested *H. suaveolens* in the lab against the pests *Sitophilus oryzae*, *Sitophilus zeamais*, and *Callosobruchus maculatus* that live on stored goods. He found that the highest concentration (100 mg/ml) caused the most deaths after the full exposure time. Also, previous studies indicated that capsaicin exhibited a repellent effect on agricultural pests. de Paula Marchiori et al. [65] indicated a significant insecticidal efficacy of the aqueous extract of *C. frutescens* against pink hibiscus mealybug, *Maconellicoccus hirsutus*, as mortality rates exceeded 70% even at minimal doses of the extract. The death rate can be ascribed to the secondary chemicals found in the *C. frutescens* extract.

Digestion is an important part of the insect body and the deeper understanding of how digestive enzymes function is essential to develop methods for insect control. So, it's crucial to know how plant chemicals are processed and how insect enzymes help both to neutralize harmful plant substances and keep cells stable[66]. The results showed that the secondary substances in both *M. domestica* and *Cx. pipiens* larvae, along with the extract from *C. annuum*, significantly lowered the activity of all four digestive enzymes: lipase, α-amylase, invertase, and protease. This reduction in enzyme activity could affect the catalysis of carbohydrates [67]. The decrease in enzyme activity might be due to the harmful effects of certain plant compounds on the gut cells that make α-amylase, which could also block the sites where proteases work, stopping them from binding to their substrates [67]. Proteases in Diptera larvae (e.g., trypsin-like enzymes in *M. domestica*) function optimally in alkaline environments. Plant secondary metabolites like phenolics may acidify the midgut, destabilizing enzyme structure [68]. Compounds may suppress gene expression or disrupt zymogen activation, thereby decreasing the production of protease [69].

The greater inhibition in *M. domestica* (60% versus 29%) aligns with its need for high-protein diets (such as decaying organic matter), which demands strong proteolytic activity. In contrast, *Cx. pipiens* larvae feed on microorganisms and detritus, which may require less proteolytic specialization [70]. The reduction in enzyme activity could be a consequence of the cytotoxic effect of plant compounds on epithelial cells of the gut that are responsible for the synthesis of α-amylase [71]. Cytotoxicity appears to include membrane damage by causing coagulation of the cytoplasm [72] thereby damaging its lipid and protein contents [73] or disrupting cell membrane leading to leakage of macromolecules and ultimately cell lysis [74]. Some plant EOs are also known to form stable complexes with digestive enzymes, making dissociation difficult. Babu and Subrahmanyam [75] outlined that significant enzyme inhibition or stimulation above the control would result in metabolic imbalance, growth impairment, and housefly mortality. A decrease in such digestive enzyme's activities by the plant-derived essential oils on *M. domestica* was previously reported [75, 76]. According to Mulla and Tamhane [77] found that the extract of *C. annuum* (CanDef-20) caused a decrease in the size of larvae and pupae, slowed down their development, and greatly reduced the ability to reproduce in the pest *Helicoverpa armigera*. Different types of lipase, serine endopeptidase, glutathione S-transferase, cadherin, alkaline phosphatase, and aminopeptidases were increased as a way to help during digestion. There are more reports that also showed decreasing of digestive enzyme activities after treatment with various plants extract [78, 79].

For the antimicrobial activity test, we used six different pathogenic microbes to find out how well the *C. annuum* petroleum ether extracts killed them. The diameters of the inhibition zones served to express the results of antimicrobial activity. Our data showed that the petroleum ether extract from *C. annuum* killed most of the tested microbes, except for one. We can reasonably disclose the divergence in the extent of the inhibition area among the diverse groups of microbes, whether bacteria or fungi. The highest inhibition zone was observed for *Staphylococcus aureus*, *Candida albicans*, *Klebsiella pneumoniae*, and *Salmonella typhi*. *Bacillus subtilis*, on the other hand, showed the lowest inhibition zone, measuring 21 mm. No antibacterial activity was recorded for *Penicillium glabrum*.

Natural products constituted a sustainable source for new, potent, and eco-friendly solutions for many concurrent problems affecting the environment and human health. The current study involved preparing four extracts from the fruits of *Capsicum annum*, each with a different solvent polarity. The four extracts were analyzed using UPLC/MS in order to compare their phytochemical profiles. Eighty components were identified and quantified as detailed in the results section. The main parts of most of the extracts were flavonoids, phenolic acids, and capsaicinoids. Other related components included methoxyphenols, fatty acids, sophorolipids, triterpenoids, lignans, and lignins. Numerous published articles previously reported the isolated or tentatively identified components from the genus *Capsicum*. From various capsicum samples, capsaicinoids (nordihydrocapsaicin, capsaicin, dihydrocapsaicin, homocapsaicin, and homodihydrocapsaicin) were identified. Ethanol was used as an extracting solvent in a liquid–liquid extraction process to extract capsaicinoids. The limits of detection for each capsaicinoid were 0.15, 0.05, 0.06, 0.2, and 0.1 µg/g for nordihydrocapsaicin, capsaicin, dihydrocapsaicin, homocapsaicin, and homodihydrocapsaicin, in that order. The linear ranges were 0.5–50 µg/g, and the correlation coefficient (R2) was greater than 0.999 in the best possible test conditions [80].

Red pepper pods (*Capsicum annuum*) were used to extract carotenoids and carotenoid esters without saponification. Based on their retention durations, four non-esterified, eleven mono-, and seventeen diesters were identified from the 42 compounds found. Major and minor carotenoids and their esters were characterized using both positive and negative ion mode techniques. Most of the red pepper extracts were made up of capsanthin, which is linked to lauric, palmitic, and myristic acids. Furthermore, researchers tentatively found one zeaxanthin monoester and three b-cryptoxanthins in red pepper pods for the first time. Additionally, we distinguished the two regioisomers using the unique fragmentation patterns of capsanthin-laurate-myristate and capsanthin-myristate-palmitate [81].

Sweet and hot pepper ethanol extracts were separated into fractions with varying levels of lipophilicity. Following drying, the extracts and fractions were examined for their chemical makeup, cytotoxic activity against PC-3 and HTC-116 cells, and anti-radical activity in the DPPH radical system. Using the LC-QTOF-MS technology, a thorough qualitative examination of the fractions was carried out. The chemical makeup of pepper fractions did not always reflect their biological activity. The portion of sweet pepper that eluted with 40% methanol had the strongest antiradical activity. A comparable fraction from hot pepper had the highest total concentration of phenolic chemicals and the largest cytotoxic effect on the PC-3 tumor line. Of the 53 chemicals found by the LC–MS study, four were unique to hot pepper and six were unique to sweet pepper [82]. The phenol profiling of 28 chill genotypes using LC–MS in conjunction with ACCMS 1 (P1) and ACS 18–08 (P2) was the subject of another investigation. The samples of chili leaves were taken three months following transplantation. The 20 phenolic acids used as standards were ferulic acid, cinnamic acid, syringaldehyde, fraxetin, 4-hydroxy cinnamaldehyde, aminobenzoic acid, catechin hydrate, sinapic acid, pyrocatechol, methylumbelliferone, umbelliferon, quercetin, coumaric acid, caffeic acid, chlorogenic acid, ferulic acid, cinnamic acid, syringaldehyde, fraxetin, 4-hydroxy cinnamaldehyde, aminobenzoic acid, catechin hydrate, sinapic acid, and epigallocatechin gallate. Six of these phenolic acids were found in detectable amounts in the chili leaves: quercetin, caffeic acid, ferulic acid, sinapic acid, gallic acid, and epigallocatechin gallate. The current study found that the amounts of ferulic acid, caffeic acid, epigallocatechin gallate, sinapic acid, and gallic acid were lower in parent ACCMS 1 than in parent ACS 18–08. The amounts were 0.0075 ppm for ferulic acid, 0.0740 ppm for caffeic acid, 0.0119 ppm for epigallocatechin gallate, 0.0083 ppm for gallic acid, and 0.1470 ppm for each. Quercetin, a single phenolic acid, was only found in ACCMS 1 and was absent from ACS 18–08 [83].

The American serpentine leaf miner fly, *Liriomyza trifolii* (Burgess), is a well-known and dangerous pest worldwide. The Solanaceae are among the 21 plant groups that this insect attacks. *Capsicum annuum* (Solanaceae), a mature sweet pepper, exhibits resistance to this leaf miner fly. The sweet pepper leaf's ovipositional deterrent against the fly species is the basis for this resistance. The ovipositional deterrent against this insect species was found to be luteolin-7-*O*-*beta*-D-apiofuranosyl-(1–>2)-*beta*-D-glucopyranoside, which was isolated *via* bioassay-guided fractionation. This substance completely prevented female *L. trifolii* from depositing their eggs on a host plant leaf [84]. It's important to note that luteolin-pentosyl-hexoside was one of the main substances we think we identified in this study. It may have something to do with the fact that *C. annum* fruit extracts were able to kill insects.

Clustered heat maps are one of the advanced illustrative methods that represent the analytical data in a more defined and easily visualized form. The results of the UPLC/MS analysis were constructed into two heat maps for the main metabolites in the positive and negative ion modes. There are two different heat maps that show the main parts in the two modes. This is because some parts of the capsicum, like capsaicinoids, are better absorbed in the positive mode, while flavonoids and phenolic acids are more common in the negative mode. Thus, the phytochemical profile was different for each extract upon selecting its key components in each mode.

In the presented research work, the molecular docking studies aimed to rationalize the insecticide activity observed

practically. The investigation of the interaction diagrams of the docked compounds at the oleate binding site (2FLJ) showed that all of them (except for (6*E*)-*n*-(4-hydroxy-3-methoxybenzyl)-8-methyl-6-nonenamide) could bind with amino acids Arg 108 and Arg 128, just like the oleate reference compound. Noteworthy, (6*E*)-*n*-(4-hydroxy-3-methoxybenzyl)-8-methyl-6-nonenamide showed an altered interaction pattern where it exhibited pi-pi stacking with Arg 108, hydrogen bonding with Arg 128, and additional hydrogen bonding with Gln 98.

Moreover, the docking results showed that all docked compounds exhibited comparable CDOCKER interaction energies compared to those of reference compounds on their specified targets. Among the docked compounds, three compounds showed better C-DOCKER interaction energies than oleate, whereas 9-octadecenoic acid showed comparable results to that of oleate. However, only 2-hydroxy-3-[(9*E*)-9-octadecenoyl oxy]propyl (9*E*)-9-octadecenoate failed to fit into the oleate active site due to its bulky structure.

The docking results also showed that all the compounds could fit into the digestive lysosome active site of *M. domestica* (2H5Z). The interaction energies of all the compounds were similar to those seen in the reference compound Chitotetraose. Interestingly, out of all the docked compounds, (6*E*)-*n*-(4-hydroxy-3-methoxybenzyl)-8-methyl-6-nonenamide had the best interaction mode. It exhibited a hydrogen bond with Gln 100, pi-pi stacking with Trp 61, and another hydrogen bond with Gln 99. Based on what we know so far, the petroleum ether extract may kill insects because of the interaction of its main components with essential amino acids at the target sites of both *Cx. pipiens* and *M. domestica*. This lets them do their job and affect the insects' vital functions that they need to stay alive.

Conclusion

Chili pepper (*Capsicum annuum* L.) is a popular food and medicine plant. It contains many bioactive phytochemicals that could be used against insects, microbes, and other medicinal aspects. All four plant extracts were tested against mosquito and housefly larvae and showed significant insecticidal and antimicrobial activities. Moreover, the results reveal CAPe and CAM extracts are clearly toxic to insects. The findings about how effectively the extracts kill mosquito and fly larvae match the chemical details obtained from UPLC/MS, GC–MS, and molecular docking studies. The plant analyses showed that there are high levels of flavonoids, phenolic acids, and capsaicinoids, which matches what is already known about the *Capsicum* genus. Furthermore, the negative ion mode had many flavonoids and phenolic acids, while the positive ion mode mostly had capsaicinoids. Capsaicinoids, sophorolipids, triterpenoids, and phenolic acids were present in larger quantities in the methanol extract than in the petroleum ether extract, which contained flavonoids, triterpenoids, and fatty acids. This finding suggests that more research should be done on the antibacterial, anti-inflammatory, and anticancer properties of the bioactive parts of *C. annuum* fruits. More research should be done on how these leaf extracts might work against other medically important insects or through bioassays to kill or scare them away. Perhaps they could replace the harmful or toxic chemicals used to kill or scare medical or veterinary pests.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s11686-025-01066-3.

Author Contributions Conceptualization, MMB, EAE, AHA, RMM, MHA, HSG, AS, MEG, HFA; methodology, MMB, EAE, AHA, RMM, MHA, HSG, AS, MEG, HFA; validation, EAE, MMB, RMM and MEG; formal analysis, MMB, EAE, AHA, RMM, MHA, HSG, AS, MEG, HFA; investigation, MMB, EAE, AHA, RMM, MHA, HSG, AS, MEG, HFA; resources, MMB, EAE, AHA, RMM, MHA, HSG, AS, MEG, HFA; data curation, MMB, EAE, AHA, RMM, MHA, HSG, AS, MEG, HFA; writing—original draft preparation, MMB, EAE, AHA, RMM, MHA, HSG, AS, MEG, HFA; writing—review and editing, MMB, EAE, AHA, RMM, MHA, HSG, AS, MEG, HFA and; All authors have read and agreed to the published version of the manuscript.

Funding None.

Availability of data and materials The datasets used and/or analyzed during the current study are available from the corresponding author upon reasonable request.

Declarations

Competing interests The authors declare no competing interests.

Ethical statement The Ethics Committee of the Faculty of Science, Benha University, approved the work protocol (Code: BUFS-REC-2025–339 Ent). The study was conducted in accordance with local legislation and institutional requirements, and we confirm that a commitment to institutional guidelines for invertebrate experiments would be appreciated.

Consent for publication Not applicable.

References

1. Cuthbert RN, Darriet F, Chabrerie O, Lenoir J, Courchamp F, Claeys C, Robert V, Jourdain F, Ulmer R, Diagne C (2023) Invasive hematophagous arthropods and associated diseases in a changing world. Parasit Vectors 16(1):291
2. Socha W, Kwasnik M, Larska M, Rola J, Rozek W (2022) Vector-borne viral diseases as a current threat for human and animal health—One Health perspective. J Clin Med 11(11):3026
3. Nebbak A, Almeras L, Parola P, Bitam I (2022) Mosquito vectors (Diptera: Culicidae) and mosquito-borne diseases in North Africa. Insects 13(10):962

4. Mohamed D, Darwish A, Aboelela H, Baz M, Moharam A (2024) Biocontrol efficacy of some essential oils as larvicides and inhibitors of the emergence of adult *Musca domestica*. Parasitologists United J 17(2):105–111
5. Baz MM, Alfagham AT, Al-Shuraym LA, Moharam AF (2024) Efficacy and Comparative Toxicity of Phytochemical Compounds Extracted from Aromatic Perennial Trees and Herbs against Vector Borne *Culex pipiens* (Diptera: Culicidae) and *Hyalomma dromedarii* (Acari: Ixodidae) as Green Insecticides. Pakistan Veterinary Journal 44(1).
6. Şengül Demirak MŞ, Canpolat E (2022) Plant-based bioinsecticides for mosquito control: Impact on insecticide resistance and disease transmission. Insects 13(2):162
7. Baz MM, El-Shourbagy NM, Alkhaibari AM, Gattan HS, Alruhaili MH, Selim A, Radwan IT (2024) Larvicidal activity of *Acacia nilotica* extracts against *Culex pipiens* and their suggested mode of action by molecular simulation docking. Sci Rep 14(1):6248
8. Nathan SS, Kalaivani K, Murugan K, Chung PG (2005) The toxicity and physiological effect of neem limonoids on *Cnaphalocrocis medinalis* (Guenée) the rice leaffolder. Pestic Biochem Physiol 81(2):113–122
9. Sayada N, Tine S, Soltani N (2008) Evaluation of a botanical insecticide, lavender (*Lavandula angustifolia* (M.)) essential oil as toxicant, repellent and antifeedant against lesser grain borer (*Rhyzopertha dominica* (F.)). Appl Ecol Environ Res 20:1301–1324
10. Ahmed SS, Yousery A, Shaalan MG, Tarek A (2023) Phytochemical Investigation of the Neem Oil and Its Larvicidal Activity Against the Mosquito Vector *Culex pipiens* (L.). Egypt J Aquat Biol Fisher 27(6)
11. Hemingway J, Ranson H (2000) Insecticide resistance in insect vectors of human disease. Annu Rev Entomol 45(1):371–391
12. Sofi MA, Nanda A, Sofi MA, Maduraiveeran R, Nazir S, Siddiqui N, Nadeem A, Shah ZA, Rehman MU (2022) Larvicidal activity of *Artemisia absinthium* extracts with special reference to inhibition of detoxifying enzymes in larvae of *Aedes aegypti* L. J King Saud University-Sci 34(7):102248
13. de la Luz Sánchez-Estrada M, Aguirre-Becerra H, Feregrino-Pérez AA (2024) Bioactive compounds and biological activity in edible insects: A review. Heliyon. https://doi.org/10.1016/j.heliyon.2024.e24045
14. Hostettmann K, Chinyanganya F, Maillard M, Wolfender J (1996) Chemistry, biological, and pharmacological properties of African medicinal plants, University of Zimbabwe Publications
15. Asadollahi A, Khoobdel M, Zahraei-Ramazani A, Azarmi S, Mosawi SH (2019) Effectiveness of plant-based repellents against different *Anopheles* species: a systematic. Malar J. https://doi.org/10.1186/s12936-019-3064-8
16. Dewick PM (2002) Medicinal natural products: a biosynthetic approach, John Wiley & Sons
17. Abubakar M, Oneeb M, Rashid M, Ashraf K, Chisti GA, Awan F, Sarwar N-u-A (2024) In vitro anthelmintic efficacy of three plant extracts against various developmental stages of *Haemonchus contortus*. Pakistan Veterin J 44(2)
18. Hillary VE, Ceasar SA, Ignacimuthu S (2024) Efficacy of plant products in controlling disease vector mosquitoes, a review. Entomol Exp Appl 172(3):195–214
19. Bello SM (2015) Newspaper coverage of health issues in Nigeria: the frequency of reporting malaria, HIV/AIDS and polio and the effect of seeking health information on the health behaviours of newspaper readers
20. Bhutia ND, Seth T, Shende VD, Dutta S, Chattopadhyay A (2015) Estimation of heterosis, dominance effect and genetic control of fresh fruit yield, quality and leaf curl disease severity traits of chilli pepper (*Capsicum annuum* L.). Sci Hortic 182:47–55
21. Lu M, Ho C-T, Huang Q (2017) Extraction, bioavailability, and bioefficacy of capsaicinoids. J Food Drug Anal 25(1):27–36
22. Asnin L, Park S (2015) Isolation and analysis of bioactive compounds in *Capsicum peppers*. Crit Rev Food Sci Nutr 55(2):254–289
23. Singh IP, Mase N, Tanwar AK, Sengar N, Chatterjee O (2024) Chemical Diversity and Functionality of Capsaicinoids. CRC Press, Peppers, pp 40–64
24. Maokam C, Techawongstien S, Chanthai S (2014) Determination of major and minor capsaicinoids in different varieties of the Capsicum fruits using GC-MS and their inhibition effect of the chilli extract on [*alpha*]-amylase activity. Int Food Res J 21(6):2237
25. Luján-Méndez F, Roldán-Padrón O, Castro-Ruíz JE, López-Martínez J, García-Gasca T (2023) Capsaicinoids and their effects on cancer: the "Double-Edged Sword" postulate from the molecular scale. Cells 12(21):2573
26. Sganzerla M, Coutinho JP, de Melo AMT, Godoy HT (2014) Fast method for capsaicinoids analysis from Capsicum chinense fruits. Food Res Int 64:718–725
27. Lorenzoni R, Barreto F, Contri RV, de Araújo BV, Pohlman AR, Dalla Costa T, Guterres SS (2019) Rapid and sensitive LC-MS/MS method for simultaneous quantification of capsaicin and dihydrocapsaicin in microdialysis samples following dermal application. J Pharm Biomed Anal 173:126–133
28. Baz M (2013) Strategies for mosquito control [PhD thesis]. Faculty of Science, Benha University
29. Baz MM, El-Tabakh MA, Selim A, Alasmari SM, Alkhaibari AM, Alruhaili MH, Gattan HS, Abdelkhalek HF (2025) Chemical composition and bio-efficacy of agro-waste plant extracts and their potential as bioinsecticides against *Culex pipiens* mosquitoes. Parasitol Int 104:102968
30. Larvicides M (2005) Guidelines for laboratory and field testing of mosquito larvicides. Google Scholar
31. Khater HF, Geden CJ (2019) Efficacy and repellency of some essential oils and their blends against larval and adult house flies, *Musca domestica* L. (Diptera: Muscidae). J Vect Ecol 44(2):256–263
32. Choi S-J, Hwang J-M, Kim S-I (2003) A colorimetric microplate assay method for high throughput analysis of lipase activity. BMB Rep 36(4):417–420
33. Ishaaya I, Swirski E (1976) Trehalase, invertase, and amylase activities in the black scale, *Saissetia oleae*, and their relation to host adaptability. J Insect Physiol 22(7):1025–1029
34. Magaldi S, Mata-Essayag S, De Capriles CH, Pérez C, Colella M, Olaizola C, Ontiveros Y (2004) Well diffusion for antifungal susceptibility testing. Int J Infect Dis 8(1):39–45
35. Elhawary E, Mostafa N, Shehata A, Labib R (2021) Comparative study of selected Rosa varieties' metabolites through UPLC-ESI-MS/MS, chemometrics and investigation of their insecticidal activity against *Culex pipiens* L. Jordan J Pharmaceut Sci 14(4)
36. Yagi S, Zengin G, Eldahshan OA, Singab ANB, Selvi S, Cetiz MV, Rodrigues MJ, Custodio L, Dall'Acqua S, Elhawary EA (2024) Functional constituents of *Colchicum lingulatum* Boiss. & Spruner subsp. Rigescens K. Perss. Extracts and their biological activities with different perspectives. Food Biosci 60 104496
37. El-Tabakh MA, Elhawary EA, Hwihy HM, Darweesh KF, Shaapan RM, Ghazala EA, Mokhtar MM, Waheeb HO, Emam DE, Bakr NA (2023) UPLC/ESI/MS profiling of red algae *Galaxaura rugosa* extracts and its activity against malaria mosquito vector, *Anopheles pharoensis*, with reference to *Danio rerio* and *Daphnia magna* as bioindicators. Malar J 22(1):368
38. Nilofar, Bahadırlı NP, Elhawary EA, Eldahshan O, Singab AN, Saka E, Cespedes-Acuna CL, Andruch V, Kalyniukova A, Zengin G (2024) Exploring the chemical composition and biological effects of four *Salvia* hybrids: an innovative perspective on functional yields. eFood 5(5): e70012.

39. Baz MM, Selim AM, Radwan IT, Alkhaibari AM, Gattan HS, Alruhaili MH, Alasmari SM, Gad ME (2024) Evaluating larvicidal, ovicidal and growth inhibiting activity of five medicinal plant extracts on *Culex pipiens* (Diptera: Culicidae), the West Nile virus vector. Sci Rep 14(1):19660
40. Baz MM, Selim A, Radwan IT, Alkhaibari AM, Khater HF (2022) Larvicidal and adulticidal effects of some Egyptian oils against *Culex pipiens*. Sci Rep 12(1):4406
41. Baz MM, Selim AM, Radwan IT, Khater HF (2022) Plant oils in the fight against the West Nile Vector, *Culex pipiens*. Int J Trop Insect Sci 42(3):2373–2380
42. Abd Elmohsen M, Selim A, Abd Elmoneim AE (2019) Prevalence and molecular characterization of Lumpy Skin Disease in cattle during period 2016–2017. Benha Veterin Med J 37(1):172–175
43. Selim A, Said Ahmed S, Galila E (2019) Prevalence and molecular detection of *Ehrlichia canis* in dogs. Benha Veterin Med J 37(1):169–171
44. Ni Z-J, Wang X, Shen Y, Thakur K, Han J, Zhang J-G, Hu F, Wei Z-J (2021) Recent updates on the chemistry, bioactivities, mode of action, and industrial applications of plant essential oils. Trends Food Sci Technol 110:78–89
45. Mostafa RM, Baz MM, Ebeed HT, Essawy HS, Dawwam GE, Darwish AB, Selim A, El-Shourbagy NM (2024) Biological effects of *Bougainvillea glabra, Delonix regia, Lantana camara*, and *Platycladus orientalis* extracts and their possible metabolomics therapeutics against the West Nile virus vector, *Culex pipiens* (Diptera: Culicidae). Microb Pathog 195:106870
46. Kumar J, Ramlal A, Mallick D, Mishra V (2021) An overview of some biopesticides and their importance in plant protection for commercial acceptance. Plants 10(6):1185
47. Ayilara MS, Adeleke BS, Akinola SA, Fayose CA, Adeyemi UT, Gbadegesin LA, Omole RK, Johnson RM, Uthman QO, Babalola OO (2023) Biopesticides as a promising alternative to synthetic pesticides: a case for microbial pesticides, phytopesticides, and nanobiopesticides. Front Microbiol 14:1040901
48. Marrone PG (2019) Pesticidal natural products–status and future potential. Pest Manag Sci 75(9):2325–2340
49. Tudi M, Daniel Ruan H, Wang L, Lyu J, Sadler R, Connell D, Chu C, Phung DT (2021) Agriculture development, pesticide application and its impact on the environment. Int J Environ Res Public Health 18(3):1112
50. Onen H, Luzala MM, Kigozi S, Sikumbili RM, Muanga C-JK, Zola EN, Wendji SN, Buya AB, Balciunaitiene A, Viškelis J (2023) Mosquito-borne diseases and their control strategies: an overview focused on green synthesized plant-based metallic nanoparticles. Insects 14(3):221
51. Chandra G, Bhattacharjee I (2024) Mosquito-Borne Human Diseases, Mosquitoes: Biology. Springer, Pathogenicity and Management, pp 257–286
52. Madhumathy A, Aivazi A, Vijayan V (2007) Larvicidal efficacy of *Capsicum annum* against *Anopheles stephensi* and *Culex quinquefasciatus*. J Vector Borne Dis 44(3):223
53. Rajapaksha W, De Silva W, Weeraratne T (2024) Comparative evaluation of the effect of phytochemicals of garlic (*Allium sativum*) ethanolic extract against *Aedes albopictus* and *Culex quinquefasciatus* mosquitoes in Sri Lanka. Ceylon J Sci 53:161–167
54. Elhawary EA, Gad ME, Hegazy MM, Mostafa RM, Gattan HS, Alruhaili MH, Selim AM, Mashlawi AM, Alkhaibari AM, Alasmari SM (2025) Study of the effect of dryness and storage on *Ceratonia siliqua* L. stem extracts and evaluation of their insecticidal activity. Scient Rep 15(1):11123
55. Farahat NM, Khaled AS, Hussein MA, Zyaan OH (2021) Biological and histological alterations in the larvae of *Culex pipiens* L (Diptera: Culicidae) induced by imidcaloproid and tannic acid. Egypt Acad J Biol Sci A Entomol 14(1):243–254
56. Izah S, Etim N, Ilerhunmwuwa I, Silas G (2019) Evaluation of crude and ethanolic extracts of *Capsicum frutescens* var. minima fruit against some common bacterial pathogens. Int J Complement Altern Med 12(3):105–108
57. Haldar KM, Ghosh P, Chandra G (2014) Larvicidal, adulticidal, repellency and smoke toxic efficacy of *Ficus krishnae* against *Anopheles stephensi* Liston and *Culex vishnui* group mosquitoes. Asian Pacific J Trop Dis 4:S214–S220
58. Samuel M, Oliver SV, Coetzee M, Brooke BD (2016) The larvicidal effects of black pepper (*Piper nigrum* L.) and piperine against insecticide resistant and susceptible strains of *Anopheles malaria* vector mosquitoes. Parasit Vectors 9:1–9
59. Pam V, Odey S, Ombugadu A, Uzoigwe N, Maikenti J, Adejoh V, Ahmed H, Aimankhu P, Aliyu A, Ayuba S (2021) Larvicidal activity of the leaf extracts and powder of *Millettia aboensis* against larvae of *Anopheles gambiae* sl collected from Lafia, Nasarawa State, Nigeria. Biomed J Scient Techn Res 39(2):31103–31109
60. Kholhring Lalchhandama KL (2011) Mosquitocidal activity of *Millettia pachycarpa* on the larvae and eggs of *Aedis aegypti*
61. Chukwujekwu J, Smith P, Coombes P, Mulholland D, Van Staden J (2005) Antiplasmodial diterpenoid from the leaves of *Hyptis suaveolens*. J Ethnopharmacol 102(2):295–297
62. Erdoğan P (2023) Insecticidal effect of different plants extracts against Wheat Weevil, *Sitophilus granarius* (L., 1985) (Coleoptera: Curculionidae). Am J Entomol 7(3):94–99
63. Li B, Yang M, Shi R, Ye M (2019) Insecticidal activity of natural capsaicinoids against several agricultural insects. Natur Product Commun 14(7):1934578X19862695
64. Olotuah O (2013) Laboratory evaluation of pesticidal activities of *Hyptis suaveolens* in pest management. Int J Agric Res. https://doi.org/10.3923/ijar.2013.101.106
65. de Paula Marchiori JJ, Holtz AM, Piffer M, Aguiar RL, Botti JMC, Franzin ML, de Souza OV, Furno PS, Fontes BdOM, de Paula GM (2023) Aqueous extract of chili pepper in the management of the pink hibiscus mealybug. Pseudococcidae, Hemiptera
66. Maqbool SB, Riazuddin S, Loc NT, Gatehouse AM, Gatehouse JA, Christou P (2001) Expression of multiple insecticidal genes confers broad resistance against a range of different rice pests. Mol Breeding 7:85–93
67. Kazzazi M, Bandani AR, Hosseinkhani S (2005) Biochemical characterization of α-amylase of the Sunn pest. Eurygaster integriceps Entomol Sci 8(4):371–377
68. Heil M, Büchler R, Boland W (2005) Quantification of invertase activity in ants under field conditions. J Chem Ecol 31:431–43
69. Santana CC, Barbosa LA, Diniz Basílio Júnior I, Gomes do Nascimento T, Dornelas CB, Grillo LA (2017) Lipase activity in the larval midgut of *Rhynchophorus palmarum*: biochemical characterization and the effects of reducing agents. Insects 8(3):100
70. Octavio L, Daniel R, Francislete RM, Maria F (2002) Plant amylase inhibitors and their interaction with insect amylase. Eur J Biochem 269:397–412
71. Hichri F, Omri A, Hossan ASM, Ben Jannet H (2019) Alpha-glucosidase and amylase inhibitory effects of *Eruca vesicaria* subsp. longirostris essential oils: synthesis of new 1, 2, 4-triazole-thiol derivatives and 1, 3, 4-thiadiazole with potential inhibitory activity. Pharmaceut Biol 57(1):564–570
72. Gustafson L, Chew M, Wyllie W (1998) Effects of tea tree oil on *Escherichia coli*. Lett Appl Microbiol 26(3):194–198
73. Ultee A, Kets EP, Alberda M, Hoekstra FA, Smid EJ (2000) Adaptation of the food-borne pathogen *Bacillus cereus* to carvacrol. Arch Microbiol 174:233–238
74. Oussalah M, Caillet S, Salmiéri S, Saucier L, Lacroix M (2007) Antimicrobial effects of alginate-based films containing essential oils on *Listeria monocytogenes* and *Salmonella typhimurium* present in bologna and ham. J Food Prot 70(4):901–908

75. Babu SR, Subrahmanyam B (2010) Bio-potency of serine proteinase inhibitors from *Acacia senegal* seeds on digestive proteinases, larval growth and development of *Helicoverpa armigera* (Hübner). Pestic Biochem Physiol 98(3):349–358
76. Chintalchere JM, Dar MA, Pandit RS (2020) Biocontrol efficacy of bay essential oil against housefly, *Musca domestica* (Diptera: Muscidae). J Basic Appl Zool 81:1–12
77. Mulla JA, Tamhane VA (2023) Novel insights into plant defensin ingestion induced metabolic responses in the polyphagous insect pest *Helicoverpa armigera*. Sci Rep 13(1):3151
78. Magierowicz K, Górska-Drabik E, Sempruch C (2020) The effect of *Tanacetum vulgare* essential oil and its main components on some ecological and physiological parameters of *Acrobasis advenella* (Zinck.)(Lepidoptera: Pyralidae). Pestic Biochem Physiol 162:105–112
79. Hussein HS, Salem MZ, Soliman AM, Eldesouky SE (2023) Comparative study of three plant-derived extracts as new management strategies against *Spodoptera littoralis* (Boisd.) (Lepidoptera: Noctuidae). Scient Rep 13(1):3542
80. Alothman ZA, Wabaidur SM, Khan MR, Ghafar AA, Habila MA, Ahmed YBH (2012) Determination of capsaicinoids in *Capsicum* species using ultra performance liquid chromatography-mass spectrometry. J Sep Sci 35(21):2892–2896
81. Schweiggert U, Kammerer DR, Carle R, Schieber A (2005) Characterization of carotenoids and carotenoid esters in red pepper pods (*Capsicum annuum* L) by high-performance liquid chromatography/atmospheric pressure chemical ionization mass spectrometry. Rapid Commun Mass Spectrom 19(18):2617–2628
82. Chilczuk B, Marciniak B, Kontek R, Materska M (2021) Diversity of the chemical profile and biological activity of *Capsicum annuum* L. extracts in relation to their lipophilicity. Molecules 26(17):5215
83. Pacifico S, Galasso S, Piccolella S, Kretschmer N, Pan S-P, Marciano S, Bauer R, Monaco P (2015) Seasonal variation in phenolic composition and antioxidant and anti-inflammatory activities of *Calamintha nepeta* (L.) Savi. Food Res Int 69:121–132
84. Kashiwagi T, Horibata Y, Mekuria DB, Tebayashi S-I, Kim C-S (2005) Ovipositional deterrent in the sweet pepper, *Capsicum annuum*, at the mature stage against *Liriomyza trifolii* (Burgess). Biosci Biotechnol Biochem 69(10):1831–1835

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