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Chemical composition and bio-efficacy of agro-waste plant extracts and their potential as bioinsecticides against *Culex pipiens* mosquitoes

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ABSTRACT

Mosquitoes are considered one of the most lethal creatures on the planet and are responsible for millions of fatalities annually through the transmission of several diseases to humans. Green trash is commonly employed in agricultural fertilizer manufacturing and microbial bioprocesses for energy production. However, there is limited information available on the conversion of green waste into biocides. This study investigates the viability of utilizing green waste as a new biopesticide against *Culex pipiens* mosquito larvae. The current study found that plant extracts from *Punica granatum* (98.4 % mortality), *Citrus sinensis* (92 % mortality), *Brassica oleracea* (88 % mortality), *Oryza sativa* (81.6 % mortality), and *Colocasia esculenta* (53.6 % mortality) were very good at killing *Cx. pipiens* larvae 24 h post-treatment. The LC_{50} values were 314.43, 370.72, 465.59, 666.67, and 1798.03 ppm for *P. granatum, C. sinensis, B. oleracea, O. sativa,* and *C. esculenta*, respectively. All plant extracts, particularly *P. granatum* extract (14.93 and 41.87 U/g), showed a significant reduction in acid and alkaline phosphate activity. Additionally, pomegranate extract showed a significant decrease (90 %) in field larval density, with a stability of up to five days post-treatment. GC–MS results showed more chemical classes, such as terpenes, esters, fatty acids, alkanes, and phenolic compounds. HPLC analysis revealed that the analyzed extracts had a high concentration of phenolic and flavonoid components. Moreover, there are many variations among these plants in the amount of each compound. The docking interaction showed a simulation of the atomic-level interaction between a protein and a small molecule through the binding site of target proteins, explaining the most critical elements influencing the enzyme's activity or inhibitions. The study's findings showed that the various phytochemicals found in agro-waste plants had high larvicidal activity and provide a safe and efficient substitute to conventional pesticides for pest management, as well as a potential future in biotechnology.

1. Introduction

Mosquitoes have posed a significant danger to human and animal health for an extended period, and this concern persists. Mosquitoes can spread to different geographical areas and new settings due to urbanization, global trade, and travel, placing alot of people in danger of the diseases they carry [1–3]. In 2019, the world reported 56 million cases of dengue fever and 229 million cases of malaria, resulting in 409 thousand deaths. Over the past 50 years, outbreaks in new nations have estimated an almost thirty-fold increase in dengue incidence worldwide [4]. Climate change is predicted to raise the occurance of mosquito-borne illnesses despite continuous attempts to reduce disease, posing an even larger risk to human health [5].

There have been efforts to control mosquitoes at the larval and adult

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stages. Insecticides on clothes, indoor sprays, and bed nets are the most prevalent methods of keeping mosquitoes away from humans. In many regions, particularly endemic areas, the use of synthetic pesticides gives clear hope of restricting disease transmission; nonetheless, mosquito resistance to insecticides has become a major barrier to reducing the disease burden [6–10]. Therefore, there is widespread recognition that pesticide resistance significantly increases the risk of not preventing mosquito invasion and disease spread. Above all, pesticides are costly, hard to get by, and highly harmful [11].

The scientific community continues to pay more attention to natural materials because they are less harmful, more effective than synthetic materials, and a realistic alternative to pesticides. Since bioinsecticides have fewer harmful effects on the environment and human health, their use has proven to be an ideal alternative [12].

Agricultural waste refers to natural organic elements lost, wasted, or disposed of [13]. Although the Food and Agriculture Organization (FAO) noted the rise in food losses in Egypt, which consequently led to a rise in food waste [6], many studies have shown that some agricultural residues are effective for industrial use and controlling insects [14,15].

The study of Visakh et al. [16] revealed that turmeric (*Curcuma longa* L.) is an essential medicinal plant, spice, culinary colourant, and cosmetic element. After collecting the roots, they tested turmeric leaf waste as a pesticide against two common stored grain insects (*Tribolium castaneum* and *Rhyzopertha dominica*). The study's findings showed that essential oils isolated from turmeric leaf waste have promising potential as safe botanical pesticides against stored grain insects, as well as wonderful antioxidant qualities. Many studies have proven the importance of green waste, such as study of Panzella et al. [17], which included the most innovative and promising techniques for extracting phenolic compounds from food and agricultural waste; these techniques mostly depend on the use of organic solvents like methanol, acetone, or ethanol. The necessity for environmentally friendly, sustainable methods that produce phenolic-rich extracts with minimal negative effects on the environment was underlined.

It is believed that using some agricultural waste materials can help control mosquitoes, discover novel effective substances, and lower the costs associated with manufacturing antimicrobial agents, natural pesticides, and other agricultural products. Using agricultural waste as a renewable and cost-effective energy source can benefit several industrial applications [18,19]. As a result, many studies seek to discover new elements and effectiveness in seemingly worthless green waste that, when displayed and their contents revealed, appear to be a natural treasure full of numerous medical and industrial benefits, as we are attempting to demonstrate in our current work. The composition of the selected plant waste is novel, emphasising the importance of this green waste.

Cabbages (*Brassica oleracea*) are rich in several substances, including vitamins (A, C, K, and B6), polyphenols (sinapic acid derivatives, flavonoids and chlorogenic), carotenoids, minerals (manganese and potassium, selenium), and nitrogen-sulfur derivatives (glucosinolates and isothiocyanates) [20]. Several of these chemicals are generally recognized in the literature as having anticarcinogenic, antibacterial, anti-inflammatory, and antidiabetic effects [21].

The annual herbaceous plant *Colocasia esculenta* (L.) Shott, commonly referred to as "taro," belongs to the Araceae family and may be used as a source of botanical pest control [22]. The tropical crop *C. esculenta* (L), frequently referred to as taro, is mainly grown for its tubers, or corms. However, it also contains underdeveloped and underutilized leaves and stems that may be useful. According to Oriyomi, Fagbohun, Oyedeji and Agboola [23], a plant's ability to demonstrate biotoxicity against pests is impacted by the development of idioblasts, specialized cells with acrid and irritating tastes, and the plant's resilience to pest attack.

Oranges and pomegranate are considered two of the most abundant crops in the Egyptian areas, in addition to their peel. Researchers have conducted analyses of the chemical components and their biological effects in the peel of various fruits, including orange (*Citrus sinensis*) and pomegranate (*Punica granatum*). Orange and pomegranate peels, juices, pulps, and seeds contain a variety of polyphenols and antioxidants [24]. The primary agricultural waste from rice production is rice husk. The principal components are polyphenols with established antioxidant effects [25].

In this work, we evaluated the agriculture waste extracts from taro and cabbage and taro leaves (*Brassica oleracea* and *Colocasia esculenta*), orange (*Citrus sinensis*) and pomegranate (*Punica granatum*) peels, and rice (*Oriza sativa*) and wheat brans (*Triticum aestivum*) against mosquito larvae as safe green pesticides. We also examined the effects of these extracts on the enzyme aspects of insects.

2. Materials and methods

2.1. Plant materials and analysis

2.1.1. Plant collection

Brassica oleracea (CA), *Citrus sinensis* (OO), *Colocasia esculenta* (CE), *Oryza sativa* (RA), *Punica granatum* (PO), *and Triticum aestivum* (TA) were gathered from different farmlands in faculty of Agriculture, Qalyubia Governorate, Egypt, during July to November 2023 (Table 1). *Oryza sativa* and *Triticum aestivum* were obtained from Benha Mills companies in Qalyubiya Governorate, Egypt. The Department of Botany and Plant Taxonomy at the Agricultural Research Center in Giza, Egypt, was responsible for plant identification. The plant components were dried in the shade for three to seven days at room temperature until they were entirely dry, and their weight dropped. Th desiccated tissues were pulverised using a stainless-steel electric blender and stored in sealed containers to avoid moisture exposure until needed.

2.1.2. Plant extraction

The plant extracts were obtained by bathing 25 g of dried plant powder in 100 to 150 ml of methanol solvent in a 250 ml conical flask at 27 ± 2 °C. 25 g of plant components were extracted with warm water (50 °C) at room temperature to create an aqueous extract. The extraction procedure was completed after 48 h, and the solution was filtered via a Buchner funnel and Whatman No. 1 filter paper [26]. Using a rotary evaporator, concentrate the extracts and store them.

2.2. Phytochemical analysis

2.2.1. GC/MS analysis

A Thermo Scientific Trace GC Ultra/ISQ (Thermo Fisher, Germany) Single Quadrupole MS, TG-5MS fused silica capillary column (0.1 mm, 0.251 mm, and 30 m film thickness) was used for the GC/MS analysis. An electron ionisation device with an ionisation energy of 70 eV was utilized for GC/MS detection. The carrier gas was hydrogen, which moved at a steady pace of 1 ml/min. The temperatures of the injector and MS transfer line were adjusted to 280 °C. After reaching 50 °C and holding it for two minutes, the temperature was raised to 150 °C at a rate of 7 °C per minute, 270 °C at a rate of 5 °C per minute, and ultimately 310 °C at a rate of 3.5 °C per minute, which was kept for ten minutes. A percent relative peak area was utilized to explore the quantification of every component that was detected. By comparing each sample's mass

Table 1
List of plant species and plant parts tested against *Cx. pipiens* larvae.

No.	Botanical Name	Family	Common Name	Part used
1	*Brassica oleracea*	Brassicaceae	Cabbage	leaf
2	*Citrus sinensis*	Rutaceae	Sweet orange	peel
3	*Colocasia esculenta*	Araceae	Wild taro	leaf
4	*Oriza sativa L.*	Poaceae	Rice	hull
5	*Punica granatum*	Lythraceae	Pomegranate	peel
6	*Triticum aestivum*	Poaceae	Common wheat	hull

spectrum and retention duration to the NIST and WILLY library data that was acquired from the GC/MS apparatus, the compounds were taken to be what they were. The identification process employed mass spectra and a computer search of user-generated reference libraries. Single-ion chromatographic reconstruction was utilized to verify peak homogeneity. The only information available when no matching spectra could be found was the mass spectral fragmentation and structural type of the relevant component. To verify GC retention durations, reference substances were co-chromatographed whenever possible [27].

2.2.2. Polyphenol contents concentration determination by the HPLC

An HPLC system (Agilent Series 1100) (Agilent, USA) was utilized to analyse the flavonoid and phenolic components in plant extracts from *C. sinensis, P. granatum, O. sativa,* and *B. oleracea.* The apparatus comprised of a UV/Vis detector, two LC pumps (series 1100), a solvent degasser, an auto-sampling injector, and ChemStation software.

For phenolic acids, the UV/Vis detector was set to 250 nm, and for flavonoids, at 360 nm. The analysis was conducted on a C18 column measuring 125 mm ñ 4.60 mm with particle size of 5 μm. A gradient mobile phase comprising two solvents, acetic acid and methanol in water (1,25), was utilized to separate the phenolic acids. During the first three minutes of the gradient program, the concentration was set at 100 % B. After that, five minutes were spent using an eluent A that contained 50 %. This was followed by a subsequent 2 min increase in the concentration of A, followed by a subsequent 5 min decrease to 50 % at a detecting wavelength of 250 nm. Isocratic elution was used to separate flavonoids using a mobile phase consisting of two solvents: acetonitrile (A) and 0.2 % (*v*/v) aqueous formic acid (B). The solvent flow rate was 1 mi/min, and the separation was carried out at a temperature of 25 °C. The injection volumes were 25 μL [28].

2.2.3. Molecular docking

The compounds' structure was generated in the PDB file format using the output from the Gaussian 09 software. The crystallographic data for GPT (PDB ID: 2HUU), β-esterase (PDB ID: 3O9M), acid phosphatase (PDB ID: 1WAR), and alkaline phosphatase (PDB ID: 1K7H) were obtained from the Protein Data Bank at http://www.rcsb.org.pdb. Molecular docking investigations were conducted using the MOE 2015 program.

2.3. Mosquito larvicidal assay

2.3.1. Rearing of Culex pipiens

In the insectary, *Cx pipiens* larvae were grown in controlled settings with a temperature of 27 ± 2 °C, humidity of 75 ± 5 %, and a light/dark cycle of 12:12 h. The ground bread and fish food (Tetramin) were mixed in a 1:3 ratio to feed the larvae. The pupae were transferred from the pans to a cup of dechlorinated water, and the adult insects emerged from the 35 × 35 × 40 cm screened cages. Periodically, adult mosquitoes were given a 10 % sugar solution to drink, while females feeding on blood of a hamster. The larvae and pupae stages were available regularly for the studies and kept in the same laboratory.

2.3.2. Larvicidal activity

In a lab environment, the 4th larval stage of *Cx. pipiens* was tested against aqueous and methanol extracts from the leaves of *B. oleracea, C. sinensis, C. esculenta, O. sativa, P. granatum*, and *T. aestivum*. The 4th larval instar was exposed to dosages of 62.5, 125, 250, 500, 1000, and 1500 ppm (1 g/1000 ml distilled water). A glass beaker was filled with 250 ml of distilled water and twenty larvae per concentration. Each concentration was tested in three replicates. Mortalities were documented at 24, and 48 h following the initial exposure according to WHO guidelines [29] and post-treatment.

2.3.3. Mosquito larvae biochemical studies

GPT catalyzes the transfer of amino groups from D and L alanine to α-ketoacid (α-ketoglutaric acid), producing L-glutamate and pyruvic acid. Pyruvate undergoes a reaction with 2,4-dinitrophenylhydrazine to produce pyruvate hydrazine, which generates a brown color in an alkaline environment that may be quantified using spectrophotometry. GOT and GPT levels were measured calorimetrically using the Reitman and Frankel [30] model. The reaction mixture included 1 ml of phosphate buffer (pH 7.2) solution, 0.2 mM α-ketoglutaric acid, and 200 mM L-aspartate and incubated for precisely 30 min. Dispense 1 mlof 0.001 M 2,4-dinitrophenylhydrazine. Wait for a minimum of 30 min. Subsequently, 10 ml of 0.4 Normal sodium hydroxide solution were introduced. The spectrophotometer measures the optical density of the brown color produced after 5 min at a wavelength of 520 nm. Enzyme activity is quantified in units per gram of body weight. β-esterase activity was measured calorimetrically following the procedure van Asperen [31] outlined with α-naphthyl acetate and β-naphthyl acetate as substrates. Naphthol formed from substrate hydrolysis can be detected by adding a diazo blue sodium lauryl sulfate solution to the enzyme source. As a result, α-naphthol has a bright blue purple color and β-naphthol has a deep red-purple color. Next, the colors are measured using spectrophotometry, with α-naphthol being measured at 600 nm and β-naphthol at 555 nm.

Alkaline phosphatase was assessed following the procedure outlined by Powell and Smith [32]. Enzymes break down disodium phenyl phosphate to produce phenol, which then combines with 4-amino antipyrine in this process. The addition of potassium ferricyanide produces the characteristic color associated with this procedure. The reaction mixture contains 1 ml of carbonate buffer (pH 4.5 to 10.4), 1 ml of 0.01 M disodium phenyl phosphate (substrate), and 0.1 mL of sample and incubated at 37 °C for 30 min. At the end of the incubation period, 0.8 mL of 0.5 N NaOH was added to stop the reaction. Add 1.2 mL of 0.5 N NaHCO3, 1 mL of 4-amino antipyrine solution (1 %), and 1 mL of potassium ferricyanide (0.5 %). The color was measured immediately at a wavelength of 510 nm. Enzyme activity is assessed in units (U), with one unit capable of hydrolysing 1.0 mol of *p*-nitrophenyl phosphate per minute at 37 °C and pH 10.4.

2.3.4. Effect of sublethal concentrations on larval longevity and survival

In this experiment, LC50 concentrations of the plant extracts *C. sinensis* (253.19 ppm), *P. granatum* (208.39 ppm), *O. sativa* (461.12 ppm), and *B. oleracea* (1335.92 ppm) were applied to 25 mosquito larvae in the 3rd instar. The solution was prepared in 100 ml of water. After being left there for 48 h, 12 groups of mosquitoes (300 larvae) received treatment, while three groups (75 larvae) served as controls. The control was dechlorinated water. The number of dead and moribund larvae was counted 48 h after treatment to determine mortality, according to the WHO [29]. Using a pipette, live larvae were extracted 48 h post-treatment. After that, wire gauze was used to transfer them to plastic cups filled with 100 ml of distilled water. Following that, the larvae were fed a little quantity of dry bread until they developed into pupae, which then became adults.

2.3.5. Field evaluation of larvicides

The field testing of *C. sinensis, P. granatum, O. sativa,* and *B. oleracea* extracts against larval and pupal mosquito populations was conducted Using LC_{95} X2 concentration, in standing water ditches (average 170 m × 1.25 m and 0.55 m deep) in Saad village, Qalybia Governorate, Egypt, where the water was stagnant, and density of mosquito was high. Only the control site utilized dechlorinated water. For every treatment, three replicates were employed. Every day for a week, samples of mosquitoes were taken before and after treatment at each location. At every larvicide location, fourth instar larvae were gathered in field water using a larval dipper (450 ml) in order to count and analyse the samples.

2.4. Statistical analysis

The data was coded and inputted via the statistical software SPSS

V.22, for doing the Probit analyses to calculate the lethal concentration (LC) values [33]. The data was tested to verify it met the criteria for parametric tests. Continuous variables were tested for normality using the Shapiro-Wilk and Kolmogorov-Smirnov tests. The data were displayed as the mean and standard deviation. ANOVA analyses were conducted for experimental groups (Control, CA, RA, OO, and PO) about enzymatic activity, namely GPT (μ/g/min), β-esterase (μg/g/min), acid phosphatase (U/g), and alkaline phosphatase (U/g). The study was conducted with a minimum of three replicates for each group. Post-hoc analysis was performed using a Tukey pairwise comparison. Statistical significance was determined at a *P*-value of less than 0.05. The analysis is now accessible through MiniTab V14. Data were displayed graphically using R Studio version 2022.02.4.

3. Results

3.1. Mosquito larvicidal activity

Using *C. sinensis, P. granatum, O. sativa, T. aestivum, C. esculenta,* and *B. oleracea* plant extracts, the study evaluated their effects on *Cx. pipiens* larvae in their 4th instar. Most plant extracts evaluated in the study exhibited significant insecticidal effects on mosquito larvae, *Cx. pipiens*, following varying exposure durations. At 24 h post-treatment (PT), the mortality rate (MO%) of *Cx. pipiens* treated with 2000 ppm methanol extracts of *C. sinensis, P. granatum, O. sativa, T. aestivum, C. esculenta,* and *B. oleracea* was, 92, 98.4, 84, 47.2, 53.6, 88 % (MO%) (Table 2) with LC_{50} (50 %, median lethal concentration) = 370.72, 314.43, 666.67, 2502.84, 1798.03 and 465.59 ppm, respectively after 24 h treatment (Table 3). After 48 h of PT, the maximum larval mortality was observed; at 2000 ppm, it was 100 % in the methanol extracts for *C. sinensis* and *P. granatum* and 90, 95, 65.6, and 55.2 % for *B. oleracea, O. sativa, C. esculenta*, and *T. aestivum*, respectively. It is evident from Tables 2,3 and Fig. 1 that the LC_{50} values of methanol of *P. granatum* (208.39 ppm), *C. sinensis* (253.19 ppm), *C. esculenta* (335.92 ppm), and *O. sativa* (461.12 ppm), were the most effective plant extracts against the 4th larval instar of *Cx. pipiens*, 48 h PT.

3.2. Biochemical activity of the tested plant extracts on Cx. pipiens

The biochemical changes in the activity of glutamic pyruvic transaminase (GPT), β-esterase, and acid and alkaline phosphatases (ACP and ALP) of the larvae of *Cx. pipiens* at 24 h post-treatment with the LC_{50} of the tested plant methanol extracts are listed in Table 4 and illustrated in Fig. 2. CA extract shows its role in inhibiting GPT, while RA extract affects the activity of β-esterase. At the same time, PO extract shows a significant inhibition ($P < 0.05$) for both acid and alkaline phosphatases. *Oryza sativa* (rice) hulls significantly reduced the activity of the enzymes compared to untreated larvae. According to *C. aurantium* (orange) peels, the LC_{50} values significantly affected all enzymes. In contrast, GPT and β-esterase did not show a statistical difference between the treated and controlled insects.

3.3. Sublethal effect of plant extracts on survival of mosquito larvae

The proportion of *Cx. pipiens* larvae that survived till adulthood was significantly affected after 24 h of exposure to the LC_{50} values of *C. sinensis* (253.19 ppm), *P. granatum* (208.39 ppm), *O. sativa* (461.12 ppm), and *B. oleracea* (1335.92 ppm). In the control group, there was no mortality seen. All plant extracts subjected to LC_{50} concentrations after 48 h had significantly lower proportions of mosquito larvae that survived and matured into pupae than the control group (Fig. 3a). Furthermore, following treatment with plant extracts, the percentage of pupae that effectively matured into adults was much lower than in the control group (Fig. 3b). Overall, there was a substantial decrease in the survival rates of larvae ($F = 13.242$; df. =2, 57; $P < 0.001$) following a 48-h exposure to the $L_{C5}0$ concentrations of *P. granatum* (32 %) and *C. sinensis* (53.3 %). The survival rate seen in the control group, which was 94.7 %, was much lower than these rates (Fig. 3a).

3.4. Larvicidal field evaluation

At larval breeding locations, the larvicides of *C. sinensis, P. granatum, O. sativa,* and *B. oleracea* extracts were evaluated in the field using LC_{95} X2 (6255.32, 4501.84, 12,082.22, and 7907.72 ppm, respectively). Dechlorinated water was used at the control location, where results showed lower larval densities in traded ditches in Saaed village, post-treatments. The larval reduction % reached 84, 90, 78, and 60 %, respectively, over 24 h for *C. sinensis, P. granatum, O. sativa,* and *B. oleracea*, with stability up to five days PT for *P. granatum* (Fig. 4).

3.5. Phytochemical activity

3.5.1. GC–MS data analysis

GC–MS analysis was used to assist in the metabolic study of the four extracts. Using methanol solvent, the results of our study's GC–MS analysis allowed us to identify a number of substances in the leaves of *B. oleracea, C. sinensis, O. sativa,* and *P. granatum,* including terpenes, phenols, esters, fatty acids, alkane, ketone, steroids, and aliphatic amine (Tables 5–8). *Brassica oleracea* extract contained 9 compounds (Table 5), of which *B. oleracea* showed an abundance of hexadecanoic acid, 2,3-hydroxypropyl ester (29.31 %), hexadecanoic acid, methyl ester (19.95 %), and linoleic acid ethyl ester (18.52 %). *Citrus sinensis* extract had 11 compounds; the greatest concentrations of the investigated compounds were hexadecanoic acid, 2,3-hydroxypropyl ester (20.51

Table 2
Efficacy of *Citrus sinensis, Punica granatum, Oriza sativa, Triticum aestivum, Colocasia esculenta, and Brassica oleracea* extracts on *Cx. pipiens* larvae, 24 and 48 h post-treatment.

Time (hr)	Plant type	Concentration (ppm) (mean ± SE).						
		0.0	62.5	125	250	500	1000	2000
24	*C. sinensis*	0 ± 0^{aG}	8.8 ± 1.50^{bF}	19.2 ± 2.33^{bE}	40.8 ± 1.96^{bD}	56.8 ± 3.44^{bC}	76.0 ± 1.79^{bB}	92.0 ± 3.79^{bA}
	P. granatum	0 ± 0^{aG}	10.4 ± 0.98^{aF}	22.4 ± 0.98^{aE}	44.8 ± 2.33^{aD}	60.8 ± 2.33^{aC}	79.2 ± 1.96^{aB}	98.4 ± 0.98^{aA}
	O. sativa	0 ± 0^{aG}	4.0 ± 1.79^{dF}	10.4 ± 2.04^{eE}	25.6 ± 2.04^{dD}	38.4 ± 1.60^{dC}	60.0 ± 1.79^{dB}	81.6 ± 3.71^{dA}
	T. aestivum	0 ± 0^{aG}	2.4 ± 0.98^{eF}	4.8 ± 0.80^{fE}	9.6 ± 2.040^{fD}	18.4 ± 1.60^{fC}	28.0 ± 2.83^{fB}	47.2 ± 1.96^{fA}
	C. esculenta	0 ± 0^{aG}	4.0 ± 1.26^{dF}	6.4 ± 0.98^{eE}	12.0 ± 1.26^{dD}	23.2 ± 1.50^{eC}	36.8 ± 1.50^{eB}	53.6 ± 2.04^{eA}
	B. oleracea	0 ± 0^{aG}	7.2 ± 0.80^{cF}	16.0 ± 1.26^{cE}	29.6 ± 2.04^{cD}	50.4 ± 1.60^{cC}	72.8 ± 1.50^{cB}	88.0 ± 2.83^{cA}
48	*C. sinensis*	0 ± 0^{aG}	11.2 ± 1.50^{bF}	24.8 ± 1.50^{bE}	50.4 ± 3.25^{bD}	70.4 ± 2.71^{bC}	88.0 ± 1.26^{bB}	100 ± 0.00^{aA}
	P. granatum	0 ± 0^{aG}	14.4 ± 1.60^{aF}	32.8 ± 3.44^{aE}	58.4 ± 2.04^{aD}	80.0 ± 3.35^{aC}	92.8 ± 2.33^{aB}	100 ± 0.00^{aA}
	O. sativa	0 ± 0^{aG}	6.4 ± 0.98^{cF}	13.6 ± 2.40^{dE}	32.8 ± 1.50^{dD}	51.2 ± 3.44^{dC}	70.4 ± 2.71^{dB}	90.4 ± 2.71^{cA}
	T. aestivum	0 ± 0^{aG}	3.2 ± 0.80^{eF}	6.4 ± 0.98^{eE}	12.0 ± 2.83^{fD}	21.6 ± 2.71^{fC}	35.2 ± 2.65^{fB}	55.2 ± 2.33^{eA}
	C. esculenta	0 ± 0^{aG}	4.0 ± 1.79^{eF}	7.2 ± 0.80^{fE}	13.6 ± 2.04^{eD}	26.4 ± 2.04^{eC}	48.0 ± 2.83^{eB}	65.6 ± 2.71^{dA}
	B. oleracea	0 ± 0^{aG}	8.0 ± 1.26^{cF}	21.6 ± 2.04^{cE}	45.6 ± 2.71^{cD}	62.4 ± 2.40^{cC}	84.0 ± 2.83^{cB}	95.2 ± 2.33^{bA}

a, b & c: There is no significant difference (P > 0.05) in the same superscript letter between any two media within the same column. A, B & C: There is no significant difference ($P > 0.05$) between any two means within the same row with the same superscript letter.

Table 3
Lethal concentrations (ppm) of *Citrus sinensis, Punica granatum, Oriza sativa, Triticum aestivum, Colocasia esculenta,* and *Brassica oleracea* methanol extracts against *Cx. pipiens*, 24 and 48 h post-treatment.

Time (hr)	Plant	LC_{50} (Low-Up.)	LC_{90} (Low-Up.)	LC_{95} (Low-Up.)	Slope ± SE	X^2 (Sig.)
24	*C. sinensis*	370.72 (318.90–431.39)	1952.82 (1516.14–2697.58)	3127.66 (2308.75–4633.92)	1.776 ± 0.129	1.122 (0.890)
	P. granatum	314.43 (273.46–360.78)	1457.33 (1177.62–1896.51)	2250.92 (1745.52–3098.10)	1.924 ± 0.127	4.668 (0.323)
	O. sativa	666.67 (568.61–794.08)	3712.82 (2729.32–5564.18)	6041.11 (4192.38–9812.90)	1.718 ± 0.133	1.179 (0.881)
	T. aestivum	2502.84 (1798.19–4033.67)	24,911.5 (12,301.7–171,012.88)	47,785.58 (21,050.32–171,012.88)	1.284 ± 0.146	0.584 (0.964)
	C. esculenta	1798.03 (1356.79–2640.04)	17,627.78 (9483.87–44,451.18)	33.669.25 (16,298.66–99,935.33)	1.292 ± 0.135	0.634 (0.959)
	B. oleracea	465.59 (400.53–544.37)	2465.09 (1886.08–3478.10)	3953.86 (2872.04–5995.03)	1.770 ± 0.130	0.665 (0.955)
48	*C. sinensis*	253.19 (221.03–288.80)	1000.98 (823.68–1274.73)	1477.93 (1171.59–1982.16)	2.146 ± 0.147	3.950 (0.412)
	P. granatum	208.39 (180.46–239.32)	865.38 (701.11–1130.23)	1295.65 (1006.37–1796.38)	2.072 ± 0.154	6.590 (0.159)
	O. sativa	461.12 (398.45–536.34)	2286.61 (17,772.70–3161.88)	3598.08 (2658.04–5323.53)	1.843 ± 0.133	1.425 (0.839)
	T. aestivum	1775.87 (1353.80–2563.69)	15,991.36 (8855.50–38,374.16)	29,816.32 (14,938.15–83,426.38)	1.342 ± 0.138	0.699 (0.951)
	C. esculenta	1147.30 (936.73–1471.74)	7922.31 (5146.94–14,450.47)	13,700.49 (8245.54–27,937.30)	1.527 ± 0.137	1.511 (0.824)
	B. oleracea	335.92 (286.50–394.23)	1852.70 (1408.14–2652.89)	3006.21 (2159.69–4663.47)	1.728 ± 0.136	6.280 (0.179)

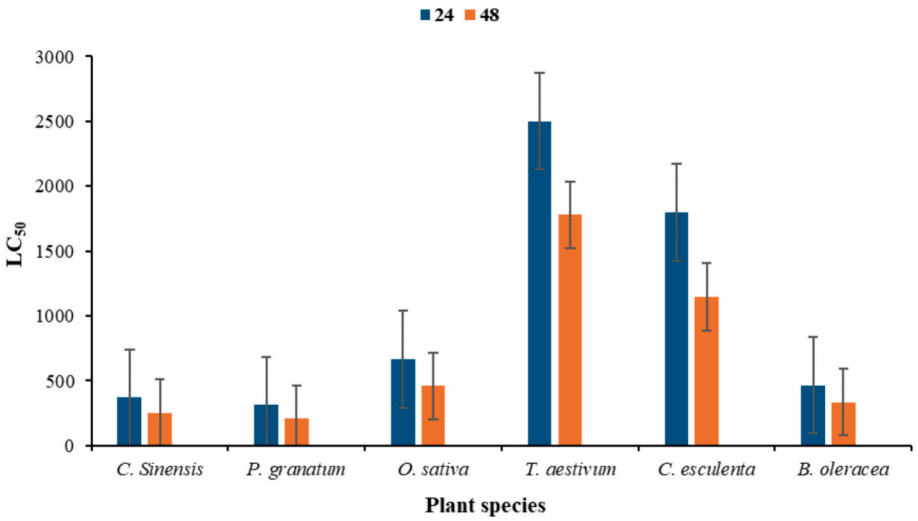

Fig. 1. The LC_{50} of larval mortalities induced by *Citrus sinensis, Punica granatum, Oriza sativa, Triticum aestivum, Colocasia esculenta,* and *Brassica oleracea* extracts against 4th larval instars of *Cx. pipiens*, 48 h post-exposure.

Table 4
Determination of acid and alkaline phosphatase, β-esterases, GST enzymes 4th larval instar of *Cx. pipiens* treated with LC_{50} of *Brassica oleracea, Citrus sinensis, Oriza sativa* and *Punica granatum.*

Enzymes	Control	*B. oleracea* (CA)	*O. sativa* (RA)	*C. sinensis* (OO)	*P. granatum* (PO)
GPT (μ/g/min)	209.06 ± 1.69^{a}	8.85 ± 2.85^{d}	136.25 ± 4.53^{c}	205.57 ± 4.92^{a}	159.87 ± 2.18^{b}
β -esterase (μg/g/min)	11 ± 0.44^{a}	8.56 ± 0.48^{b}	5.1 ± 0.33^{c}	10.65 ± 1.1^{a}	6.78 ± 0.34^{c}
Acid phosphatase (U/g)	29.47 ± 0.07^{a}	16.89 ± 0.06^{d}	18.82 ± 0.02^{c}	22.73 ± 0.06^{b}	14.93 ± 0.01^{e}
Alkaline phosphatase (U/g)	135.82 ± 4.92^{a}	105.83 ± 1.69^{b}	62.34 ± 0.39^{d}	82.12 ± 1.68^{c}	41.87 ± 0.71^{e}

This means that not sharing a letter in a raw is significantly different. CA: *B. oleracea*; RA: *O. sativa*; OO: *C. sinensis*; PO: *P. granatum.*

%), 9-octadecenoic acid (z)- (15.81 %), 3,5-heptadienal, 2-ethylidene-6-methyl (13.27 %), and 9,12,15-octadecatrienoic acid, 2-[(trimethylsilyl) oxy]-1-[[(trimethylsilyl)oxy]methyl]ethyl ester, (z,z,z)- (10.86 %) (Table 6). *Oryza sativa* extract had 12 compounds: caryophyllene oxide (37.70 %), 1,6,10-dodecatrien-3-ol, 3,7,11-trimethyl- (28.34 %), and hexadecanoic acid, 2,3-hydroxypropyl ester (10.10 %), which had the greatest concentrations of the detected compounds (Table 7). While in *Punica granatum* extract, 11 compounds were detected: desulphosinigrin (18.26 %), 9-octadecenoic acid (z)- (17.70 %), 9,12-octadecadienoyl chloride (z) (16.33 %), and hexadecanoic acid, 2,3-hydroxypropyl ester (12.64 %) (Table 8).

3.5.2. HPLC analysis

Table 9, and Fig. 5 show the recorded concentrations of different active phenolic and flavonoid compounds in four tested extracts: *B. oleracea* (CA), *C. sinensis* (OO), *P. granatum* (PO), and *O. sativa* (RA). Data showed that CA extract was characterized by Quercetin concentration, while OO extract highlighted its content from gallic; on the other hand, Po contains a significant amount of P-Coumaric. Finally, the RA extract shows that it begins with the most abundant compound in the extract.

3.5.3. Docking investigation (docking on the enzyme receptor)

The docking procedure was initially validated by redocking the co-crystallized ligand Alanine against GPT (**PDB ID:** 2HUU), Benzoic acid against β-esterase (**PDB ID:** 3O9M), and 2-acetamido-2-deoxy-beta-D-glucopyranose against Acid phosphatase (**PDB ID:** 1WAR), and Alkaline phosphatase (**PDB ID:** 1K7H) in the enzyme binding pocket with an energy score (S) = −3.5339, −4.5838, −4.8903, and − 4.078 kcal/mol respectively. The docking energy score of the docked tested compounds Quercetin against GPT (**PDB ID:** 2HUU), Apigenin against β -esterase (**PDB ID:** 3O9M), and p-Coumaric against Acid phosphatase (**PDB ID:**

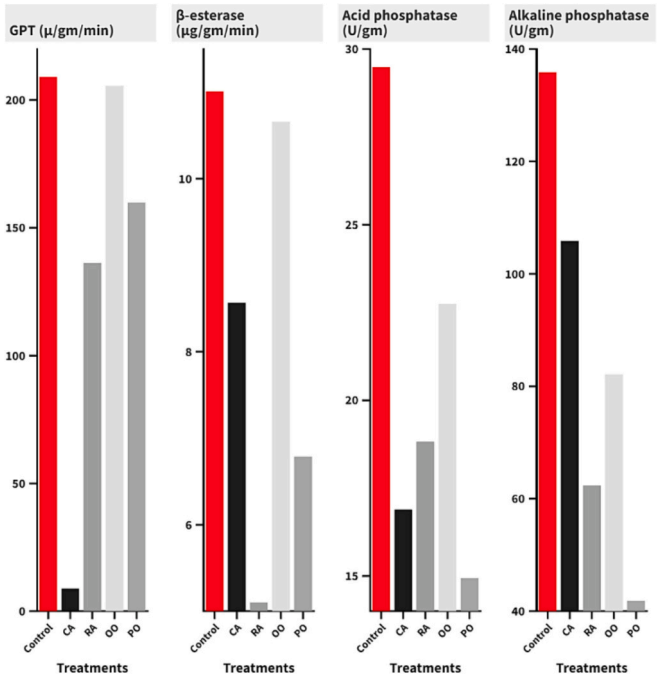

Fig. 2. Determination of acid and alkaline phosphatase, β-esterases, GST enzymes for 4th larval mosquito.

1WAR), and Alkaline phosphatase (**PDB ID:** 1K7H) found to be −5.079, −6.1145, −5.0348 and −4.3119 kcal/mol respectively, which are more than this of the co-crystallized ligand as shown in Table 10. The greater the engagement, the lower the energy score. As a result, the evaluated compound is more engaged with the tested enzyme, which explains the observed inhibition. These results are in line with the in-vivo assay's experimental results.

The similarity of experimental data with theoretical data is relatively remarkable compared to data produced through theoretical calculations. Interaction is recognized as the most crucial factor regulating the biological activity of compounds against enzymes (Table 10 and Fig. 6).

4. Discussion

Extensive research has demonstrated the toxicity of synthetic pesticides often used in commercial settings, impacting not only people and other animals as well as non-target plants, and the environment. Furthermore, there is growing apprehension regarding the emergence of pest resistance to these artificial pesticides. Biopesticides, referred to as pesticides originating from natural sources such as fungi, bacteria, plants, animals, and certain minerals, are emerging as alternatives to conventional pesticides and are garnering growing interest as environmentally friendly and safer insecticides employed in pest control [11].

Within this category, plant-based biopesticides from agricultural waste represent a very modest but essential subset of biopesticides. These wastes provide potential environmentally friendly alternatives to synthetic chemical pesticides. To enhance food productivity, sustainable agriculture requires the implementation of innovative solutions in addition to expanding the agricultural area, which results in many forms of agricultural waste or green waste (GW), and this green waste has emerged as a prevalent type of organic waste, especially with the growth of urban areas [34]. Which is collected from the fields after planting or during cultivation in the form of agricultural waste consisting of different components of plants such as seeds, roots, leaves, stems, bark, resin, fruits, and fruit peels.

The current research showed that plant extracts from *P. granatum, C. sinensis, O. sativa,* and *B. oleracea* were very good at killing *Cx. pipiens* larvae. On the other hand, plant extracts from *T. aestivum* and *C. esculenta* were not as good at killing mosquito larvae. In a study parallel to ours, El-Maghraby et al. [35] evaluated the toxicity of some agricultural waste extracts (apricot kernel, eucalyptol, corn, white liquor, black liquor and rice bran) against mosquito larvae, where the black liquor was effective in controlling *Cx. pipiens*.

A previous study examined some natural agricultural and industrial waste products as natural insecticides against mosquito larvae. These included extracts from apricot pits, Lipton tea, burnt rice straw, date palm kernels, and an extract from cigarette filters. The results concluded that cigarette filters (100 %), palm kernel (90 %) and Lipton tea (97 %) mortality were the most effective in killing mosquito larvae [15].

Many previous studies have examined the efficacy of plant extracts derived from local plants in Egypt and different regions of the world [26,36,37], with particular emphasis on their potential toxicological effects on diverse insect species. This study targets to evaluate the utilization of plant wastes and convert them into valuable resources that are effective as insecticides against many pests. The examined extracts showed differences in mosquito larval mortality rates between different types of plant and industrial waste extracts. These differences are due to the potency of the extracts tested and often to the primary components of each extract.

The above emphasizes the importance of agricultural waste as a natural pesticide. The importance of agricultural waste in many areas of industry has also been monitored. GW has been used primarily for fertilization for an extended period [38]. In addition, it has been used as a substrate for synthesizing char, biogas, and bioethanol in recent studies [39,40]. However, GW is a promising energy source for microbial processes, leading to the synthesis of commercially viable products such as polyaromatic hydrocarbons (PHAs), enzymes, and lipids [41]. In a recent study by Yuan et al. [42], GW served as the substrate for biohydrogen creation.

Numerous plant extracts and oils exhibit larvicidal, ovicidal, pupaecidal, and repellant properties against several mosquito species. The repellent activity of plant extracts or essential oils derived from plants belonging to the *Poaceae, Rutaceae, Lamiaceae,* and *Myrtaceae* families has been widely recognized [43]. Due to their low toxicity, the U.S. Environmental Protection Agency (US EPA) has recommended commercially available essential oils derived from eucalyptus, lemon and citronella as repellent components for topical application. One instance of an active constituent in the lemon eucalyptus plant is P-menthane-3,8 diol (PMD), which is accountable for the mosquito-repellent properties [44].

Phosphatases and transaminases are highly valuable for diagnosis because they are crucial in various insect body processes. They are involved in producing food, fibrous proteins, and the growth and maturation of insect eggs [45]. Significant inhibition of GPT, ACP, and AlP activity of *Cx. pipiens* were treated with the LC_{50} concentrations of all tested extracts except *Citrus sinensis*, which showed a negligible decrease compared to GPT. This means that various physiological functions of the insect body, such as growth, development, and reproduction, are inhibited, ultimately leading to death. The current results come in accordance with previous studies [46,47] that various types of stress, diseases, and toxic chemicals significantly decrease ALP activity. Furthermore, it was consistent with previous results [42,48] showing the inhibitory effect of some plant oils on ACP and ALP in treated Khapra beetle larvae, *Trogoderma ranarium* and grasshopper nymphs *Eupropocnemis plorans*, respectively. Previous studies reported that insecticide use can have several sublethal effects on enzyme activities in insect [45]. Therefore, all tested extracts were suspected to have promising insecticidal activity.

Studies suggest that esterase plays a crucial role as a detoxifying enzyme in insects. Esterase is vital in detoxifying and metabolizing many external and internal toxins, making it essential for insects [49]. The larvae of *Choristoneura rosaceana* treated with *M. azedarach* oil exhibited a significant reduction in β -CE activity [50]. Koodalingam,

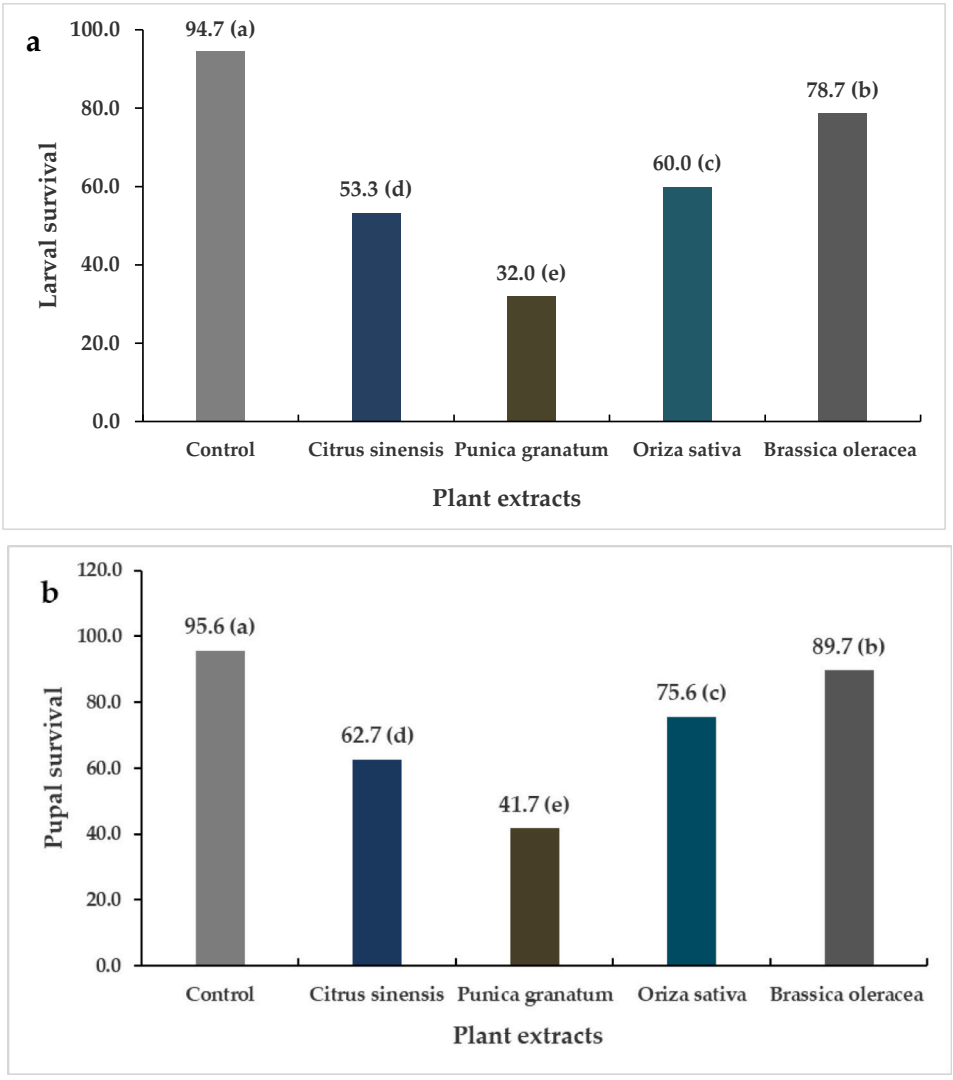

Fig. 3. Larval (a) and pupal (b) survival (%) of *Cx. pipiens* mosquitoes that remained alive after 48 h exposure of 3rd instar larvae to LC_{50} concentrations of plant extracts. Percentages in a column followed by a different letter are significantly different ($p < 0.05$).

Mullainadhan and Arumugam [51] demonstrated that releasing *Aedes aegypti* larvae to harvest soapnut, Sapindus emarginatus, led to a substantial decrease in β-CE activity, with no impact on α-CE activity. Mujeeb and Shakoori [52] demonstrated that Fury, a synthetic pyrethroid, hinders the carboxylesterase (CE) activity in all developmental stages of the red flour beetle, *T. castaneum*. This finding is consistent with Taha et al. [53], who observed a notable reduction in enzyme activity in *T. absoluta* when exposed to cloves, cumin, garlic, and dill extracts. Ali et al. [54] showed that extracts from *C. colocynthis* and *M. azedarach* significantly inhibited β-carboxylesterase activities in *Trogoderma granarium* Everts, *Tribolium castaneum* (Herbst), and *Sitophilus granarius (L.)*. The results showed a significant suppression of α -CE and β -CE activity in *S. granaries*, *T. castaneum* and *T. granarium,* and when exposed to a 20 % concentration. Dose-dependent responses of beta-carotene ester activity.

Plant extracts showed a significant effect on the survival and growth rates of *Cx. pipiens* after 48 h exposure to LC_{50} concentrations of plant extracts, coupled with the failure of adult emergence compared to untreated groups, indicating a possible growth inhibitory activity that interferes with the moulting process. The pomegranate plant had the greatest share in inhibiting or reducing the survival of larvae until they reached the adult stage. Numerous plant species' secondary metabolites exhibit growth-inhibiting effects on several mosquito species' developmental phases, including delayed death, notably during moulting, and increased growth of larvae and pupae [55,56]. Shaalan et al. [57] stated that exposure to these substances usually results in morphological anomalies in mosquitoes, such as lack of melanin in the larval stages, immature pupae with a long abdomen, dead larvae, and pupa intermedia with pupae head and larval abdomen (larval pupae). The presence of folded wings in adulthood during pupal moulting and the appearance of being unable to break free from the pupal exoskeleton indicate that metamorphosis is inhibited, maybe as a result of hormone disruption or disruption of chitin synthesis during the moulting process [55]. The prolonged development of larvae and pupae following exposure to secondary metabolites suggests disruption of the normal hormonal activity of metabolic processes (endocrine mechanism) during the developing phases [58]. Nevertheless, the treatment with oregano oil did not have an impact on the growth of the larvae that survived to pupa and, ultimately, to adulthood. This may be because other substances were present in smaller amounts.

Most plant larvicidal agents contain chemicals that are directly toxic to mosquitoes when added to the water in which they breed, but some have growth-inhibitory effects similar to insect growth regulators, making mosquitoes less able to breed and reproduce [59]. For example, survival of *Anopheles gambiae* larvae was reduced by 60–95 % when exposed to pyridone alkaloids derived from sesquiterpene lactones and

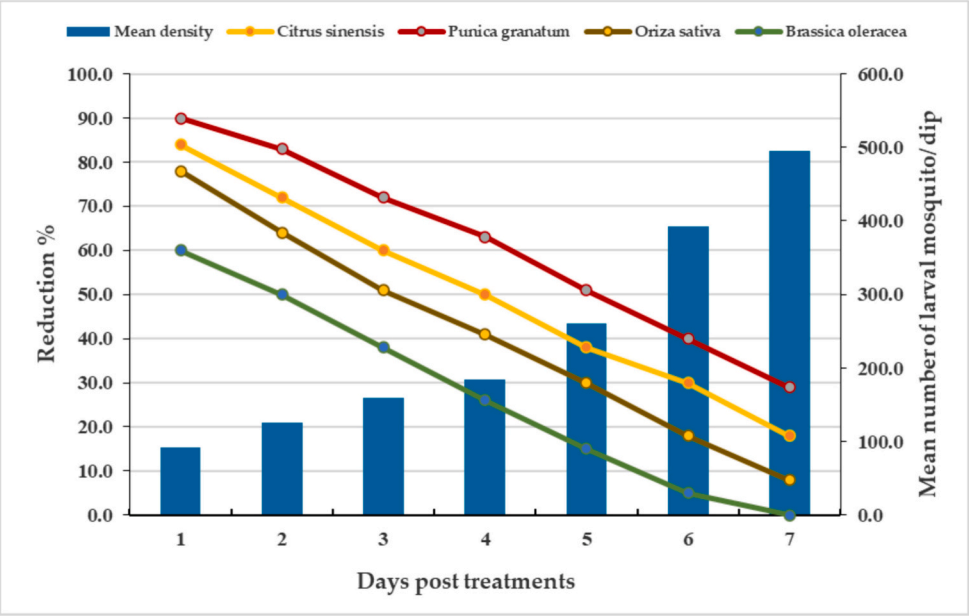

Fig. 4. Field evaluation for larvicidal efficacy of *Citrus sinensis, Punica granatum, Oriza sativa, and Brassica oleracea* extracts treated at a dose of LC95 X2 (6255.32, 4501.84, 12,082.22 and 7907.72 ppm respectively, in larval breeding sites.

Table 5
The major chemical constituents of *Brassica oleracea* extracts.

No	RT	Area %	Compound Nam	M. F.	M. W
			Fatty acid and Esters		
1	4.02	4.16	9-Octadecenoic acid (z)-	$C_{18}H_{34}O_2$	282
2	5.10	5.19	10-Heptadecen-8-yonic acid, methyl ester, €-	$C_{18}H_{30}O_2$	278
3	25.61	9.75	Cyclopropanebutanoic acid, 2-[[2-[[2-[(2-pentyl cyclopropyl)methyl] cyclopropyl]methyl]cyclopropyl] methyl]-, methyl ester	$C_{25}H_{42}O_2$	374
4	26.45	29.31	Hexadecanoic acid, 2,3-hydroxy-propyl ester	$C_{19}H_{38}O_4$	330
5	28.61	19.95	Hexadecanoic acid, methyl ester	$C_{17}H_{30}O_2$	226
6	28.92	3.23	6,9,12,15-Docosatetraenoic acid, methyl ester	$C_{23}H_{38}O_2$	346
7	29.65	18.52	Linoleic acid ethyl ester	$C_{20}H_{36}O_2$	308
			Phenol		
8	11.56	3.87	p-Coumaric (4-hydroxycinnamic)	$C_9H_8O_3$	164
9	13.43	5.92	2,5,5,8a-Tetramethyl-1,2,3,5,6,7,8,8a-octahedron-1-naphthalenol	$C_{14}H_{24}O$	208

Table 6
The major chemical constituents of *Citrus sinensis* extracts.

No	RT	Area %	Compound Nam	M. F.	M. W
			Terpene (Monoterpene and Sesquiterpene)		
1	11.83	13.27	3,5-Heptadienal, 2-ethylidene-6-methyl-	$C_{10}H_{14}O$	150
			Alkanes		
2	6.96	6.19	Dotriacontane	$C_{32}H_{66}$	450
3	9.31	2.25	Cis-2-phenyl-1, 3-dioxolane-4-methyl octadic-9, 12, 15-tridentate	$C_{28}H_{40}O_4$	440
			Phenol		
4	5.08	8.40	Hi-oleic safflower oil	$C_{21}H_{22}O_{11}$	450
			Fatty acid and Esters		
5	25.61	5.88	Pentadecanoic acid, 14-methyl-, methyl ester	$C_{17}H_{34}O_2$	270
6	26.45	20.51	Hexadecanoic acid, 2,3-hydroxy-propyl ester	$C_{19}H_{38}O_4$	330
7	28.62	3.87	9,12,15-Octadecatrienoic acid, 2,3-dihydroxypropyl ester, (z,z,z)-	$C_{21}H_{36}O_4$	352
8	28.79	9.70	9-Octadecenoic acid (z)-	$C_{18}H_{34}O_2$	282
9	29.36	3.26	Methyl-9,9,10,10-d4-octadecanoate	$C_{19}H_{34}D_4O_2$	302
10	29.63	15.81	9-Octadecenoic acid (z)-	$C_{18}H_{34}O_2$	282
11	30.11	10.86	9,12,15-Octadecatrienoic acid, 2-[(trimethylsilyl)oxy]-1-[[(trimethylsilyl)oxy]methyl]ethyl ester, (z,z,z)-	$C_{27}H_{52}O_4Si_2$	496

Ricinus communis obtained from *Tithonia diversifolia*, with LC_{50} values of 0.18 mg/ml and 0.33 mg/ml, respectively [40]. Previous studies by Pradeepa et al. [60] demonstrated the action of naphthoisoquinolines derived from *Plumbago zeylanica* and *Lantana viburnoides* against *An. gambiae* and *An. arabiensis* larvae. Research on chemicals that regulate insect growth and essential oils as plant-derived mosquito control agents is widespread due to their inhibitory factors.

Study data recorded the ability of plant extracts (*C. sinensis, P. granatum, O. sativa,* and *B. oleracea*) to lower the mosquito larvae's density in ditches. Among them, the pomegranate extract had a clear effect, and its effect continued to lower the mosquito larvae's density in the field for up to five days. Among the studies that agree with us and are parallel is the study of Radwan et al. [61], which pointed out the power of fennel oil and green tea to reduce mosquito larvae to 96.2 % for 24 h while remaining stable for five and seven days, respectively. Some studies have observed the lethal effect of plant extracts or essential oils on laboratory and field strains of *Cx. pipiens* larvae. Researchers have discovered that these oils negatively impact pupal and adult emergence rates, leading to abnormalities in larval development [3,62,63].

Plants are living entities that synthesize a variety of compounds called secondary metabolites. The pharmacological effects of therapeutic plants are due to secondary metabolites like alkaloids, flavonoids, carbohydrates, tannins, terpenoids and saponins. Various bioactive compounds included in botanical insecticides might cause harmful effects on pests or creatures that come into contact with them [64,65].

Our data showed that GC–MS analysis in our study led to the identification of various compounds such as terpenes, esters, fatty acids, alkanes, ketones, aliphatic amines, steroids, and phenols in the leaves of

Table 7
The major chemical constituents of *Oryza sativa* extracts.

No	RT	Area %	Compound Nam	M. F.	M. W
			Terpene (Monoterpene and Sesquiterpene)		
1	5.08	2.37	p-Cymene	$C_{10}H_{14}$	134
2	15.33	5.95	a-Humulene	$C_{15}H_{24}$	204
3	17.88	28.34	1,6,10-Dodecatrien-3-ol, 3,7,11-trimethyl-	$C_{15}H_{26}O$	222
4	19.68	37.70	Caryophyllene oxide	$C_{15}H_{24}O$	220
			Phenol		
5	17.68	1.86	Nerolidol-epoxy acetate	$C_{17}H_{28}O_4$	296
			Alkane		
6	6.95	1.03	Dodecane	$C_{12}H_{26}$	170
			Fatty acid and Esters		
7	17.55	3.86	Cis-5,8,11,14,17-eicosapentaenoic acid	$C_{20}H_{30}O_2$	302
8	21.81	1.22	Androstane-17-one, 3-ethyl-3-hydroxy-, (5à)-	$C_{21}H_{34}O_2$	318
9	26.54	10.10	Hexadecanoic acid, 2,3-dihydroxypropyl ester	$C_{19}H_{38}O_4$	330
10	28.81	3.47	10-Octadecenoic acid, methyl ester	$C_{19}H_{36}O_2$	296
11	29.64	2.86	Linoleic acid ethyl ester	$C_{20}H_{36}O_2$	308
12	35.79	1.24	9,12,15-Octadecatrienoic acid, 2,3-bis[(trimethylsilyl)oxy]propyl ester, (z,z,z)-	$C_{27}H_{52}O_4Si_2$	496

Table 8
The major chemical constituents of *Punica granatum* extracts.

No	RT	Area %	Compound Nam	M. F.	M. W
			Phenol		
1	4.11	16.33	9,12-Octadecadienoyl chloride, (z,z)-	$C_{18}H_{31}ClO$	298
2	4.69	4.61	17-Octadecynoic acid	$C_{18}H_{32}O_2$	280
3	15.51	8.11	3-Furan acetic acid, 4-hexyl-2,5-dihydro-2,5-dioxo-	$C_{12}H_{16}O_5$	240
4	17.95	18.26	Desulphosinigrin	$C_{10}H_{17}NO_6S$	279
5	6.96	3.30	2,2,3,3,4,4 Hexadeutero octadecanal	$C_{18}H_{30}D_6O$	274
			Fatty acid and Esters		
6	7.69	5.72	Cyclohexane carboxylic acid, 2-hydroxy-, ethyl ester	$C_9H_{16}O_3$	172
7	15.04	4.16	à-D-Galactopyranose, 6-o-(trimethylsilyl)-, cyclic 1,2:3,4-bis (methyl boronate)	$C_{11}H_{22}B_2O_6Si$	300
8	25.60	3.76	Methyl-9,9,10,10-d4-octadecanoate	$C_{19}H_{34}D_4O_2$	302
9	26.39	12.64	Hexadecanoic acid, 2,3-dihydroxypropyl ester	$C_{19}H_{38}O_4$	330
10	29.61	17.70	9-Octadecenoic acid (z)-	$C_{18}H_{34}O_2$	282
11	30.06	5.41	Hexadecanoic acid, 2,3-dihydroxypropyl ester	$C_{19}H_{38}O_4$	330

B. oleracea, C. sinensis, O. sativa, and P. granatum using methanol solvent. The cabbage extract stood out due to its specific presence of fat molecules with at least 16 carbons in its chain, such as hexadecanoic acid and linoleic acid ethyl ester. These phytochemical compounds are saturated fatty acids that are more prevalent in plants, animals, and microbes.

On the other hand, the pomegranate and orange extracts stood out due to their diverse composition of fatty acid, phenol, and terpene components. Several higher plant organs, such as grains, legumes, nuts, fruits, vegetables, and legumes, contain phenolic compounds, a broad family of secondary metabolites. These days, research and applications pertaining to the intrinsic anti-inflammatory, antibacterial, anticarcinogenic, and antioxidant capabilities of phenolic compounds are highly desired. These substances can be divided into numerous types; the primary categories are phenolic acids, flavonoids, stilbenes, tannins, and lignans [66].

Table 9
HPLC analysis of phenolic and flavonoid compounds in *Brassica oleracea, Citrus sinensis, Punica granatum,* and *Oriza sativa* extracts. ND not found.

No	Compound	CA	OO	PO	RA
1	Querestin	750.10	448.52	540.63	184.05
2	Kampferol	498.02	204.44	184.06	155.19
3	Luteolin	250.01	380.78	539.41	ND
4	Apegenin	210.13	89.58	178.36	605.16
5	Catechin	280.20	320.09	420.70	450.02
6	Naringin	ND	215.68	192.05	150.47
7	Rutin	ND	330.85	201.88	580.96
8	Chlorogenic	90.23	410.59	ND	510.22
9	p-Coumaric	ND	112.32	656.08	ND
10	Catechol	350.63	ND	250.56	ND
11	Syringenic	300.96	105.04	210.45	430.08
12	Cinnamic	270.42	430.89	ND	ND
13	Caffeic	210.70	136.54	510.85	188.79
14	Pyrogallol	100.05	ND	ND	439.77
15	Ferulic	95.26	322.44	490.47	75.04
16	Salicylic	115.52	ND	ND	ND
17	Gallic	ND	625.07	201.45	ND

Despite this, the study's findings support the effectiveness of rice straw due to its high terpene and other chemical content. Neem extracts, orange or citrus oils, and essential oils isolated from *Chenopodium ambrosioides* and other plants are examples of terpene-based biopesticides [67]. According to Raina et al. [68], the main component of orange or citrus oils is D-limonene, which is known to be harmful to a variety of insect pest species.

A broad spectrum of plant species contains phytochemical components that function as antioxidants. Furthermore, apart from well-known antioxidants like vitamins C and E and B-carotene, there are several phytochemicals, including quercetin and various flavonoids [69].

Caryophyllene and caryophyllene oxide were the main monoterpenes and sesquiterpenes found in the studied *O. sativa* leaf extracts. The primary ingredients, which include caryophyllene oxide, eucalyptol and caryophyllene (also known as caryophyllene), may be responsible for the insecticidal action. This finding fits with what Zoubiri and Baaliouamer [70] found. They also said the main parts, like b-caryophyllene and caryophyllene oxide, could kill insects. Furthermore, research has shown that caryophyllene oxide, spathulenol, and germacrene-D can help fight cancer, reduce inflammation, kill insects and pests, and kill bacteria [71].

Polyphenols, or phenolic compounds, are a broad class of substances that include phenolic acids, vibrant anthocyanins, basic flavonoids and intricant flavonoids. Plant defense mechanisms commonly link phenolic compounds. Phenolic metabolites are essential for many biological processes, such as adding attractive compounds to help pollination, coloration to help plants hide and protect themselves from herbivores, and antibacterial and antifungal properties [64,72].

Current studies show that *P. granatum* extract contains many phenolic compounds, including P-Coumaric, Caffeic, and Ferulic. This explains the high toxicity of the extract against mosquito larvae. *P. granatum* MgO-NPs showed hopeful effect in inhibiting the growth of pathogens and inhibiting the development of *Cx. quinquefasciatus* larvae into adults [73]. A study by Pavela [74] looked at how harmful thirteen phenols and eight phenolic acids were to adult of *M. domestica* adults and larvae of *Cx quinquefasciatus*. The study revealed that, except for salicylic acid, the phenolic acids exhibited moderate toxicity, where mortality reached 100 % for 24 h following treatment.

A wide range of medical and veterinary diseases have been addressed through the utilization of medicinal plants following the recognition of their therapeutic capabilities [75–77]. Pharmaceuticals, particularly those with anti-cancer and antibacterial properties, should be derived from natural substances from plants and animals. In human illness treatment, contemporary medicine has superseded its earlier, more antiquated antecedent [42]. Medicinal plant use has increased significantly in recent years, especially in industrialised countries, with the

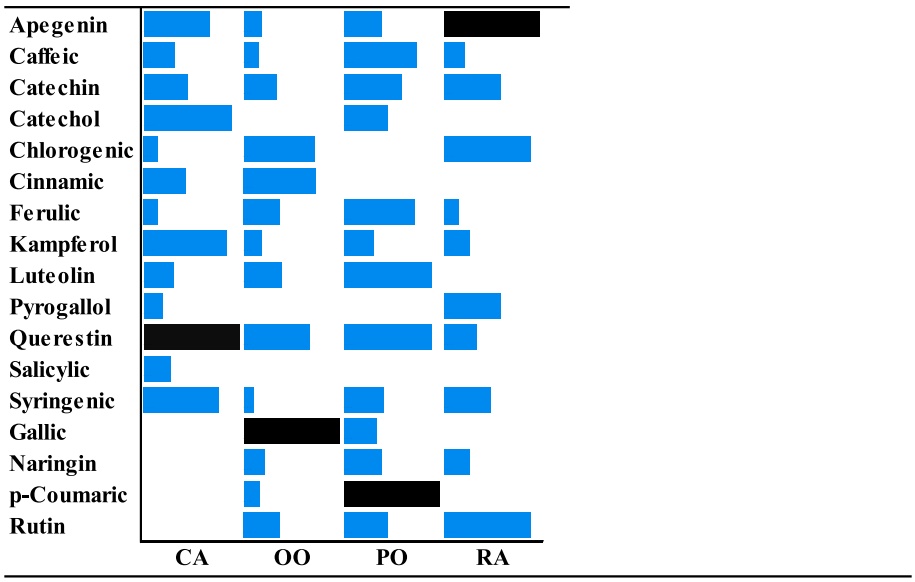

Fig. 5. Polyphenols and flavonoids detection by HPLC in *Brassica oleracea* (CA), *Citrus sinensis* (OO), *Punica granatum* (PO), and *Oryza sativa* (RA).

Table 10
Docking interaction data calculations of co-crystallized GPT (**PDB ID:** 2HUU) against Quercetin, β -esterase (**PDB ID:** 3O9M) against Apigenin, and Acid phosphatase (**PDB ID:** 1WAR), and Alkaline phosphatase (**PDB ID:** 1K7H) against p-Coumaric in the enzyme binding pocket with the active site of the receptor of those enzymes.

Enzyme	Compounds	Energy score (S) (Kcal/mol)	Affinity Bond strength (Kcal/mol)	Affinity Bond length (in A° from the main residue)	Amino acid	Ligand	Interaction
GPT	Ligand (Alanine)	−3.5339	−0.8	3.11	ARG 368	O 8	H-acceptor
	Quercetin	−5.079	−2.6	2.9	ASN 19	O 31	H-donor
			−1.5	4.96	ALA 30	6-ring	pi-H
	Ligand (Benzoic acid)	-4.5838	−1.2	3.83	TRP 82	6-ring	pi-pi
β-esterase (PDB ID: 3O9M)			−5	2.85	ASP 70	O 25	H-donor
	Apigenin	−6.1145	−2.5	2.78	SER 198	O 14	H-acceptor
			−3.4	3.02	HIS 438	O 14	H-acceptor
			−0.6	3.88	PHE 329	6-ring	pi-H
	Ligand (2-acetamido-2-deoxy-beta-D-glucopyranose)	-4.8903	−0.7	2.99	ASP 156	O 21	H-acceptor
			−4.5	2.89	ASN 142	O 28	H-acceptor
Acid phosphatase (PDB ID: 1WAR)			−1.4	2.9	THR 138	O 16	H-donor
	p-Coumaric	−5.0348	−2.6	2.91	HIS 92	O 19	H-donor
			−2.2	2.96	ASN 142	O 18	H-acceptor
			−0.8	3.93	THR 138	6-ring	pi-H
Alkaline phosphatase (PDB ID: 1K7H)	Ligand (2-acetamido-2-deoxy-beta-D-glucopyranose)	−4.078	−0.8	3.29	ARG 406	O 28	H-acceptor
	p-Coumaric	−4.3119	−4.1	2.96	THR 388	O 18	H-acceptor
Enzyme			2D		3D		

purpose of curing diseases and improving health [78]. Several developed nations, such as China, United Kingdom, France and Germany, currently employ medicinal plant extracts as prescription medications [79]. Paclitaxel and morphine, which are widely used medications, are obtained from plants, and make up approximately 25 % of all prescriptions approved by the European Medical Agency (EMA) and/or the FDA [80].

5. Conclusion

Mosquitoes pose a threat to human health because they are major vectors for many deadly pathogens. Synthetic insecticides are widely used to control mosquito-borne diseases, but due to their toxicity to the environment and non-target organisms, a safer, biodegradable, less toxic, and more effective alternative for mosquito management is required. The study data showed that agro-waste plant extracts not only kill mosquito larvae but also reveal the presence of several biologically active organic compounds through GC–MS and HPLC analysis. Studies should focus on exploring and trying to exploit plant wastes on a large scale as a safe and reliable natural pesticide to reduce mosquito hazards and stop mosquito-borne diseases.

Consent for publication

Not applicable.

Availability of data and materials

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

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CRediT authorship contribution statement

Mohamed A.M. El-Tabakh: Writing – original draft, Visualization, Methodology, Investigation, Data curation, Conceptualization. **Abdelfattah Selim:** Writing – original draft, Visualization, Software, Funding

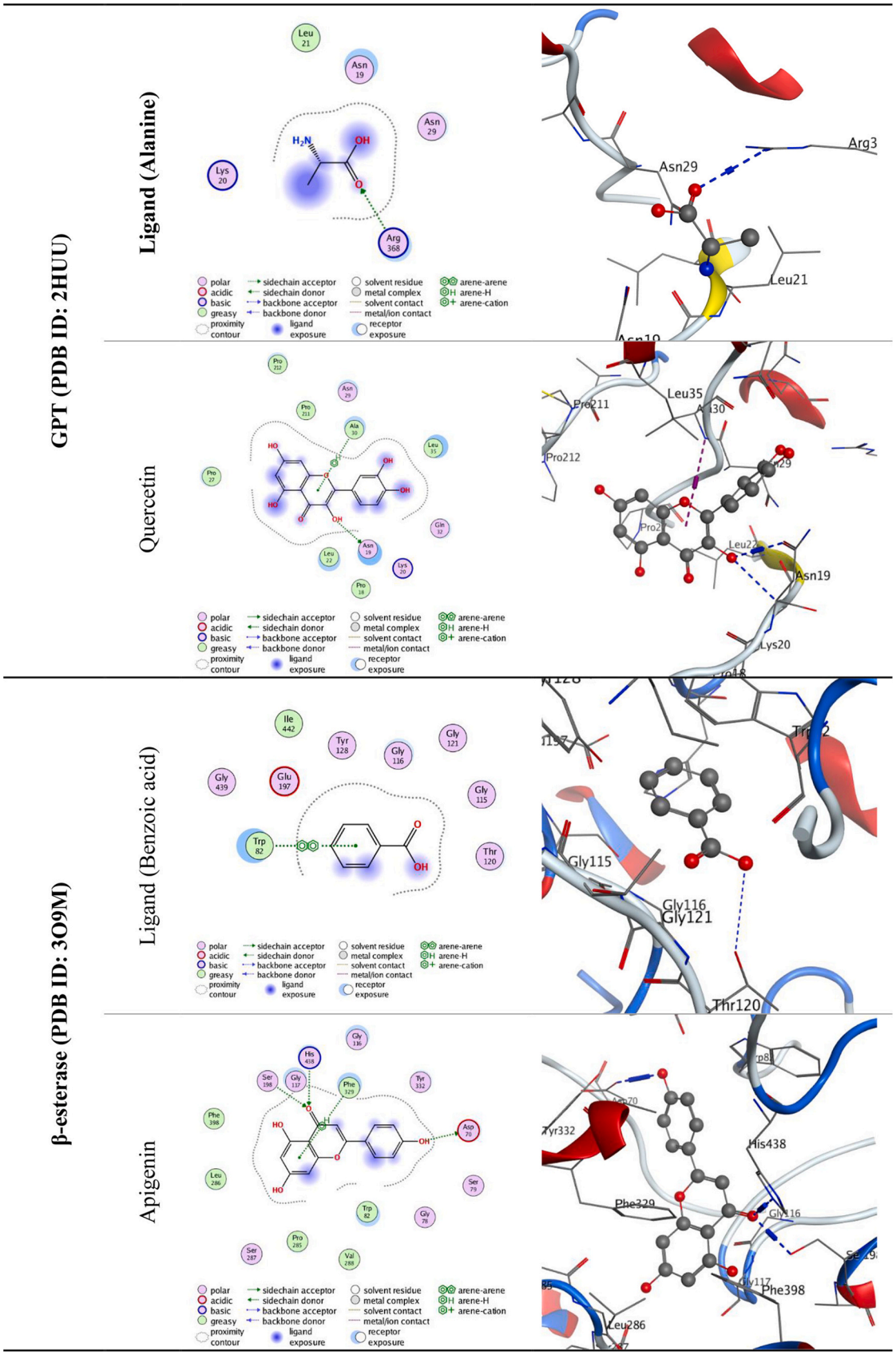

Fig. 6. Docking interaction data calculations of co-crystallized GPT (**PDB ID:** 2HUU) against Quercetin, β -esterase (**PDB ID:** 3O9M) against Apigenin, and Acid phosphatase (**PDB ID:** 1WAR), and Alkaline phosphatase (**PDB ID:** 1K7H) against p-Coumaric in the enzyme binding pocket with the active site of the receptor of those enzymes.

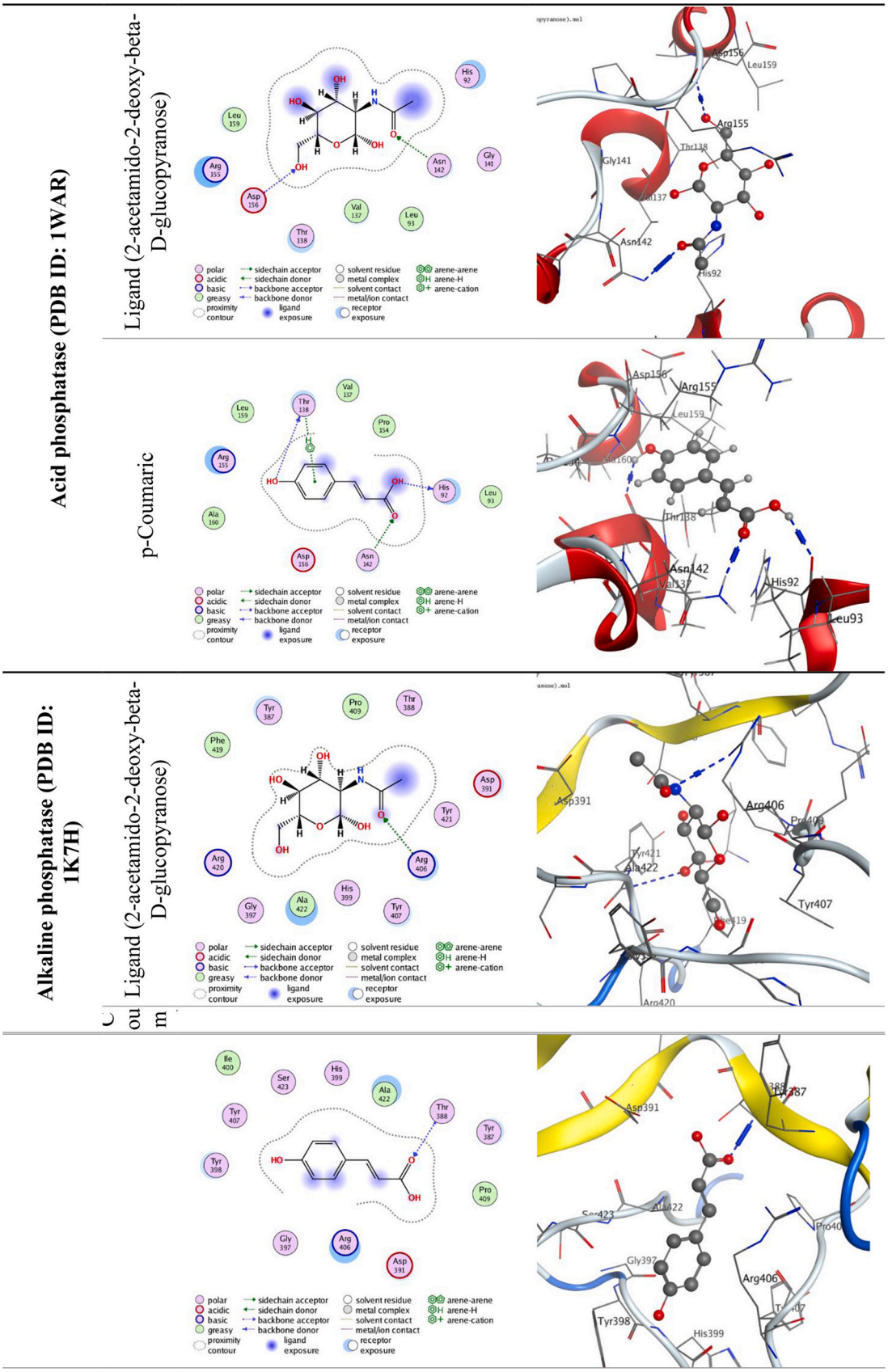

Fig. 6. (*continued*).

acquisition, Data curation, Conceptualization. **Saeed M. Alasmari:** Writing – original draft, Visualization, Validation, Project administration, Methodology, Formal analysis, Conceptualization. **Abeer Mousa Alkhaibari:** Conceptualization, Formal analysis, Investigation, Methodology, Validation, Visualization, Writing – original draft. **Mohammed H. Alruhaili:** Writing – original draft, Supervision, Methodology, Formal analysis, Data curation, Conceptualization. **Hattan S. Gattan:** Writing – original draft, Visualization, Supervision, Methodology, Investigation, Conceptualization. **Heba F. Abdelkhalek:** Writing – original draft, Validation, Software, Methodology, Formal analysis, Data curation.

Ethical consideration

The study was carried out according to the guidelines of the declaration of Benha University and approved by the Ethics Committee of the Faculty of Science, Benha University (Code: BUFS-REC-2024-255Ent).

Declaration of competing interest

There are no conflicts of interest declared by the authors.

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