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Larvicidal and adulticidal effects of combined hydroethanolic extracts of clove flower buds and garlic bulbs on *Anopheles gambiae*

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Abstract

Background Insecticides are toxic substances, though not necessarily toxins, intentionally released into the environment to kill or manage insect populations. These chemical toxins pose significant risks to human and animal health due to their direct effects and their impact as environmental pollutants. To mitigate these chemical hazards, the use of phytocompounds with superior larvicidal or mosquitocidal effects, low toxicity levels in mammals, and short-term environmental persistence may be a preferable alternative.

Methods This study aimed to evaluate the larvicidal and adulticidal effects of the combined hydroethanolic extracts of garlic and clove on *Anopheles gambiae*. The experiment included four treatment groups: garlic, clove, combined extract (garlic + clove), and deltamethrin (standard insecticide). At the end of the 24 h experiment, the knocked-down adult mosquitoes were homogenized and used for biochemical assays.

Results The combined garlic-clove extract significantly increased ($p < 0.05$) the larval mortality rate compared to the single garlic and clove extracts. Similarly, the combined extracts of garlic-clove significantly increased ($p < 0.05$) mortality of adult mosquitoes compared to deltamethrin and the individual garlic and clove extract. The garlic-clove combined extracts showed a significant decrease ($p < 0.05$) in the activity of acetylcholine esterase and Na–k ATPase compared with the deltamethrin group. Furthermore, the docking interaction between AChE and voltage gated ion channel with the GC–MS identified compounds of the extracts showed a higher binding affinity with caryophyllene, estrone, morellinol compared to deltamethrin.

Conclusions The efficacy of the garlic-clove extract as a larvicide and adulticide has been confirmed through biochemical analyses and in silico studies. This lethal effect is likely due to the inhibition of crucial enzymes that facilitate essential processes such as signal transduction and energy production. Moreover, the biocidal properties of these extracts stem from the alteration of vital metabolic pathways, influenced by a range of bioactive compounds found in both garlic and clove.

Keywords Insecticides, Plant extracts, *Anopheles*, Acetylcholinesterase, Gas chromatography-mass spectrometry, Molecular docking simulation

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Background

Controlling mosquito populations is crucial for the effective management of malaria infections in endemic regions. Mosquito bites transmit *Plasmodium* parasites, which can lead to fatal malaria in infants under 5 years old [1]. Mosquitoes can transmit the Zika virus, causing fatal damage to fetal brain cells during early development in the womb [2]. *Anopheles* mosquitoes, vectors of malaria, pose a significant risk to global health [3]. Mosquito proliferation is curbed by the use of insecticides, which can mediate the death of mosquitoes through different mechanisms. Therefore, understanding mosquito life cycles is essential for curbing the menace they cause as it helps identify the most effective stage in their development for control efforts. This breakthrough in the control of mosquito menace aligns with the World Health Organization's (WHO) Sustainable Development Goal to ensure Good Health and Well-Being. Previous studies have highlighted the potential of natural products such as garlic and cloves, which are rich in secondary metabolites, to control mosquito populations by effectively targeting and eliminating larvae or adults. Natural insecticides are derived from plant phytochemicals, such as alkaloids, phenolics, and terpenes, which have been shown to possess insecticidal properties [4]. They may also include a variety of essential oils and extracts derived from plant leaves, stems, roots, flowers, or fruits [5]. In general, they have low toxicity levels in mammals and exhibit temporal environmental persistence. Synthetic insecticides, however, are derived from man-made materials, including chlorines, organophosphates, and carbamates [5].

Clove contains different phytochemicals such as eugenol, vanillin, tannins, acetyl eugenol, and α- and β-caryophyllene [6]. Clove contains 90% eugenol, which is approximately 15 times less toxic than neem (3% azadirachtin), 1500 times less toxic than the botanical insecticide, and 15,000 times less toxic than the organophosphate insecticide azinphosmethyl [6]. Garlic is an effective alternative plant-derived pesticide and insecticide because of its ability to effectively control pests and non-toxic effects on non-target organisms [7]. Garlic has a strong aroma due to its major component, allicin, which contributes to its beneficial properties [8]. Its oil and extracts are toxic against 3rd stage larvae of several mosquito species [9]. Natural insecticides are sourced from phytochemicals like alkaloids, phenolic compounds, and terpenes, which are known for their insecticidal properties. These also encompass various essential oils and plant extracts obtained from leaves, stems, roots, flowers, or fruits. Typically, they exhibit low mammalian toxicity and transient environmental persistence [10]. Due to the hazards associated with synthetic insecticides, research is needed to identify eco-friendly alternatives. This study assessed the larvicidal and adulticidal effects of a combined hydroethanolic extract of clove flower buds and garlic bulbs on *Anopheles* mosquitoes.

Methods

Plant materials

Mature dried cloves and garlic bulbs were gotten from the local market in Omu-Aran Kwara state on the 11th of January 2024 and a plant scientist from the University of Ilorin Kwara State's Department of Plant Biology verified their authenticity and then gave the description details UILH/001/976/2024 for garlic and UILH/003/1107/2024 for the clove.

Extraction

The cloves and garlic were cleaned and sliced into fillets and then dried in an oven at 60 °C until a constant weight was attained [11]. Subsequently, they were pulverized to a powder using an industrial blender. Approximately 100 g of each powder was macerated separately in 500 ml of 75% ethanol in an Erlenmeyer flask and swirled on a swirling machine for 24 h. Subsequently, the mixtures were drained using muslin cloth and then filtered with filter paper. The filtrates were concentrated using a rotary evaporator and subsequently the dry weight was obtained by drying at 40 °C in water bath. The dry weight is then refrigerated until needed [12].

Mosquito collection

4th instar larvae of the Kisimu strain *Anopheles* mosquito were obtained from the Molecular Entomology and Vector Control Unit at the Nigerian Institute of Medical Research (NIMR) laboratory in Yaba Lagos. Morphological features used in identifying Anopheles gambiae (Fig. 1).

Insecticidal analysis

The World Health Organization (WHO) standard procedures evaluate the larvicidal efficiency of individual and combined plant extracts [13]. The plant extracts of clove, garlic, and garlic + clove mixtures were screened at 0.5%, 1%, and 1.5% (v/v) extract concentrations for the larvicidal bioassay. Twenty late third-to early fourth-instar larvae were placed in 300 ml enamel cups with 1 ml of the suitable extract concentrations and 99 ml of distilled water. Equal volumes of water were used as a negative control. In the insectary, the experiment was run for 72 h at an ideal temperature of 27 ± 3 °C and relative humidity of 70 ± 10%, with a 12:12 light: dark photoperiod. Each experiment had five replicates. Following exposure for 24, 48, and 72 h, the number of dead larvae was counted, and the average of five replicates was used to calculate the

Fig. 1 Morphological features used in identifying *Anopheles gambiae*. The dark spot at the upper margins of the wings, common to all Anopheles species, and the elongated palps with three segments typical for the Anopheles gambiae complex

percentage of mortality. LC50 and LC90 values calculated as the standards were expressed as the average mortality of the larvae that were classified as dead, which included moribunds—those incapable of rising to the surface in each concentration of treatments [14].

Adulticidal susceptibility testing

The filter papers were impregnated with 1 ml of extract, air-dried for 15–20 min, and slotted into holding tubes. Adulticidal susceptibility tests were then performed on 2-to 3 day-old female *Anopheles* mosquitoes following the protocol outlined by the WHO [15]. Four replicates consisting of 25 mosquitoes each were exposed to extract-impregnated paper containing cloves extract, garlic extract, a mixture of cloves and garlic extract, and deltamethrin (0.05%) as a positive control. The normal control group comprised mosquitoes from the LGA exposed to untreated papers:

i. Negative control
ii. Positive control (Deltamethrin 0.05%)
iii. Group 1: Garlic (100% extract) in 4 replicates of 25 mosquitoes each
iv. Group 2: Clove (100% extract) in 4 replicates of 25 each
v. Group 3: Clove + Garlic (100% extract at equal ratios) in four replicates of 25 replicates each. For a 60 min exposure, the knockdown effect was recorded every 10 min, and mortality was evaluated 24 h after exposure [16].

Mortality count

The quantity of dead mosquitoes in the exposure and control tubes following a 24 h exposure period is known as mortality. If a mosquito cannot move, stand, or fly with coordination, it is deemed dead. The number of dead mosquitoes from each of the four replicated groups was added up, and the mortality was then expressed as a percentage of all the mosquitoes that were exposed:

$$\text{Observed mortality} = \frac{\text{Total number of dead mosquitoes} \times 100}{\text{Total number of sample size.}}$$

Preparation of homogenate

At the end of 60 min, the knockdown mosquitoes were homogenized and the homogenate was centrifuged at 3000 rpm for 10 min after which the supernatant was decanted and the residue discarded. After that, the homogenate was used for assays of enzymes.

Phytochemical identification

Quantitative analysis

The procedure described by Kumaran and Karunakaran [16] was adopted to quantify the content of flavonoids in garlic and clove plants. The procedure described by Hagerman, et al. (2000) was adopted for estimating the phenolic content of the garlic and clove plants. The procedure described by Obadoni and Ochuko [17] was adopted for the estimation of saponins in the leaf extract of garlic and clove plants. The estimation of tannins in the garlic and clove plants was carried out following the procedure described by Amadi et al. [18].

Gas chromatography-mass spectroscopy analysis (GC–MS)

Combined with an Agilean 3800/4000 gas chromatograph mass spectrometer, an Agilent splitter split/splitless was used to examine the samples chromatographically. With a BP5 (30 m × 0.25 mm × 0.25 microns) capillary column. The gas carrier used was Nitrogen. 1.0 µL volumes were injected using a splitless mode and an injector temperature of 270 °C. The temperature of the oven was increased from 80 to 200 °C at a pace of 50 °C per minute (one-minute hold). The oven temperature was maintained at 280 °C for six minutes following each analysis. The entire run time for each sample was approximately 45 min. During analytical scanning from 40 to 800 atomic mass units (amu), mass spectrometry mode was employed. The samples were injected after the injection of the blank. The samples contained organic compounds with comparison scores greater than 95% were identified utilizing the Mass Spectral Library of Wiley's NIST 08. If not, the constituents that were visible in GC–MS chromatograms were identified by using the memory background to identify the fragmentation peaks of the compounds. There was a percentage of each compound in the extract that was found in the entire sample. Following the integration of the chromatograms from the total ion count (TIC) without co-eluting peak correction, total abundance was reported. Utilizing mass spectral matching (≥ 90%) from the Wiley and NIST archives, every peak was located. Reports are limited to compounds that have a spectral matching accuracy of 90% or higher. No response factors were calculated.

Molecular docking simulation

The preparation of ligand-receptor binding configurations utilized AutoDock tool software to generate pdbqt files for both ligands and the receptor. The binding configuration entailed grid parameters of dimensions 33 × 25 × 25 for the x, y, and z axes, respectively. A grid centre at coordinates 4.055 × 0.000 × 0.000 Å, grid spacing of 1.000 Å, and exhaustiveness set at 8. Subsequently, molecular docking simulations were conducted through the AutoDock tool and Vina [19]. The binding affinities and inhibition constants of ligands docked against the receptor were computed using Eqs. 1 and 2, respectively.

$$\Delta G = -RTlnK_i \quad (1)$$

$$K_i = \exp(\Delta G/RT) \quad (2)$$

Molecular visualization

To visualize these interactions, Discovery Studio Visualizer (DS) version 4.1 was employed [20].

Analysis of the target receptor

The conformation of acetylcholinesterase (PDB ID: 5YDH) and the voltage-gated ion channel (PDB ID: 6MVVA) were assessed using a Ramachandran plot [21].

Biochemical analysis

The procedure for protein estimation was estimated according to the method of Gornall et al. [22]. $Na^+\_K^+$ ATPase activity was determined by the procedure of Ronner et al. [23] and modified by Bewaji and Bababunmi [24] was used. The activity of AChE was determined according to the method of Ellman et al. [25]. The procedure described by Tietz et al. [26] was used to assay for sodium and potassium ion concentration. GST activity was determined by the method described by Habig et al. [27].

Statistical analysis

Data was presented as mean ± SEM. The data was analysed using one-way ANOVA, followed by the Dunnett post hoc mean comparison test, and considered statistically significant at a P-value of < 0.05. SPSS Statistics 22 (Statistical Package for Social Science) (SPSS Inc., Chicago, IL, USA) was used in the statistical analyses. The LC_{50} and LC_{90} were determined using probit analysis (Fig. 1).

Results

Larvicidal activity

At 24 h, no mortality rate ($p^{>}0.05$) was recorded across the different extracts with 0.5% (v/v) extract dose compared to the control. At 48 and 72 h, there was a significant ($p < 0.05$) mortality rate compared to the control. The increase in mortality was time-dependent, with more deaths occurring at 72 h (Fig. 2A). With a 1% (v/v) dose of the extracts, at 24 h, the combined extracts showed a significant ($p < 0.05$) mortality rate compared to the control, whereas, at 24 and 72 h, all the extracts showed a significant mortality rate compared to the control (Fig. 2B). With 1.5% (v/v) dose of the extracts, clove extract, garlic extract, and combined extracts, respectively, showed a significant mortality rate compared to the control (Fig. 2C). The calculated LC 50 and LC 90 after 24 h (Table 1) are 1.824 and 2.453 for garlic extract, 2.315% and 3.257% for clove extract, and 0.994 and 1.414 for the combined extracts.

Adulticidal activity

At 10 min, there was no significant knockdown rate in adult *Anopheles* mosquitoes by clove extract compared to deltamethrin, but the combined extract showed a significant ($p < 0.05$) knockdown rate. At 15, 20, 30, 40, 50, and 60 min, the clove extract and combined extracts showed

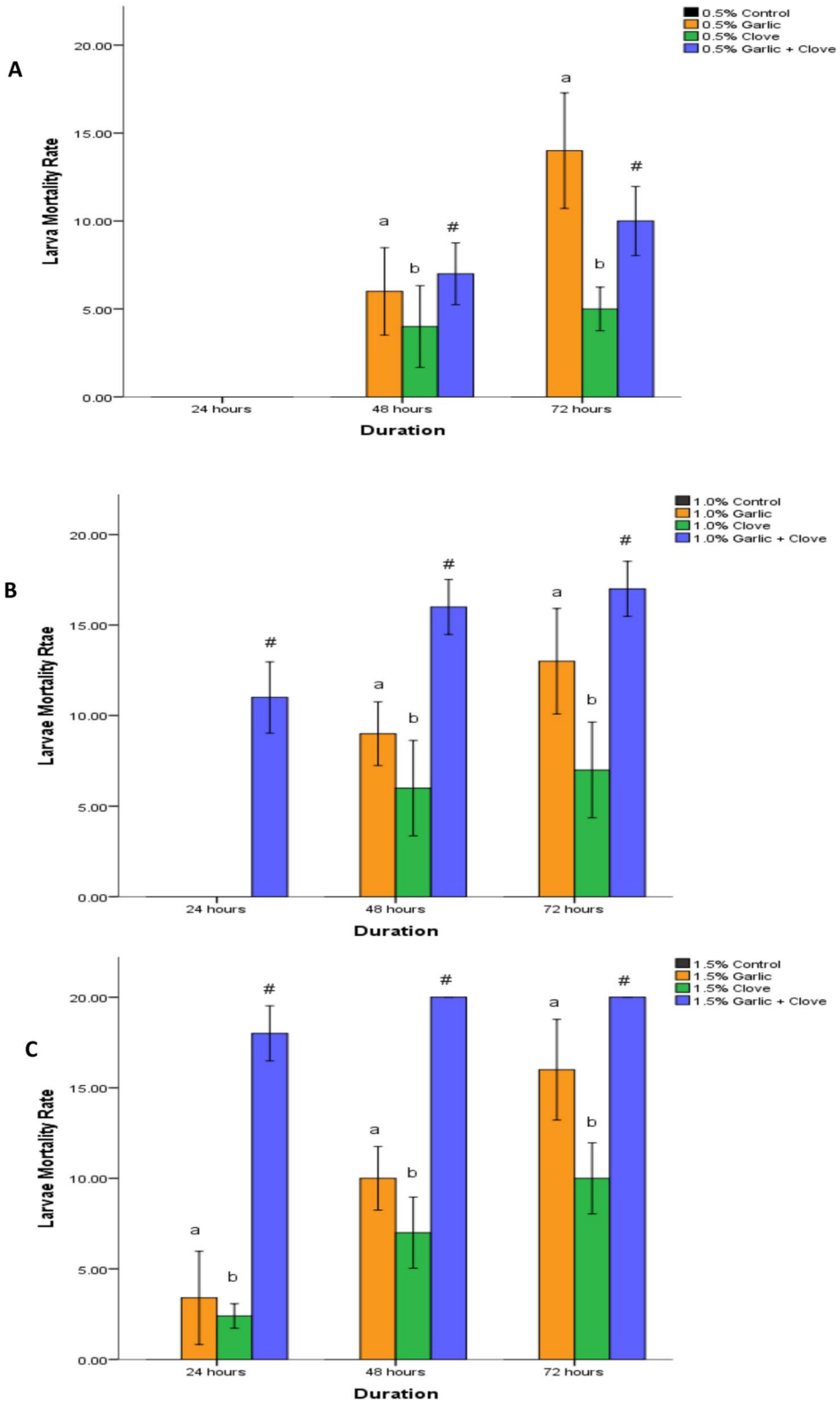


Fig. 2 Larvicidal effect of clove/garlic hydro-ethanolic extracts at 0.5% **B**, 1.5% **C**, and 1.5% **A** (v/v). Data is shown as mean ± SEM of 5 replicates (n = 20). Bars with superscripts a and b are significantly different from bars with superscript # at $p < 0.05$

Table 1 LC_{50} and LC_{90} values of larval toxicity effect of garlic/clove extracts on *Anopheles gambiae*

	Garlic (24 h)	Clove (24 h)	Garlic + Clove (24 h)
LC_{50}	1.824	2.315	0.994
LC_{90}	2.453	3.257	1.414

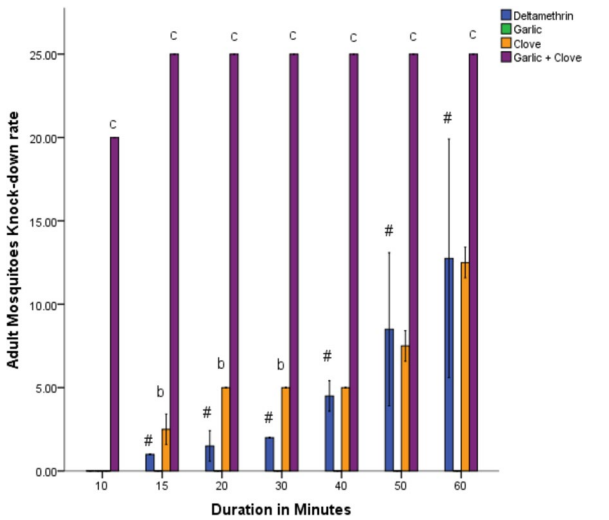


Fig. 3 Knocked-down effect of the extracts on adult mosquitoes after 60 min. Data is shown as mean ± SEM of four replicates (n = 25). Bars with varying superscripts from deltamethrin (standard) exhibit a significant difference at $p < 0.05$

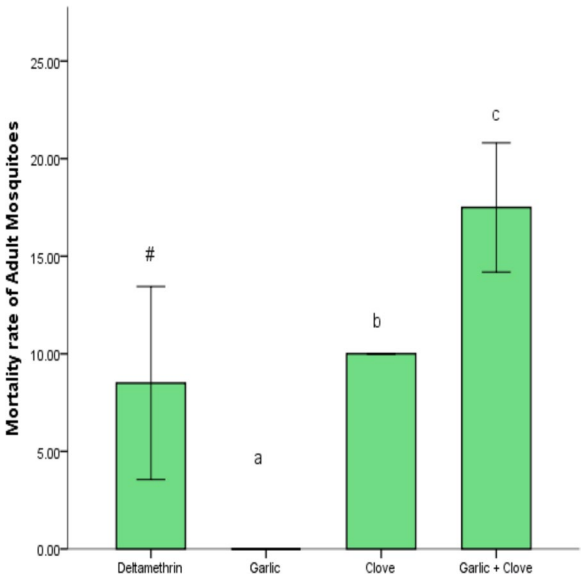


Fig. 4 Effect of extracts on mortality rate of adult mosquitoes. Data is shown as mean ± SEM of four replicates (n = 25). Bars with varying superscripts from deltamethrin (standard) exhibit a significant difference at $p < 0.05$

a significant knockdown rate of adult mosquitoes compared to deltamethrin. The knockdown effects between 15 to 60 min were time-dependent (Fig. 3). Garlic extract did not show any knockdown effects on adult mosquitoes. The mortality rate of adult mosquitoes was significantly increased ($p < 0.05$) by clove extract and combined extracts of clove and garlic compared to deltamethrin (Fig. 4). The garlic extract showed no mortality rate compared to deltamethrin (Fig. 4).

Enzyme analysis

There was a significant increase ($p < 0.05$) in potassium ion concentration in groups treated with clove extract, garlic extract, and combined extract compared to that in the deltamethrin group (Fig. 5A). Sodium ion concentration was significantly decreased ($p < 0.05$) in the group treated with clove, garlic, and combined extract compared to deltamethrin (Fig. 5B). There was a significant increase ($p < 0.05$) in acetylcholine activity in groups administered clove, garlic, and the combined extract compared to deltamethrin (Fig. 6A). Similarly, there was a significant increase ($p < 0.05$) in Na–k ATPase activity in groups administered clove, garlic, and the combined extract compared to deltamethrin (Fig. 6B). There was a significant decrease ($p < 0.05$) in glutathione-transferase activity in the group treated with garlic extract and combined extracts of clove and garlic compared to deltamethrin. In contrast, no significant change was observed in the enzyme activity in the clove-treated group (Fig. 7A). There was a significant decrease ($p < 0.05$) in protein concentration in the groups treated with clove and garlic extracts compared to the group treated with deltamethrin. There was no significant change in the group treated with the combined extracts (Fig. 7B).

Phytochemical analysis

The quantitative phytochemical evaluation shows that saponin and flavonoids are present in the garlic while saponin, tannins, phenols, and steroids are in the clove. In contrast, saponin, tannins and phenols, flavonoids, and steroids were present in the ratio 1:1 mixture of the garlic and clove (Table 2). The chromatogram of GC–MS evaluation of clove, garlic, and the combination of clove and garlic shows the presence of different compounds at different abundance and retention times (Figs. 8A–C). The GC–MS analysis of clove hydro-ethanolic extract revealed the presence of 16 compounds (Table 3) while the GC–MS constituent of garlic and clove-garlic combination showed 18 and 23 compounds respectively (Tables 4 & 5).

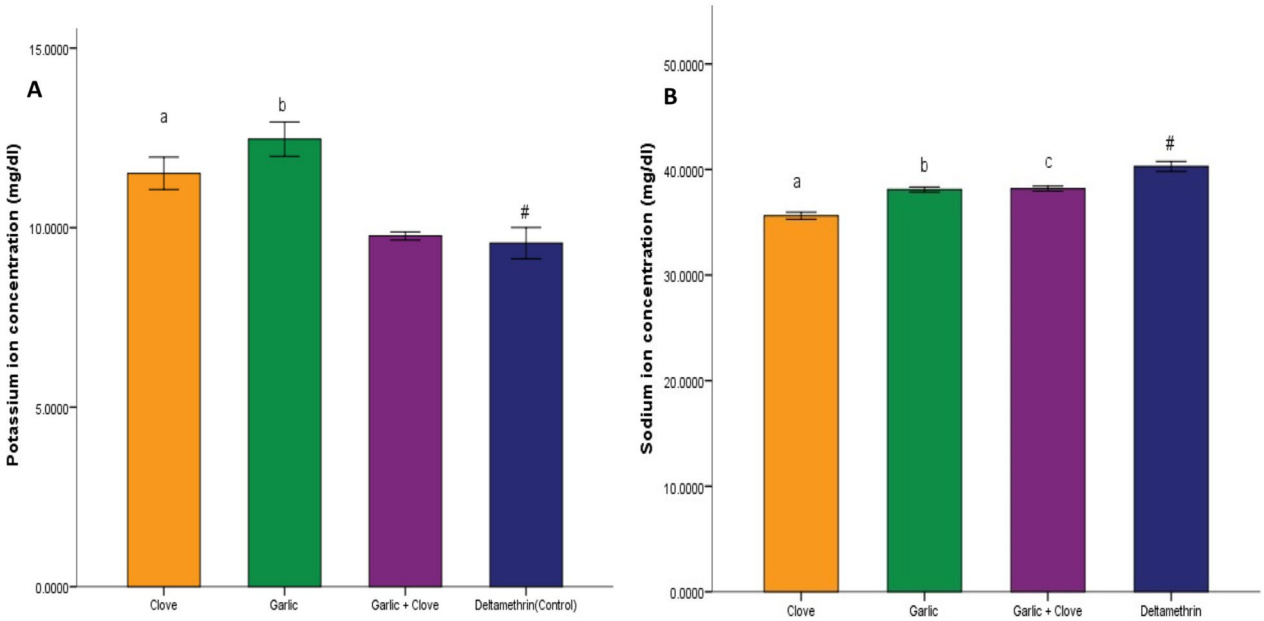


Fig. 5 Effect of clove/garlic hydroethanolic extracts on potassium ion concentration (**A**) and sodium ion concentration (**B**) in adult mosquitoes (*Anopheles gambiae*). Data is shown as mean ± SEM of four replicates (n = 25). Bars with superscripts a and b exhibit a significant difference from bar with superscript # at $p < 0.05$

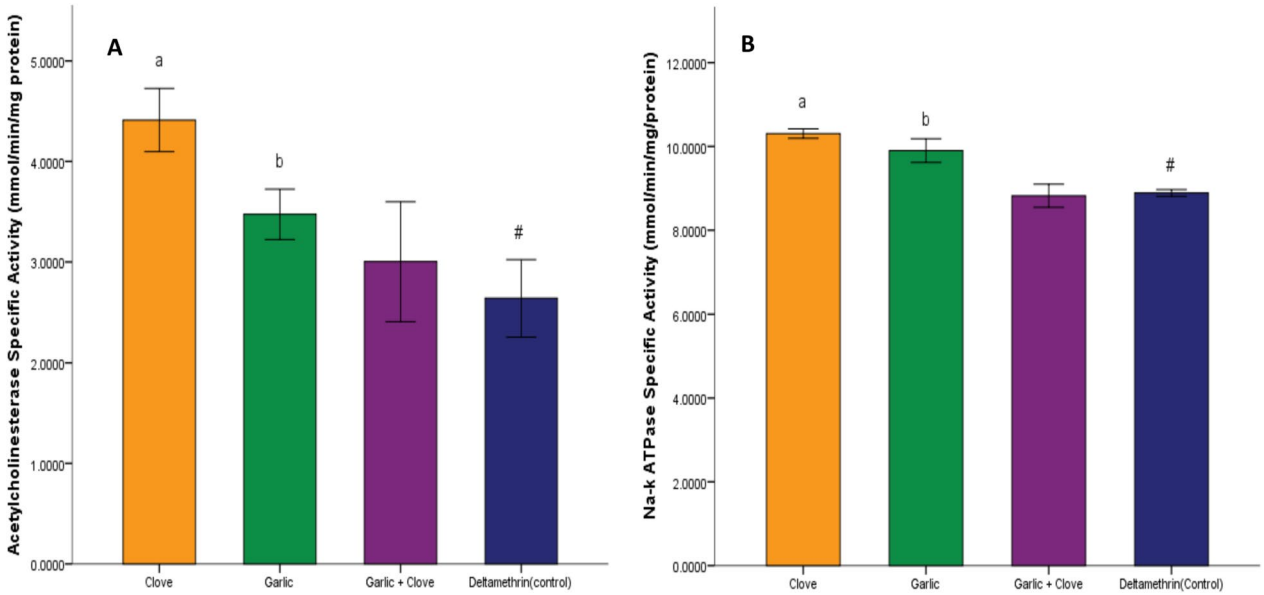


Fig. 6 Effect of clove/garlic hydro-ethanolic extracts on acetylcholinesterase activity (**A**) and Na–K ATPase activity (**B**) in adult mosquitoes (*Anopheles gambiae*). Data is shown as mean ± SEM of four replicates (n = 25). Bars with superscripts a and b exhibit a significant difference from bar with superscript # at $p < 0.05$

Molecular docking

Predicted upon their binding affinities falling below the threshold of − 8.0 kcal/mol for acetylcholinesterase and − 7.0 kcal/mol for voltage-gated sodium channels, several ligands were singled out for exclusion, as evidenced by the molecular docking outcomes meticulously depicted in Tables 6, 7, 8. However, four compounds from both proteins manifest binding affinities that are higher or close to

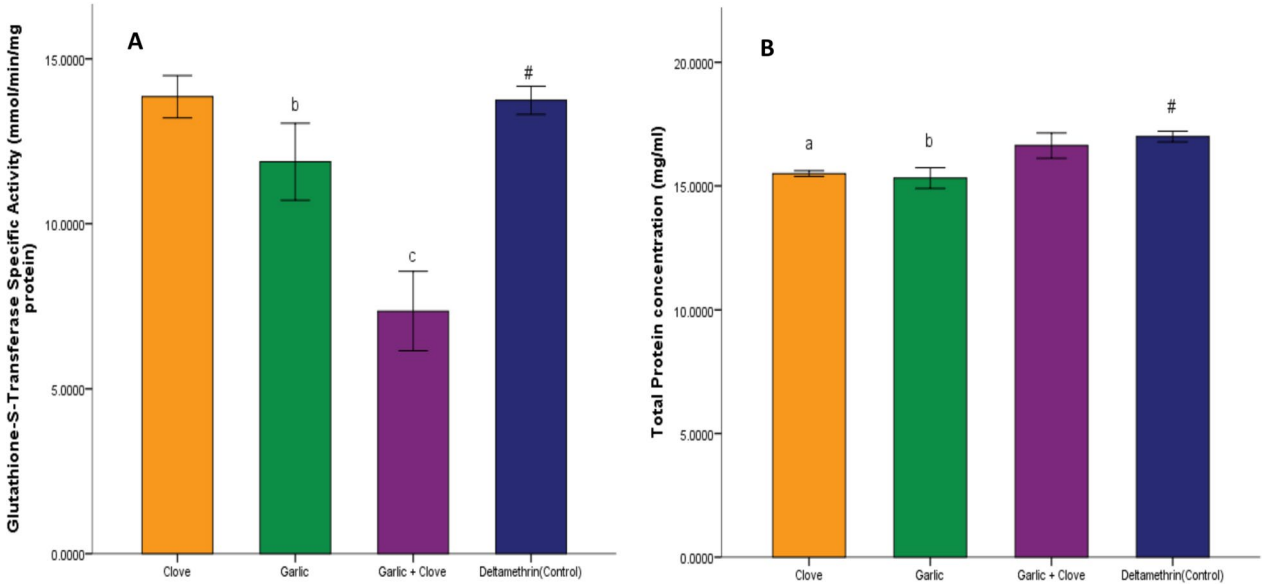


Fig. 7 Effect of clove/garlic hydro-ethanolic extracts on glutathione-S-transferase activity in adult mosquitoes (*Anopheles gambiae*). Data is shown as mean ± SEM of four replicates (n = 25). Bars with superscripts b and c exhibit a significant difference from bar with superscript # at $p < 0.05$

Table 2 Quantitative phytochemical composition of clove and garlic of hydro-ethanolic extracts in mg/100

Phytochemical	Garlic	Clove	Garlic: clove (1:1)
Saponin	0.0028 ± 0.0002	0.0086 ± 0.0002	0.01 ± 0.000
Tannins	–	25.1652 ± 0.8	15.272 ± 0.107
Phenols	–	18.4499 ± 1.06	8.5997 ± 1.898
Flavonoids	0.3729 ± 0.008	–	1.6955 ± 0.050
Steroids	–	12.81 ± 0.024	8.01 ± 0.0040

that of the reference standard (− 8.0 to − 9.4 kcal/mol). Estrone has higher binding affinities (− 9.4 kcal/mol) with both proteins than the standard drug. The conformation of acetylcholinesterase (PDB ID: 5YDH) and the Voltage-gated sodium channels (PDB ID: 6MVVA) were assessed using a Ramachandran plot (Fig. 9A–B), which revealed that 99% and 98% of their residues respectively reside within the favoured allowed regions. The target active site of acetylcholinesterase protein was found to contain amino acid residues, which include Phe490, Tyr493, and Gly44 while, that of acetylcholinesterase was identified to contain Phe1107, Val1110, Val1120, Phe1079, Trp1076 and Pro1075 as major residues involved in interactions. These were obtained from a reverse docking of the native ligand of the acetylcholinesterase complex (2-acetamido-2-deoxy-beta-D-glucopyranose (NAG)) and that of the voltage-gated sodium channels (2-Dimyristoyl-sn-glycero-3-phosphocholine) (Table 9).

Binding mode and molecular interactions of the best hit compound and the standard

During the lead optimization stage of drug discovery, the binding mode and molecular interactions involved in ligand binding to the target receptor's active site are critical. It helps to increase the chosen hit compounds' potency and effectiveness. A comparison was made between the optimal Hit compounds' molecular interactions (estrone, morellinol, caryophyllene, 1,4,7-cycloundecatriene, 1,5,9,9-tetramethyl-, Z, Z, Z-), the binding mode, and the standard reference compound (deltamethrin) for both proteins. It is noteworthy that these compounds had similar binding affinities and inhibition efficiency against the proteins. The target proteins were found to contain, among other amino residues, Phe490, Tyr493, and Gly445 (for PDB ID: 5YDH) and Phe1107, Val1110, Val1120, Phe1079, Trp1076, and Pro1075 (for PDB ID: 6MVV), according to the reverse docking analysis. Table 4 showed the interaction of estrone, morellinol, caryophyllene, 1,4,7-cycloundecatriene,1,5,9,9-tetramethyl-, Z, Z, Z- and the reference compound (deltamethrin) alongside the target's binding site of *Anopheles*' acetylcholinesterase shows that estrone formed an alkyl bond with the target receptor through Tyr282, Trp441, Val235, Tyr493, Phe490. Through the formation of a conventional hydrogen bond, morellinol and the receptor Ala391, Ser397, Pro352, Tyr558, Pro521, an alkyl bond through Val396, His559, and a Pi-Pi T shape through Tyr522. Caryophyllene also interacted with the protein to form Alkyl bonds through Tyr493, Tyr282, Ile446,

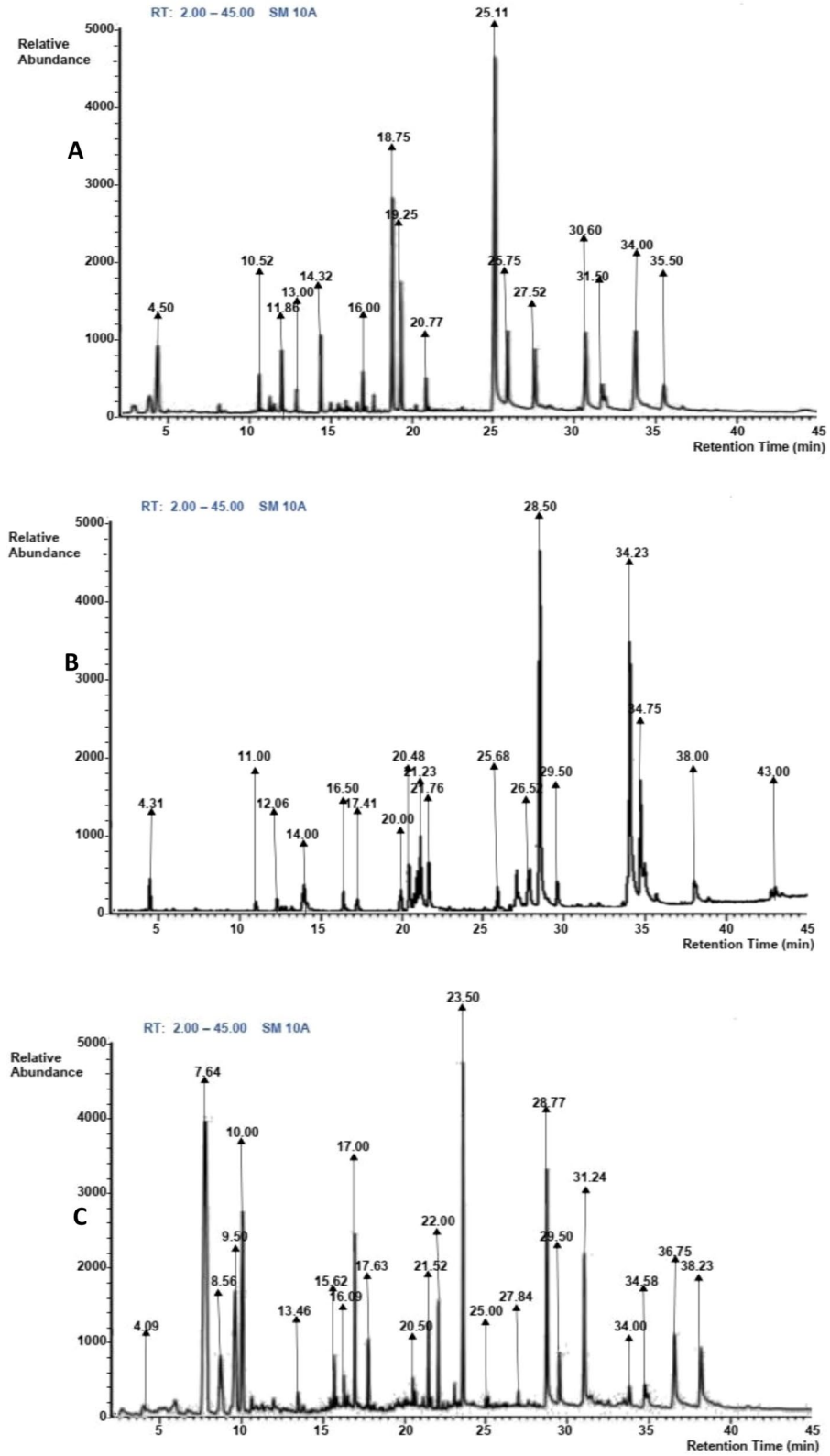


Fig. 8 A typical gas chromatogram of the chemical compounds of clove hydro-ethanolic extracts (**A**), garlic hydro-ethanolic extract (**B**), and garlic and clove hydro-ethanolic extracts (**C**)

Table 3 GC–MS chemical constituents of clove hydro-ethanolic extract

S/N	Compound	Retention time (min)	% AREA	Molecular weight (amu)
1	1,2,3-Benzenetriol	4.50	4.40	126
2	Benzaldehyde, 4-ethyl-	10.52	3.27	134
3	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	11.86	4.17	144
4	Phenol,2-methoxy-4-(methoxymethyl)	13.00	2.00	168
5	1,4,7,-Cycloundecatriene, 1,5,9,9-tetramethyl-, Z,Z,Z-	14.42	5.25	204
6	Phenol, 2-methoxy-4-(1-propenyl)-	16.00	3.00	164
7	n-Hexadecanoic acid	18.75	15.03	256
8	Caryophyllene	19.25	7.59	204
9	γ-Muurolene	20.77	2.17	204
10	Eugenol	25.11	26.07	164
11	α-Farnesene	25.75	4.51	204
12	Asarone	27.52	4.16	208
13	2′,3′,4′Trimethoxyacetophenone	30.60	6.17	210
14	Caryophyllene oxide	31.50	2.05	220
15	9,12-Octadecadienoic acid (Z,Z)-	34.00	8.21	280
16	Octadecanoic acid	35.50	1.32	284

Table 4 GC–MS chemical constituents of garlic hydro-ethanolic extract

S/N	Compound	Retention time (min)	% AREA	Molecular weight (amu)
1	Acetaldehyde	4.31	2.47	44
2	Acetic acid	11.00	1.06	60
3	Propanoic acid	12.06	1.20	744
4	4H-Pyran-4-one	14.00	3.00	96
5	3-Furaldehyde	16.50	2.25	96
6	1,2 Benzenediol	17.41	1.15	110
7	m-Toluylic acid	20.00	3.27	136
8	Gallic acid	20.48	4.59	170
9	Dihydroxyacetone	21.23	6.17	90
10	Nonanamide	21.76	5.09	157
11	Octanoic acid, 3-hydroxy-, methyl ester	25.68	3.28	174
12	Acetamide, N-tetrahydrofurfuryl-2-methoxy-	26.52	2.85	173
13	3-Vinyl-1,2-dithiacyclohex-4-ene	28.50	25.52	144
14	9,12-Octadecadienoic acid (Z,Z)-	29.50	3.95	280
15	n-Hexadecanoic acid	34.23	23.21	256
16	Estrone	34.75	6.20	270
17	Octadecanoic acid	38.00	2.69	284
18	Morellinol	43.00	2.04	546

Phe490, Tyr494, and the Pi-sigma bond through Trp441. 1,4,7- cycloundecatriene,1,5,9,9-tetramethyl-, Z, Z, Z- form a similar Pi-Alkyl bond with the target receptor through Phe449, Trp441, Tyr493, Tyr489. Similar to this, the selected reference compound binds to the protein through Ser283 to form a conventional hydrogen bond and through Tyr282, Ile231, Tyr494, and Asp233 to form an alkyl bond. Table 4 clearly shows how precisely the target receptor was contacted by estrone, caryophyllene, 1,4,7-cycloundecatriene, 1,5,9,9-tetramethyl-, Z, Z,

Table 5 GC–MS chemical constituents of garlic + clove hydro- ethanolic extracts

S/N	Compound	Retention time (min)	% AREA	Molecular weight (amu)
1	Acetic acid	4.09	1.21	60
2	Dihydroxyacetone	7.64	7.27	90
3	Propanoic acid	8.56	3.12	74
4	3-Furaldehyde	9.50	5.21	96
5	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	10.00	5.25	144
6	Phenol,2-methoxy-4-(methoxymethyl)	13.46	1.24	168
7	Phenol, 2-methoxy-4-(1-propenyl)-	15.62	3.18	164
8	Gallic Acid	16.09	2.00	170
9	3,4-Dihydroxymandelic acid	17.00	5.09	184
10	Caryophyllene	17.63	4.17	204
11	Benzyl Benzoate	20.50	1.71	212
12	Butylated Hydroxytoluene	21.52	4.11	220
13	(E)-1-(4-Hydroxy-3-methoxyphenyl)dec-3-en-5-one	22.00	5.06	276
14	Eugenol	23.50	15.28	164
15	Phentolamine	25.00	1.56	281
16	Heptadecane	27.84	1.69	240
17	n-Hexadecanoic acid	28.77	11.29	256
18	Caryophyllene oxide	29.50	2.66	220
19	9,12-Octadecadienoic acid (Z,Z)-	31.24	7.93	280
20	Octadecanoic acid	34.00	1.76	284
21	Estrone	34.58	2.92	270
22	β-Sitosterol	36.75	3.13	414
23	Morellinol	38.23	3.15	546

Table 6 Docking scores (binding affinity), hydrogen bond interactions, electrostatic/hydrophobic interactions, and the inhibition constants of the best hit and the reference compounds with acetylcholinesterase (PDB ID; 5YDH)

Ligands	Binding affinity (ΔG), kcal/mol	Human pancreatic alpha-amylase amino acids form H-bond with ligands (H-bond Distance, Å)	Electrostatic/ hydrophobicInteractions involved	Inhibition constant (K_i), μM
Estrone	− 9.4	Nil	Tyr282, Trp441, Val235, Tyr493, Phe490	0.13
Morellinol	− 9.1	Ser397, Ala391, Tyr558	Val396, Leu395, Pro521, His559, Tyr522, Pro352	0.21
Deltamethrin (Standard)	− 9.1	Ser283	Tyr282, Ile231, Tyr494, Asp233	0.21
Caryophyllene	− 8.2	Nil	Tyr493, Trp441, Tyr282, Ile446, Phe490, Tyr494	0.98
1,4,7, Cycloundecatr iene,1,5,9,9tetra methyl,Z,Z,Z-	− 8.0	Nil	Phe449, Trp441, Tyr493, Tyr489	1.37
2-acetamido2-deoxy-betaD-glu-copyranose (NAG)	− 6.4	Phe490	Tyr493, Gly445	20.45

Z-, and other chemicals through the amino residues that were found to be present at the active site. Nevertheless, none of the identified amino acid residues allowed the reference compound to interact with the active site of acetylcholinesterase. Using voltage-gated sodium channels Trp1076, Val1120, and Val1110, Estrone formed an alkyl bond with the target receptor. For these channels, it also formed a Pi-sigma bond through Phe1107 and a Pi-Pi shapes bond through Phe1079 for these channels. Morellinol and the receptor bonded via an

Table 7 Binding mode and binding interaction of the hits ligands and reference compound with acetylcholinesterase (PDB ID; 5YDH: A)

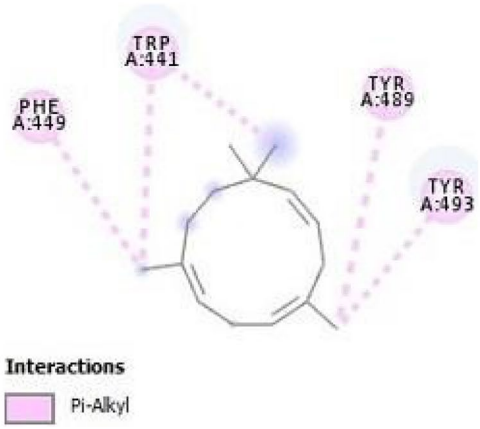
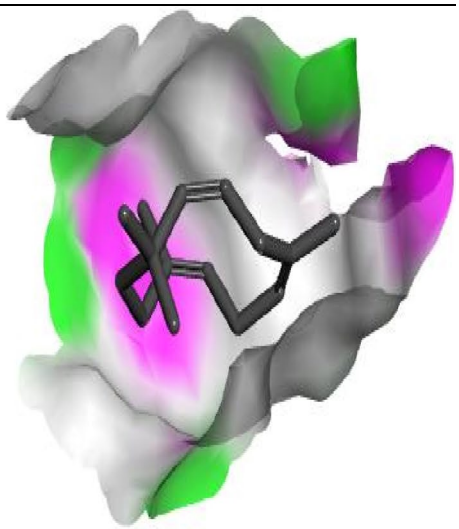
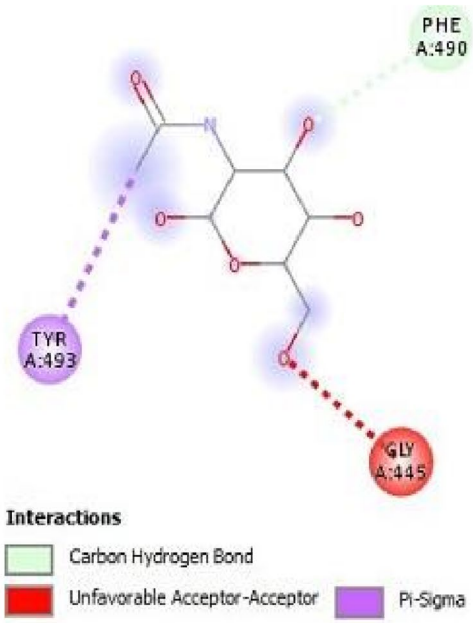
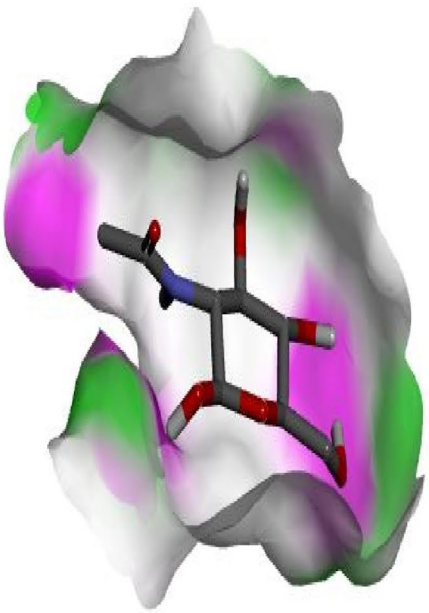
Compounds	Binding Interactions	Binding mode
1,4,7,-Cycloundecatriene,1,5,9,9-tetramethyl-,Z,Z,Z-		
2-acetamido-2deoxy-beta-Dglucopyranose (NAG)		

Table 7 (continued)

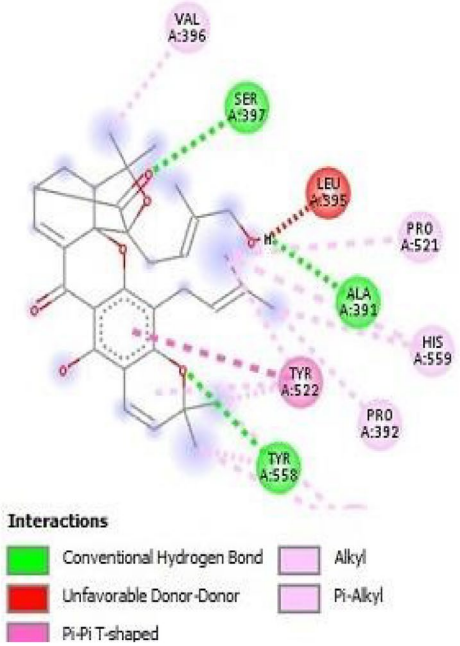
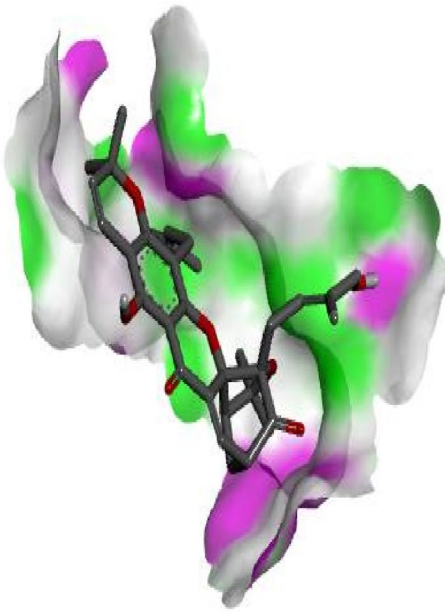
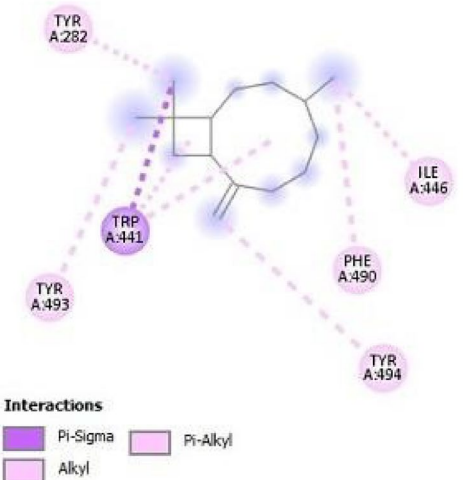
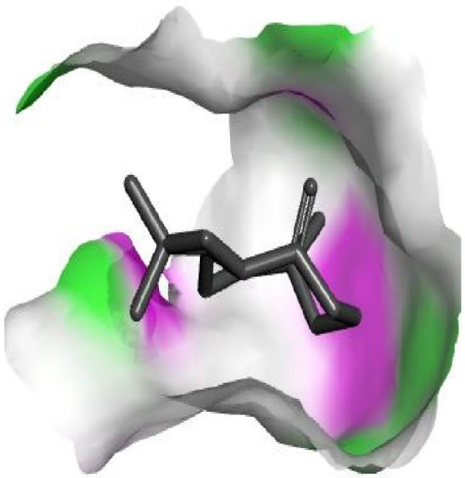
Compounds	Binding Interactions	Binding mode
Morellinol		
Caryophyllene		

Table 7 (continued)

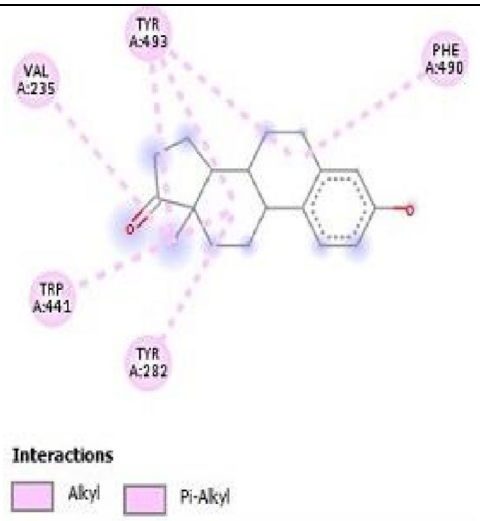
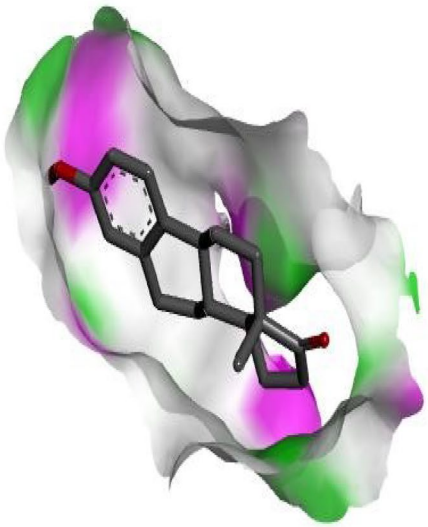
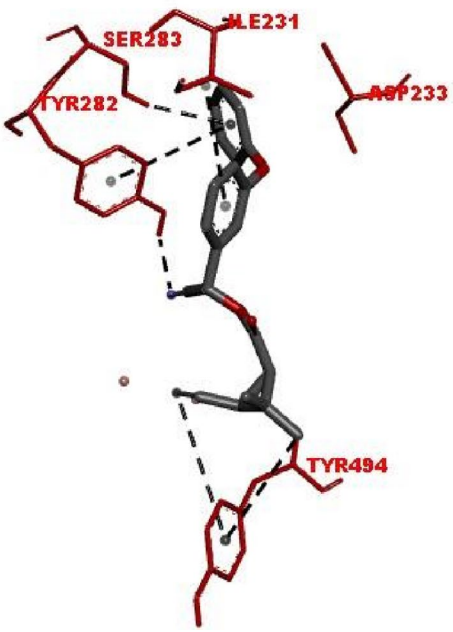
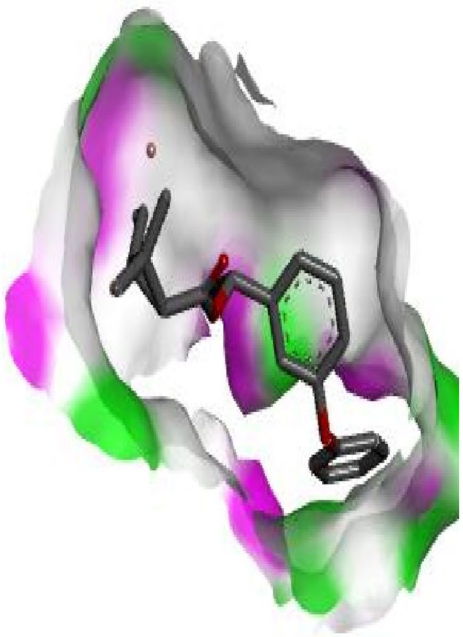
Compounds	Binding Interactions	Binding mode
Estrone		
Deltamethrin (Standard)		

Table 8 Docking scores (binding affinity), hydrogen bond interactions, electrostatic/hydrophobic interactions, and the inhibition constants of the best hit and the reference compounds with voltage-gated sodium channels (PDB ID; 6MVV)

Ligands	Binding affinity (ΔG), kcal/mol	Voltage-gated sodium channels amino acids forming H-bond with ligands (H-bond distance, Å)	Electrostatic/hydrophobic interactions involved	Inhibition constant (K_i), μM
Estrone	− 8.2	Nil	Val1120, Val1110, Trp1076, Phe1079, Phe1107	0.13
Morellinol	− 8.4	Ser397, Ala391, Tyr558	Pro1075, Phe1079, Val1120, Val1110, Phe1107, Leu1104	0.21
Deltamethrin (Standard)	− 7.9	Nil	Trp1076, Pro1075, Phe1079, Val1110, Phe1107, Val1120	0.21
Caryophyllene	− 7.1	Nil	Trp1076, Phe1079, Thr1111, Val1110, Val1120, Phe1107	0.98
1,4,7,-Cycloundecatriene,1,5,9,9-tetramethyl-,*Z,Z,Z*-	− 7.4	Nil	Phe1079, Val1110, Thr1111, Tpr1076, Pro1075, Val1120, Ile1124	1.37

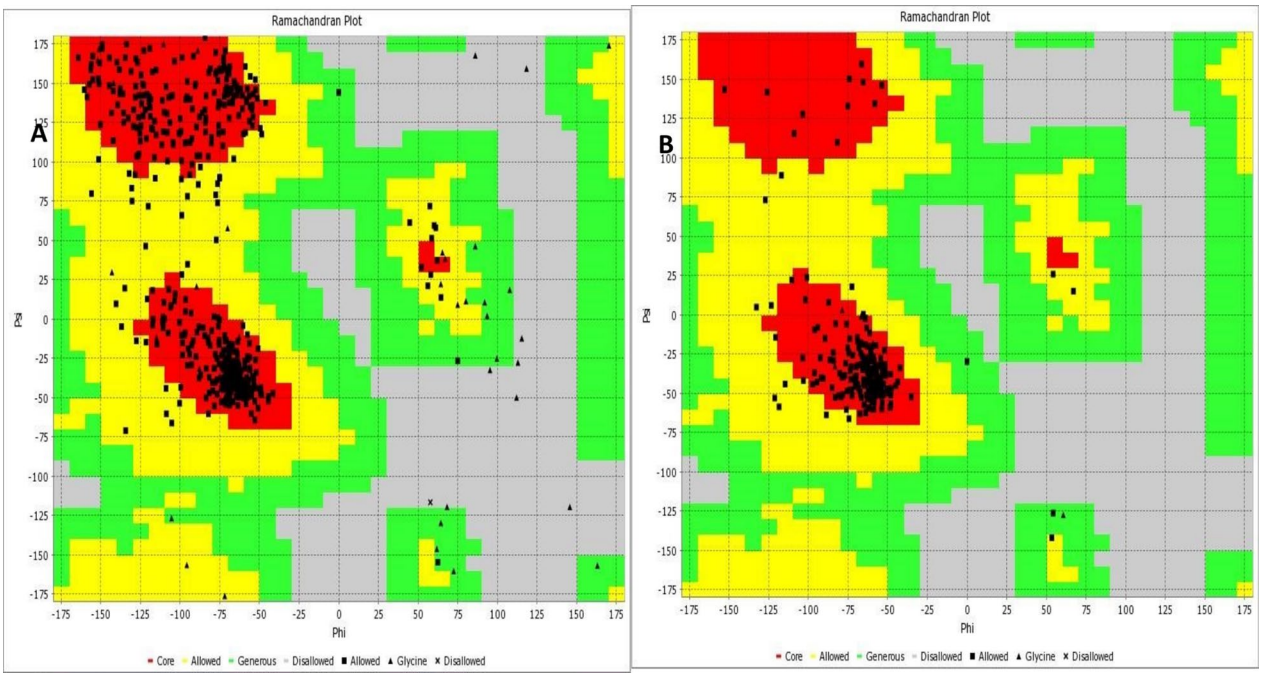


Fig. 9 Ramachandran plot of the acetylcholinesterase (PDB ID: 5YDHA) showing 99% residues in core/allowed region and Ramachandran plot of the Voltage-gated ion channel (PDB ID: 6MVVA) showing 98% residues in core/allowed region

alkyl bond through Val1110, Leu1104, and Val1120. It created Pi-sigma bonds through Phe1107, Pi-Pi stacked bonds through Phe1079, and Pro1075 also revealed an unfavourable bond. Caryophyllene also interacted with the active site to form Alkyl bonds through Trp1076, Val1110, Val1120. It formed a weak van der Waal's bond through Thr1111 and Phe1107 and lastly a Pi-sigma bond through Phe1079. 1,4,7 Cycloundecatriene,1,5,9,9-tetramethyl-, Z, Z, Z- also formed a similar weak van der Waal's bond through Thr1111, alkyl bond with the target receptor through Phe1079, Val1110, Trp1076, Pro1075, Val1120, Ile1124. Similarly, the selected reference compound formed bonds with the target protein through Phe1079, Trp1116, Pro1076, Phe1107, Val1110 and Val1120. Binding mode and binding interaction of the hit's ligands and reference compound with acetylcholinesterase and Voltage-gated sodium channels respectively (Table 7 and 9).

Table 9 Binding mode and binding interaction of the hit's ligands and reference compound with Voltage-gated sodium channels (PDB ID; 6MVV: A)

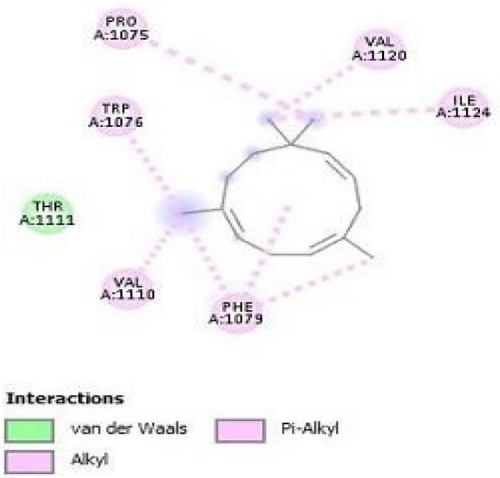
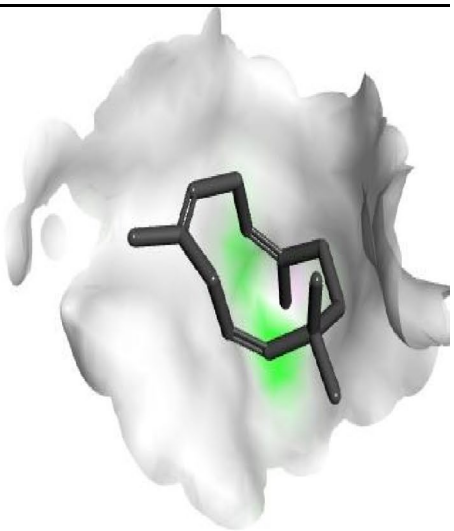
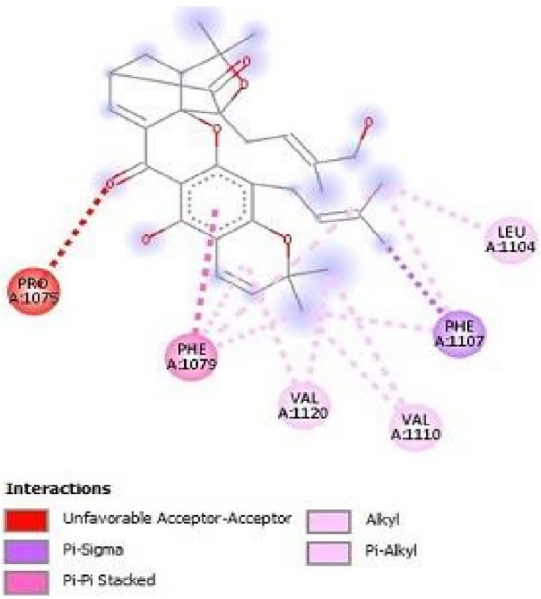
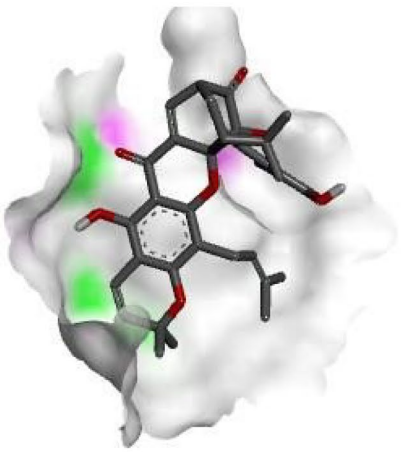
Compounds	Binding Interactions	Binding mode
1,4,7, -Cycloundecatriene,1,5,9,9 -tetramethyl -,Z,Z,Z -		
Morellinol		

Table 9 (continued)

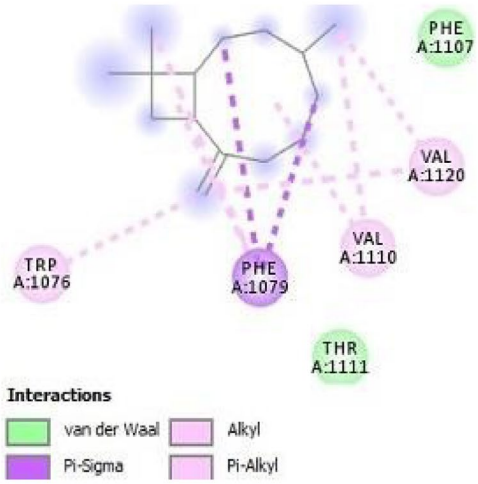
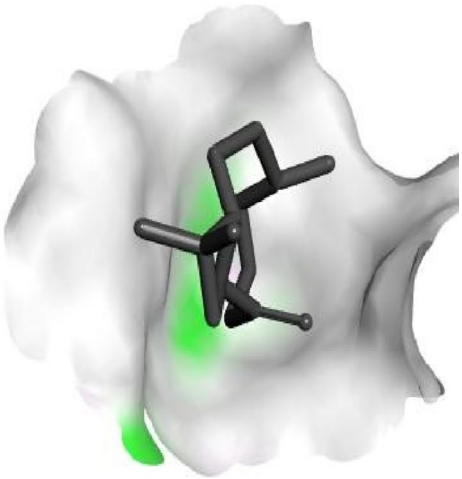
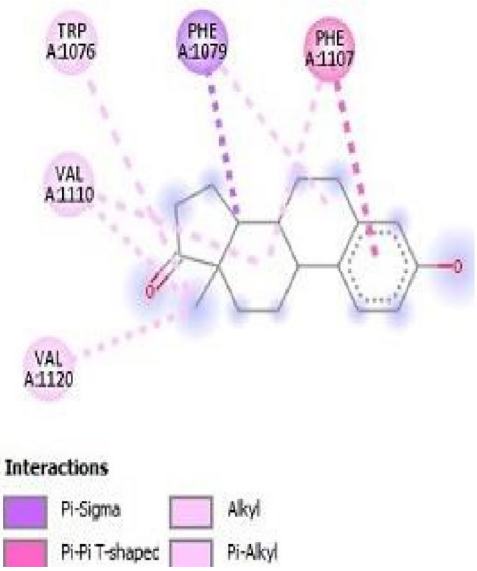
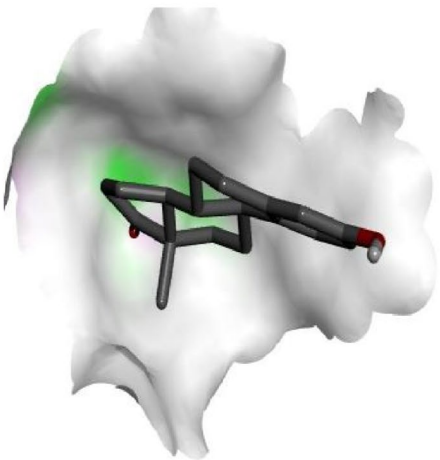
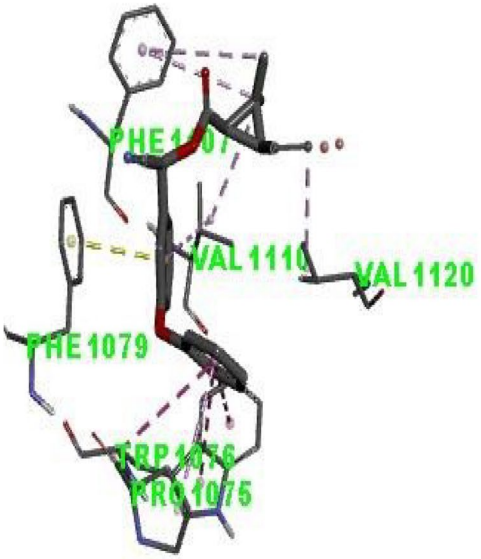
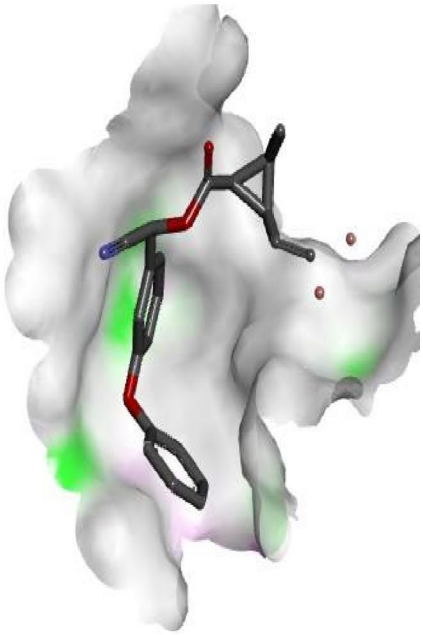
Compounds	Binding Interactions	Binding mode
Caryophyllene		
Estrone		

Table 9 (continued)

Compounds	Binding Interactions	Binding mode
Deltamethrin		

Discussion

The phytochemicals observed in the plant extracts used in this study are mostly responsible for plant systems that protect plants against insects and other parasites [28]. The larvicidal and adulticidal effects exhibited in the current study could be due to the presence of saponins, tannins, phenols, flavonoids, and steroids, as previously reported [29]. GC–MS analysis previously performed on clove in other studies showed the presence of eugenol, α-farnesene, caryophyllene, caryophyllene oxide, and 2′,3′,4′ trimethoxyacetophenone as part of its chemical constituents [30]. These compounds have been proven to be responsible for the biological, medicinal, and insecticidal abilities of cloves, with eugenol and caryophyllene identified as the major compounds responsible for their insecticidal ability [6]. Elbashir et al. [31] reported the presence of phenol, caryophyllene, 2,3-Benzentriol, eugenol, caryophyllene oxide, 1nHexadecanoic acid, and 2-methoxy-4-(2-propenyl)- acetate, which was corroborated by the results of the current study. Some of the compounds (morellinol, estrone, gallic acid, m-toluic acid, and 1,2 benzenediol) reported in this study also aligned with previous studies [32]. The current study reported some compounds that are not present in either clove or garlic extracts, which is in tandem with a previous study [33]. These compounds found which are absent in the individual extracts could be as a result of environmental factors, synergistic interactions or chemical reactions that occurred between the chemical constituents in the individual extracts leading to the formation of new compounds [34].

Redocking of the native ligand to the active site of the target protein confirmed the reliability of the docking approach. A high degree of similarity was observed between the natural binding of the native ligand and the protein, affirming the dependability of the docking methodology in concordance with the literature [35]. The literature suggests that ligands that exhibit the lowest binding affinity values when docked with the target possess a heightened propensity to inhibit the receptor [35]. The literature suggests that ligands that exhibit the lowest binding affinity values when docked with the target possess a heightened propensity to inhibit the receptor [36]. Residues in the core/favoured region are prevalent, indicating potential steric collisions during the reactions [37]. The prediction of AChE by the compounds in the extract was aligned with the biochemical experiment. Hence, the mosquitocidal effect exhibited by the extract in the current study could be the result of the inhibition of acetylcholinesterase activity. In addition, the low binding energy of the interaction between estrone and the voltage-gated sodium channel could predict alterations in the function of the protein. According to previous work, it has been

established that deltamethrin, a member of the pyrethroid class of insecticide uses inhibition of the voltage-gated sodium ion channel as its possible mechanism of action [38]. The docking experiment in this study also confirmed the inhibitory effect of deltamethrin on acetylcholine esterase and sodium voltage-gated channels. Furthermore, the study indicated that the adulticidal effect observed from the combined extract could be due to the inhibitory effects on protein functions or enzyme activity in the adult mosquitoes by certain bioactive compounds present in the extracts [39]. The highest larvicidal activity was observed with the combined clove and garlic extracts, likely due to a synergistic toxic effect on mosquito larvae. This toxicity may be mediated by the inhibition of certain enzymes in vital metabolic pathways in larvae [38]. The synergistic toxicity of the garlic-clove extract could contribute to the adulticidal effect observed for the combined extracts. Synergistic toxicity occurs when the effects of a combined extract supersede the individual effects of the extracts. This effect may also be linked to the cumulative inhibition of vital enzymes involved in the metabolism of adult *Anopheles* [38]. Similarly, mosquitoes can develop resistance against insecticides, as was reported for deltamethrin, a known member of the commonly used class of insecticides, pyrethroids. Resistance to insecticidal effects has been linked to enhanced activity of detoxification enzymes, such as glutathione-S-transferase or target site insensitivity due to point mutations in the gene for voltage-gated sodium channels [39]. GST plays an important role in the chemical detoxification of mosquitoes, leading to insecticide resistance. Therefore, elevated activity of GST could be indicative of resistance by mosquitoes against insecticides. This has been reported for several known classes of insecticides, such as organochlorides, organophosphates, and pyrethroids [40]. The decreased activity of GST reported in the current study for garlic clove combined extract might be indicative of enzyme inhibition by the bioactive compounds in the extract [28, 29]. Therefore, the larvicidal or adulticidal effects of the extracts in the present study may be attributed to the mosquito's inability to neutralize the toxicity of the insecticidal compounds found in the plant. Additionally, the nervous system, the site of action of acetylcholinesterase (AchE) enzyme, is a primary target site for many insecticides [41]. Acetylcholinesterase hydrolyses the neurotransmitter acetylcholine. Certain insecticides function by inhibiting or reducing the activity of the acetylcholinesterase enzyme. This inhibition prevents the hydrolysis of acetylcholine at its receptor, leading to an elevated concentration of the neurotransmitter in the synapse [41]. This results in continuous stimulation of the cholinergic receptors, which causes continuous neurotransmission, nerve hyperexcitation, convulsions, paralysis, and ultimately the insect's death [42]. The present study revealed that the extracts inhibit the activity of acetylcholinesterase, which might be the possible mechanism underlying their insecticidal effects [41].

The mode of the insecticidal effect of most insecticides is diverse in the nervous system. They affect the nervous system differently, including inhibiting enzymes, such as AChE or binding to keep voltage-gated sodium channels open. This open state of the sodium channel causes an uninterrupted influx of Na^+ and K^+ ions, resulting in continuous firing of the nerves, leading to muscle twitches, paralysis, and possible death of the insect [41]. Insecticides such as pyrethroids bind to voltage-gated sodium channels to inhibit inactivation and cause prolonged sodium ion inflow and potassium outflow [43]. The sodium–potassium ATPase pump is important for the transportation of ions across cell membranes, making it a vital component in nerve signaling and muscle contractions. It uses the energy released from ATP hydrolysis to pump sodium ions out of the cell and potassium ions into it to stabilize ion gradients [44]. Na–K ATPase is an important enzyme involved in the transport of Na^+ and K^+ across the cell membrane during neuronal impulse transmission [43]. This enzyme was inhibited by the combined garlic-clove extract, suggesting that this could be one of the mechanisms by which they mediate the mosquitocidal effect. Similarly, alterations in potassium and sodium ion concentrations could be linked to failure in voltage-gated channels as a result of Na–K ATPase activity [44].

Conclusion

The efficacy of the garlic-clove extract as a larvicide and adulticide has been confirmed through biochemical analyses and in silico studies. The hydroethanolic extracts of clove and garlic demonstrated significant mortality rates in both larval and adult female *Anopheles* mosquitoes. This lethal effect is likely due to the inhibition of crucial enzymes that facilitate essential processes such as signal transduction and energy production. Moreover, the biocidal properties of these extracts stem from the alteration of vital metabolic pathways, influenced by a range of bioactive compounds found in both garlic and clove. Noteworthy compounds identified through molecular docking, including estrone and caryophyllene, have shown potential interactions with acetylcholine esterase and voltage-gated channels. These interactions suggest a predictive inhibition of these proteins, indicating their

critical role in mediating the biocidal effects of the combined extracts.

Abbreviations

ACHE	Acetylcholine esterase
GC–MS	Gas chromatography-mass spectroscopy
GST	Glutathione transferase
NIST	National Institute of Standards and Technology
PBD	Protein Data Bank
WHO	World Health Organization

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Author contributions
NCO, OAB, and OBP conceived and designed the study. ETE, AAO, and LTA performed data analysis and interpretation and drafted the original manuscript. NCO, AAO,and OAB supervised and carried out the fieldwork. NCO and ETE carried out statistical analyses.

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Declarations

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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