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Research Article

Evaluation of Clove Volatile Extract Vaporizer Mats for Mosquito Control Against *Aedes aegypti*: Bioassay and Computational Analysis

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Abstract

Syzygium aromaticum contains bioactive compounds that may serve as natural alternatives to synthetic mosquito control agents. This study aimed to evaluate the mosquitocidal activity of stem, leaf, and bud extracts of *S. aromaticum*, formulated as vaporizer mats, against *Aedes aegypti* and to identify potential molecular targets of the major compounds in these extracts through computational analysis. Volatile extracts from stems, leaves, and buds were prepared by ethanol maceration and formulated into vaporizer mats at concentrations of 0.5% and 1%. A total of 525 adult *A. aegypti* (F_{330} strain) were exposed under controlled laboratory conditions in accordance with WHO guidelines. Mortality, LT_{50}, and LT_{90} values were recorded after exposure. Molecular docking was performed against odorant-binding protein AaegOBP1 and pyruvate kinase (PK1), followed by molecular dynamics simulation. Among all treatments, bud extract at 1% produced the highest mortality (33%), compared with 2% in the negative control. LT_{50} and LT_{90} values for this treatment were 1.468 minutes and 50.543 minutes, respectively, indicating prolonged biological activity. Docking analysis showed that β-caryophyllene had the strongest affinity toward AaegOBP1 (-8.4 kcal/mol), while chavicol showed the strongest interaction with PK1 (-6.5 kcal/mol). Molecular dynamics simulation confirmed stable ligand-receptor interactions with RMSF values below 3 Å. These findings indicate that *S. aromaticum* bud extract has moderate mosquitocidal potential in vaporizer mat formulation and may act through olfactory disruption and metabolic interference in *A. aegypti*.

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INTRODUCTION

The rapid spread of infectious diseases in tropical regions continues to pose major public health challenges, particularly through outbreaks of vector-borne illnesses such as dengue, yellow fever, and malaria. Consequently, effective vector control measures remain essential for suppressing mosquito populations and preventing widespread disease transmission[1].

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Among household-level interventions, the electric vaporizer mat is a widely adopted control method that operates by thermally evaporating an impregnated mat, releasing active volatile compounds into the surrounding air. This vapor-phase delivery impairs mosquito physiology while offering practical operational advantages over conventional methods, including ease of use, the absence of ash formation, and minimal aerosol residue[2].

Historically, vector control strategies have relied heavily on synthetic insecticides, primarily pyrethroids, organophosphates, and carbamates. However, the continuous and widespread application of these synthetic formulations has accelerated the emergence of insecticide resistance across wild mosquito populations[3,4]. Resistance mechanisms typically develop through enzymatic detoxification involving cytochrome P450 monooxygenases and esterases, target-site gene mutations such as knockdown resistance (*kdr*), and altered behavioral adaptations[5,6]. Furthermore, growing concerns regarding the potential environmental toxicity and human health risks associated with synthetic residues highlight an urgent need for safer, more sustainable alternatives.

Botanical insecticides, particularly volatile extracts derived from aromatic plants, have received growing attention as viable, eco-friendly alternatives. These plant-derived extracts are rich in bioactive terpenoids, phenolics, and aromatic esters that exhibit diverse physiological actions against mosquitoes, including repellent, neurotoxic, and cytotoxic properties[7,8]. Unlike synthetic insecticides that often target a single physiological site, complex botanical extracts act through multiple concurrent biochemical pathways, thereby reducing the likelihood of rapid resistance development.

Among promising botanical candidates, *Syzygium aromaticum*, or clove, a prominent species within the Myrtaceae family, synthesizes abundant bioactive phytochemicals known for their potent antioxidant, antibacterial, and insecticidal activities[9]. Different plant parts of *S. aromaticum*, including the buds, leaves, and stems, yield distinct volatile profiles characterized by major constituents such as eugenol, eugenol acetate, and β-caryophyllene[10]. While clove bud extracts demonstrate exceptionally strong antioxidant activity, the leaves and stems also contain valuable volatile fractions with significant potential for vector control[11]. Due to their inherent volatility, these phytochemicals are ideally suited for incorporation into electric vaporizer mat formulations, facilitating controlled indoor dispersion and safe application in enclosed domestic spaces.

Biological vector control utilizing natural formulations in vaporizer mats presents strategic advantages for residential mosquito management[12]. Vapor-phase dissemination enables volatile active compounds to interact directly with the mosquito olfactory system, particularly odorant-binding proteins (OBPs) housed within antennal sensilla, which play a crucial role in host location and environmental sensing[13]. Complementing empirical bioassays, computational techniques such as molecular docking and molecular dynamics (MD) simulations provide key mechanistic insights into the binding interactions between specific phytochemical ligands and mosquito target proteins, helping to elucidate potential insecticidal modes of action at the molecular level[14].

Despite extensive research on clove extracts, comparative evaluations investigating how different plant parts of *S. aromaticum* perform when formulated into thermal vaporizer mats, combined with computational target validation, remain limited. Identifying which plant part provides the most effective chemical composition is critical for optimizing natural vaporizer mat formulations. Therefore, this study aimed to evaluate the mosquitocidal activity of stem, leaf, and bud extracts from *S. aromaticum* formulated in electric vaporizer mats against *Aedes aegypti*, and to identify potential molecular targets of their major volatile compounds through computational modeling. We hypothesized that volatile extracts from distinct anatomical parts of *S. aromaticum* would exhibit differential vapor-phase biological activity against *A. aegypti*, driven by specific phytochemical interactions with mosquito olfactory and metabolic proteins. The novelty of this work lies in combining an *in vivo* vaporizer mat bioassay with *in silico* molecular docking and MD analyses against *A. aegypti* odorant-binding protein 1 (AaegOBP1) and pyruvate kinase, thereby providing comprehensive mechanistic insights into clove-derived vector control technologies.

MATERIALS AND METHODS

Materials

Fresh flower buds, leaves, and stems of *S. aromaticum* were harvested from plants cultivated without synthetic insecticides at the Institute of Tropical Diseases, Universitas Airlangga, Surabaya, Indonesia. Test mosquitoes consisted of laboratory-

reared *A. aegypti* (strain F_{330}) maintained in the insectary of the Laboratory of Entomology at the Institute of Tropical Diseases, Universitas Airlangga. 96% ethanol (analytical grade) was used as the extraction solvent and vehicle. Blank porous beer mat matrices (PT Megasari Makmur, Gunung Putri, Bogor, Indonesia) served as the vehicle carrier for the electric vaporizer mat bioassays. Distilled water was used for carrier dilutions and mosquito rearing.

Computational modeling was executed on a workstation equipped with an Intel Core i3 processor running a 64-bit Windows 11 operating system (Lenovo IdeaPad Flex 5). Protein target structures were retrieved from the RCSB Protein Data Bank (https://www.rcsb.org/). Chemical structures and ligand properties were retrieved from the PubChem database (https://pubchem.ncbi.nlm.nih.gov/). Structure preparation was conducted using PyMOL v. 3.0 (Schrödinger, LLC, New York, NY, USA). Target class predictions and drug-likeness evaluations were performed using the SwissADME server (http://www.swissadme.ch/) and the SCFBio server (http://scfbio-iitd.res.in/software/drugdesign/lipinski.jsp). Molecular docking was performed using PyRx 0.8, incorporating the AutoDock Vina (SourceForge Headquarters, San Diego, CA, USA). Conformational flexibility and Root Mean Square Fluctuation (RMSF) analysis were performed using the CABS-flex 3.0 web server (https://lcbio.pl/cabsflex3/). Non-covalent molecular interactions were visualized using BIOVIA Discovery Studio Visualizer 2021 (Dassault Systèmes BIOVIA, San Diego, CA, USA). Probit statistical modeling was performed using IBM SPSS Statistics version 26.0 (IBM Corp., Armonk, NY, USA).

Methods

Botanical extraction and sample preparation

The harvested flower buds, leaves, and stems of *S. aromaticum* were washed, cut into small segments, and shade-dried at ambient room temperature for seven days. Each dried plant part was ground into a fine powder. Extraction was conducted via cold maceration using 96 percent ethanol at a 1:10 solid-to-solvent ratio for 24 hours. The mixture was filtered, and the remaining plant residue underwent a second 24-hour re-maceration cycle with fresh 96% ethanol to maximize the recovery of volatile secondary metabolites. The combined filtrates were concentrated using a rotary evaporator set to 40-50°C, 70 rpm, and a reduced pressure of 0.7 bar until the volume was substantially reduced. Finally, the concentrated extract was dried in a hot-air oven at 40°C to yield the final volatile bioactive extract[15].

Mosquito rearing and environmental conditions

Eggs of *A. aegypti* were harvested using ovitraps placed in the insectary. The lab-strain eggs were incubated and reared under controlled optimum conditions, maintaining a relative humidity of 65-80% and a water temperature of 28-30°C. Upon hatching, larvae were fed a standard laboratory diet until reaching the third instar stage, after which they were monitored through pupation to adult emergence. Newly emerged adult mosquitoes were transferred to mesh storage cages and maintained on a 10% sucrose solution until 5 days of age. A total of 525 adult mosquitoes (275 females and 250 males) were utilized across all experimental bioassay replicates.

In vivo electric vaporizer mat bioassay

The electric vaporizer mat bioassay was conducted in accordance with World Health Organization (WHO) testing standards for household insecticide efficacy, adapted to use blank porous mat matrices[16]. *Aedes aegypti* was chosen as the test species due to its primary role as a major vector for dengue and other arboviral diseases, as well as its established status in laboratory insecticide resistance testing. Bioassays were conducted in triplicate within standard testing drums for each treatment group.

The experimental matrix detailing the extract type, applied volumetric concentrations, and sample size per test replicate is summarized in **Table I**. Two working extract concentrations were prepared on a volume-to-volume basis (v/v) in a final total carrier volume of 0.8 mL. The 0.5% formulation comprised 0.004 mL of volatile extract, 0.398 mL of 96% ethanol, and 0.398 mL of distilled water. The 1.0% formulation comprised 0.008 mL of volatile extract, 0.396 mL of 96% ethanol, and 0.396 mL of distilled water. Negative controls received 0.8 mL of the ethanol-water vehicle without extract, while positive controls utilized a commercial synthetic mat formulation. For each bioassay run, 25 adult mosquitoes were introduced into the test chamber. Treated mats were heated with a standard electric vaporizer, and mosquito knockdown and mortality were recorded at predetermined intervals (0, 5, 10, 15, 20, 25, 30, 60, 180, and 360 minutes). Final mortality was determined after

24 hours of post-exposure recovery[16]. This vapor-phase bioassay simulates real-world household exposure, reflecting how volatile plant metabolites exert biological effects via inhalation and olfactory contact in enclosed spaces.

Table I. Distribution of *A. aegypti* (Strain F_{330}) per bioassay treatment.

Type of Extract	Concentration of *S. aromaticum* Extract (%)	Number of *A. aegypti* per Application (N)
Clove Stem (CS)	0.5	25
Clove Stem (CS)	1.0	25
Clove Bud (CB)	0.5	25
Clove Bud (CB)	1.0	25
Clove Leaf (CL)	0.5	25
Clove Leaf (CL)	1.0	25
Control (-)	Ethanol 1% + Water	25
Control (+)	Commercial transfluthrin mat	25

Protein target preparation

The three-dimensional crystal structures of two key *A. aegypti* biological target receptors, odorant-binding protein 1 (AaegOBP1, PDB ID: 3K1E) and pyruvate kinase (PK1, PDB ID: 6DU6), were retrieved from the RCSB Protein Data Bank. Structural preparation and clean-up of the raw receptor files were performed using PyMOL v. 3.0. Water molecules, crystallographic artifacts, and co-crystallized native ligands were removed, missing hydrogen atoms were added, and partial atomic charges were assigned to prepare the receptor structures for docking simulations[17,18].

Phytochemical ligand selection and retrieval

Target phytochemical ligands were selected based on established chromatographic profiles of *S. aromaticum* volatile extracts reported in literature, including eugenol[19], β-caryophyllene[20], α-humulene[21], eugenol acetate[22], α-copaene[9], chavicol[23], methyl salicylate[24], 4-allylanisole[25], and benzyl acetate[26]. The three-dimensional structures of these small molecules were downloaded in SDF format from the PubChem database. Physicochemical descriptors, PubChem Compound Identifiers (CIDs), oral bioavailability scores, and synthetic accessibility scores retrieved for the study ligands are listed in **Table II**.

Table II. Bioactive phytochemicals evaluated from *S. aromaticum* volatile extract.

Compound Name	Compound CID	Bioavailability Score	Synthetic Accessibility
Eugenol	3314	0.55	1.58
β-caryophyllene	5281515	0.55	4.51
α-humulene	5281520	0.55	3.66
Eugenol acetate	7136	0.55	1.94
α-copaene	19725	0.55	4.62
Chavicol	68148	0.55	1.14
Methyl salicylate	4133	0.55	1.11
4-allylanisole	8815	0.55	1.28
Benzyl acetate	8785	0.55	1.08

Compound screening, pharmacokinetics, and drug-likeness analysis

Target receptor predictions for the identified phytochemicals were evaluated using the SwissADME web server. Drug-likeness properties and drug-candidate suitability were further calculated using the SCFBio computational server[27]. Pharmacokinetic profiles were evaluated against Lipinski's Rule of Five criteria to determine molecular suitability[28]. To satisfy Lipinski's thresholds, a candidate molecule must possess a molecular weight less than 500 Daltons, a lipophilicity value (LogP) less than 5, no more than 5 hydrogen bond donors, no more than 10 hydrogen bond acceptors, and a molar refractivity value strictly between 40 and 130[29].

Molecular docking simulations

Molecular docking experiments were carried out using PyRx 0.8 software incorporating the AutoDock Vina engine. Docking simulations were performed to evaluate the binding affinities between the optimized ligand structures and the catalytic sites of AaegOBP1 and pyruvate kinase[30]. Binding energy values were recorded in kilocalories per mole, with increasingly negative values indicating greater thermodynamic stability and stronger binding affinity of the ligand-receptor complex.

Molecular dynamics and conformational flexibility

Conformational stability and dynamic flexibility of the top-ranked ligand-receptor complexes were assessed by evaluating RMSF profiles using the CABS-flex 3.0 server. Dynamic simulation parameters, including protein backbone stiffness, global structural restraints, C-α restraint weighting, side-chain restraint weighting, trajectory cycle length, temperature range, and random number generator seed values, were kept at validated defaults to ensure accurate simulation of residue-level flexibility and complex stability under physiological thermal conditions[31].

Visualization of non-covalent molecular interactions

The non-covalent binding interactions governing the stability of docked ligand-receptor complexes were visualized using BIOVIA Discovery Studio Visualizer 2021[32]. Two-dimensional and three-dimensional interaction maps were analyzed to identify hydrogen bonds, hydrophobic contacts (including alkyl and π-alkyl interactions), and van der Waals forces, which collectively determine the binding energy and dynamic behavior of the bioactive complexes.

Data analysis

Entomological bioassay data were analyzed using probit regression analysis in IBM SPSS Statistics version 26.0 to determine lethal time median values (LT_{50} and LT_{90}) with corresponding 95% confidence intervals. Overall mosquito mortality percentages were calculated after 24 hours of exposure to the vaporizer mats and, where appropriate, were corrected for negative-control mortality.

RESULTS AND DISCUSSION

Bioassay evaluation using various concentrations of volatile extracts from *S. aromaticum* revealed distinct LT_{50} and LT_{90} values when tested against laboratory-strain F_{330} *A. aegypti* mosquitoes, analyzed via probit analysis in IBM SPSS Statistics at 95% confidence limits. The complete lethal time measurements, including specific LT_{50} and LT_{90} parameters across treatments, are summarized in **Table III**. As illustrated in **Figure 1**, the probit analysis results demonstrate clear dose-dependent and time-dependent impacts on mosquito physiological functions across all tested concentrations. The probit results demonstrate dose-dependent and time-dependent impacts on mosquito physiological functions. Across the evaluated plant parts, clove stem (CS), clove bud (CB), and clove leaf (CL) extracts at concentrations of 0.5% and 1% exhibited varying rates of acute toxicity. The negative control exhibited nominal baseline values (LT_{50} = 0.061 minutes; LT_{90} = 0.307 minutes). Higher extract concentrations increased overall mortality, whereas extended exposure duration produced progressive physiological impairment leading to delayed mortality. Detailed time-course knockdown observations are provided in the supplementary data.

Table III. Effects of extract exposure in each concentration with lethal time measurement (LT_{50} and LT_{90}).

Extract	Concentration (%)	LT_{50} (minutes)	LT_{90} (minutes)
CS	0.5	1.297	6.582
CS	1	0.772	26.589
CB	0.5	1.033	5.241
CB	1	1.468	50.543
CL	0.5	0.975	4.947
CL	1	1.507	51.911
Control (-)	0	0.061	0.307
Control (+)	1	1.197	8.132

The positive control, a commercial vaporizer mat containing synthetic pyrethroids, produced rapid knockdown activity via neurotoxic action on voltage-gated sodium channels. In contrast, *S. aromaticum* volatile extracts exhibited a slower onset of acute toxicity, as reflected by higher LT_{50} values. However, the substantially longer LT_{90} values observed in the CB and CL treatments (reaching 50.543 minutes and 51.911 minutes at 1%, respectively) indicate that clove volatile extracts deliver extended fumigant action. This extended activity suggests a sustained release of bioactive volatile constituents, such as eugenol, thereby providing prolonged protection compared to the rapid volatilization observed in synthetic formulations. Phytochemical investigations confirm that clove buds are particularly rich in eugenol, eugenol acetate, and β-caryophyllene,

which occur at higher relative abundances in buds than in leaves or stems, thereby supporting the observed bioactivity differences[20]. The prolonged LT_{50} and LT_{90} profiles of the clove bud extract indicate that mortality resulted from cumulative vapor exposure over time rather than instantaneous acute intoxication. Active plant volatile molecules are gradually released and absorbed, inducing progressive physiological disruption, a pattern consistent with vapor-phase contact studies of eugenol-rich preparations[8,22]. The corresponding overall mortality rates across all exposure groups after 24 hours are detailed in **Table IV**.

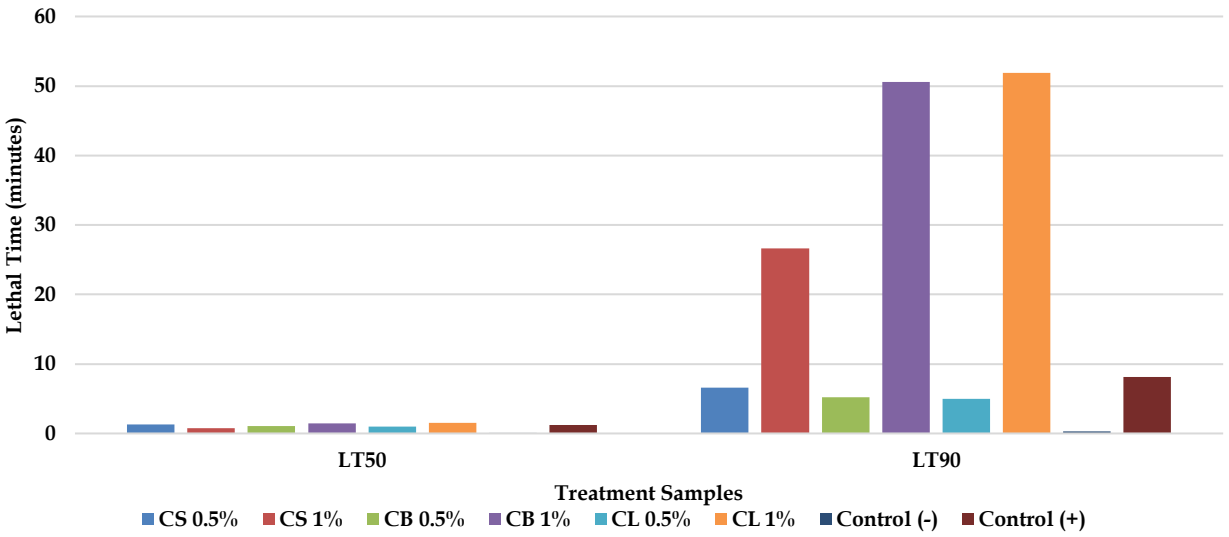


Figure 1. Lethal time concentration graph.

Table IV. Mortality percentage of *A. aegypti* in each extract exposure.

Extract	Concentration (%)	Number of *A. aegypti* per application (N)	Mortality (%)
CS	0.5	25	17
CS	1	25	22
CB	0.5	25	15
CB	1	25	33
CL	0.5	25	14
CL	1	25	29
Control (-)	0	25	2
Control (+)	1	25	13

The graphical representation of these mortality trends is shown in **Figure 2**, which highlights the distinct differences between extract types and control groups. Following 24 hours of post-exposure observation in test cages, the highest mortality rate was observed in the 1% clove bud volatile extract group (33%), whereas the lowest was recorded in the 0.5% clove leaf group (14%). All extract treatments induced markedly higher mortality than the negative control (2%), where mosquitoes maintained active movement with minimal transient disruption from the 1% ethanol-water vehicle. During vapor exposure to *S. aromaticum* extracts, treatment groups displayed progressive physiological impairment, characterized by reduced movement, impaired flight, temporary knockdown, and loss of cage attachment prior to mortality.

These responses are mediated by the major volatile components of *S. aromaticum*, including eugenol, β-caryophyllene, and α-humulene, along with secondary constituents such as betulinic acid, paramethylbenzoic acid, eucalyptolic acid, maslinic acid derivatives, and various flavonoids, tannins, and steroids[33,34]. Synthetic pyrethroids, while highly effective for immediate knockdown, face growing concerns about vector resistance due to continuous exposure. Botanical formulations with complex chemical profiles offer alternative modes of action that may help mitigate the development of resistance. Enzymatic and receptor target screening revealed that the major secondary metabolites in clove volatile extract primarily target kinase and protease pathways. In mosquitoes, pyruvate kinase (PK-1) serves as a key glycolytic enzyme in internal organs, whereas odorant-binding proteins (OBPs), such as AaegOBP1 in *A. aegypti*. act as hydrophobic olfactory receptors in sensory neurons, filtering chemical cues to regulate host-seeking and mating behavior[13,35]. The primary macromolecular target profiles predicted for each individual constituent are depicted in **Figure 3**.

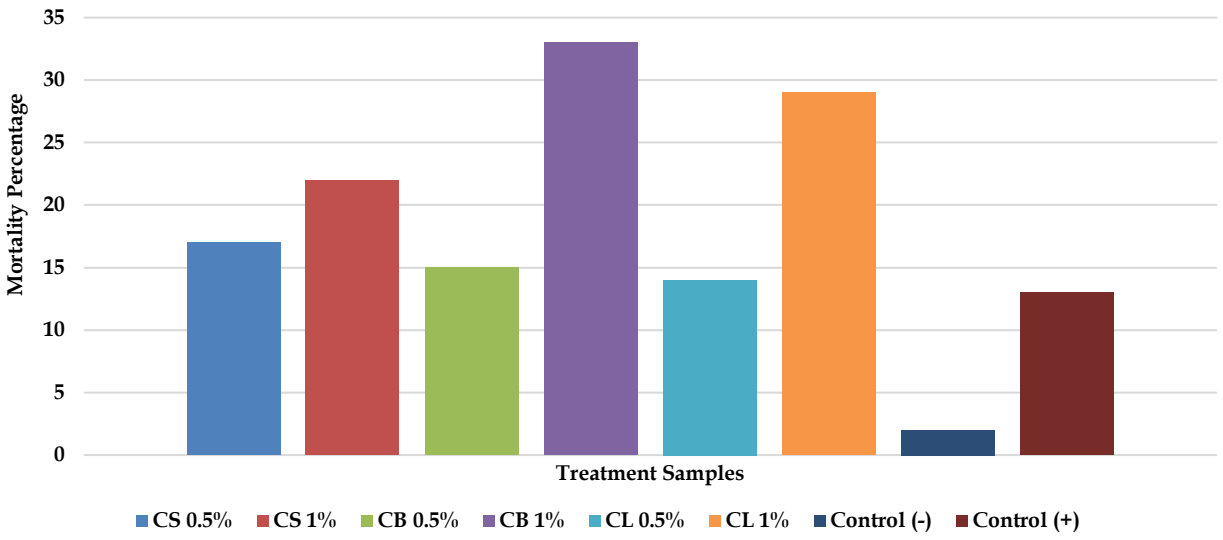


Figure 2. Mortality percentage graph.

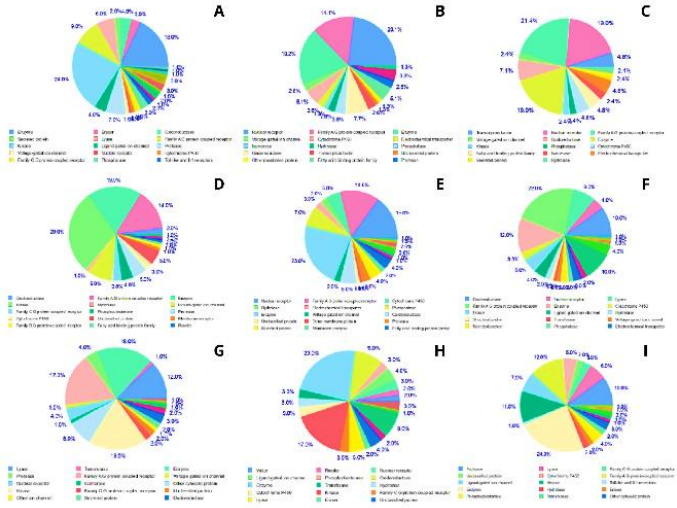


Figure 3. Target receptor of each compound: (**A**) eugenol, (**B**) β-caryophyllene, (**C**) α-humulene, (**D**) eugenol acetate, (**E**) α-copaene, (**F**) chavicol, (**G**) methyl salicylate, (**H**) 4-allylanisole, (**I**) benzyl acetate.

Physicochemical evaluation using Lipinski's Rule of Five demonstrated that the key volatile constituents exhibit drug-like properties suitable for development as active ingredients in vaporizer mat formulations, with specific parameters outlined in **Table V**. Molecular docking experiments elucidated the binding profiles of these compounds to olfactory and metabolic targets. Among the evaluated constituents, β-caryophyllene exhibited the strongest binding affinity toward AaegOBP1 (-8.4 kcal/mol). Conversely, chavicol demonstrated the highest affinity for pyruvate kinase (PK1) (-6.5 kcal/mol). The comprehensive docking scores, interaction types, and involved amino acid residues are presented in **Table VI**.

Table V. Drug-likeness compound screening analysis.

Compounds	MW	HBD	HBA	LogP	MR
Eugenol	164	1	2	2.12	48.55
β-caryophyllene	204	0	0	4.72	66.74
α-humulene	204	0	0	5.03	68.90
Eugenol acetate	206	0	3	2.34	58.20
α-copaene	204	0	0	4.27	64.51
Chavicol	134	1	1	2.12	42.00
Methyl salicylate	152	1	3	1.17	39.44
4-allylanisole	148	0	1	2.42	46.89
Benzyl acetate	150	0	2	1.74	41.91

Note: MW: Molecular weight; HBD: Hydrogen bond donors; HBA: Hydrogen bond acceptors; MR: Molar refractivity.

Table VI. The docking results of *S. aromaticum* compounds with mosquito target proteins.

Bioactive Compounds	Receptor	Binding Energy (kcal/mol)	Interaction Type	Amino Acids Involved in The Interaction
Eugenol	Odorant protein AaegOBP1	-6.8	vdw	Gly92(B), Leu73(B), His111(B), Phe15(B)
			HI	Ala88(B), Leu80(B), Leu76(B), Tyr122(B), Phe123(B)
	Pyruvate kinase (PK1)	-6.4	vdw	Leu201(B), Leu191(B), Val174(B), Val183(B), Val166(B)
			HI	Val207(B), Val157(B), Leu209(B), Leu140(B), Leu181(B), Ile138(B), Leu132(B), Ile155(B), Ile162(B)
β-caryophyllene	Odorant protein AaegOBP1	-8.4	vdw	Ala88(B), Ile87(B)
			HI	Met91(B), Met84(B), Tyr122(B), Phe123(B), Leu80(B), Phe15(B), Trp114(B), Leu76(B), Phe59(B), His111(B)
	Pyruvate kinase (PK1)	-6.2	vdw	His462(C), Phe494(C), Asn68(C), Tyr96(C), Arg41(C), Gly66(C)
			HI	Phe500(C), Val40(C), Phe39(C), Arg498(C), Phe104(C), Leu468(C)
α-humulene	Odorant protein AaegOBP1	-7.7	vdw	Ala88(B), Ile87(B), Met84(B)
			HI	Tyr122(B), Phe123(B), Met91(B), Leu80(B), His111(B), Leu76(B), Phe59(B), Phe15(B)
	Pyruvate kinase (PK1)	-6.4	vdw	His462(A), Gly66(A), Asn68(A), Arg498(A), Phe500(A), Pro105(A)
			HI	Leu468(A), Pro469(A), Phe104(A), Phe494(A)
Eugenol acetate	Odorant protein AaegOBP1	-7.0	vdw	Gly92(A), Met84(A), Tyr122(A), Leu58(A), Leu124(A), Phe59(A)
			HI	Ala88(A), Leu80(A), Leu76(A), Phe15(A)
			PHI	Ile125(A), His111(A)
	Pyruvate kinase (PK1)	-5.9	vdw	Phe500(B), Phe494(B), Val40(B), Phe39(B), Gly66(B), Asn68(B)
			HI	His462(B), Pro469(B), Pro105(B), Phe104(B)
			PHI	Arg41(B)
α-copaene	Odorant protein AaegOBP1	-6.1	vdw	Arg6(A), Ile125(A), Phe123(A), Ser41(A), Leu124(A), Asp42(A), Ile37(A), Arg23(A)
			HI	Ile38(A), Leu16(A), Pro11(A)
	Pyruvate kinase (PK1)	-6.3	vdw	Arg340(A), Arg337(A), Arg171(C), Glu298(C), Ile299(C), Ile297(C), Gly177(C), Gly296(C), Glu302(C)
			HI	Pro210(C), Ala301(C), Pro300(C)
Chavicol	Odorant protein AaegOBP1	-6.3	vdw	Leu76(B), Phe15(B), Leu124(B)
			HI	Ala88(B), Leu80(B)
			PHI	Phe123(B)
	Pyruvate kinase (PK1)	-6.5	vdw	Gly199(D), Gly198(D), Val195(D), Val172(D), Lys133(D), Leu132(D)
			HI	Leu201(D), Leu181(D), Val174(D), Ile179(D), Ile155(D), Ile138(D)
Methyl salicylate	Odorant protein AaegOBP1	-5.9	vdw	Tyr10(B), Met84(B), Ile87(B), His121(B), Phe15(B), Trp114(B)
			HI	Met91(B), Tyr122(B), Ala88(B), Leu76(B)
			PHI	Phe123(B)
	Pyruvate kinase (PK1)	-6.1	vdw	Ser517(B), Thr430(B), Gly512(B), Ile429(B), Val484(B), Arg487(B), Trp480(B)
			PHI	Thr520(B), Phe519(B), Gly518(B), Gly516(B), Thr431(B)
4-allylanisole	Odorant protein AaegOBP1	-6.5	vdw	Gly92(B), Leu73(B), Ala88(B), Phe15(B)
			HI	His111(B), Leu80(B), Leu76(B)
	Pyruvate kinase (PK1)	-6.3	vdw	Tyr159(C), Leu209(C), Cys193(C), Val174(C)
			HI	Val207(C), Ile162(C), Val157(C), Ile117(C), Leu140(C), Val172(C), Ile155(C), Ile138(C), Leu181(C)
Benzyl acetate	Odorant protein AaegOBP1	-6.4	vdw	Ala88(B), Leu73(B), His77(B)
			HI	Leu76(B), Leu80(B)
			PHI	Gly92(B), Trp114(B)
	Pyruvate kinase (PK1)	-5.8	vdw	Ile525(C), Asn527(C)
			PHI	Asn521(B)

Note: vdw: Van der Waals interaction; HI: Hydrophobic interaction; PHI: Polar hydrogen interaction.

Visualization of these top-ranked molecular docking complexes in both 2D and 3D spatial configurations is shown in **Figure 4**. The binding of β-caryophyllene within the hydrophobic cavity of AaegOBP1 involves key residues including Tyr122, Phe123, Met84, Met91, Leu80, Phe15, Trp114, Leu76, Phe59, and His111. Occupation of this non-polar pocket by a compact, bicyclic sesquiterpene disrupts the transport of volatile host cues (e.g., carbon dioxide, lactic acid, and skin odorants) to antennal olfactory receptor neurons, impairing host-seeking orientation and blood-feeding behavior[36]. Concurrently,

chavicol binding to PK1-targeting residues such as Leu201, Leu181, Val174, Ile179, Ile155, and Ile138 suggests potential inhibition of the glycolytic conversion of phosphoenolpyruvate, thereby reducing cellular ATP yield. This dual mechanism, combining sensory disruption via AaegOBP1 binding with metabolic impairment via PK1 inhibition, explains the observed *in vivo* response, in which exposed mosquitoes exhibited progressive neuromuscular weakness, loss of attachment, and delayed mortality.

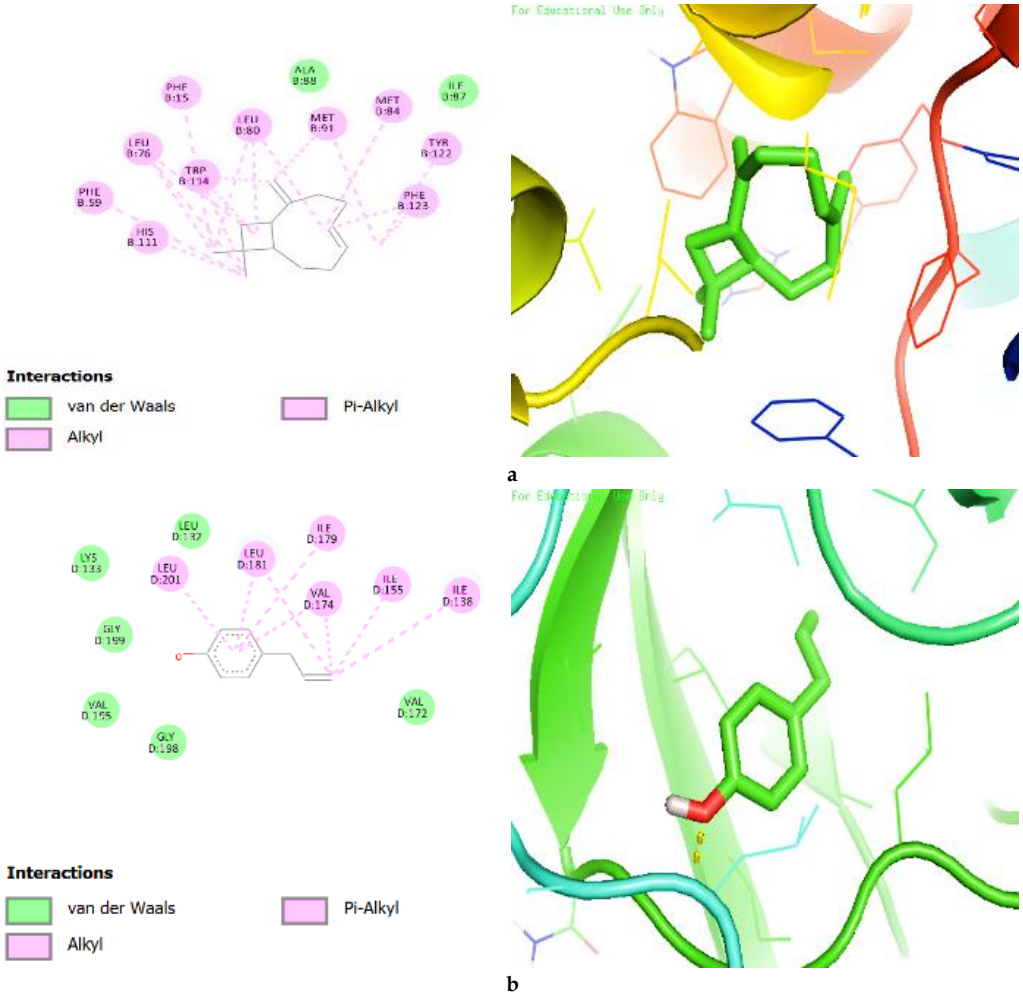
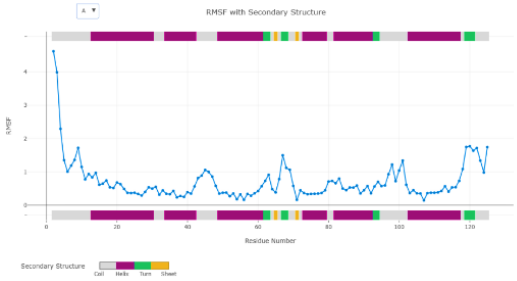
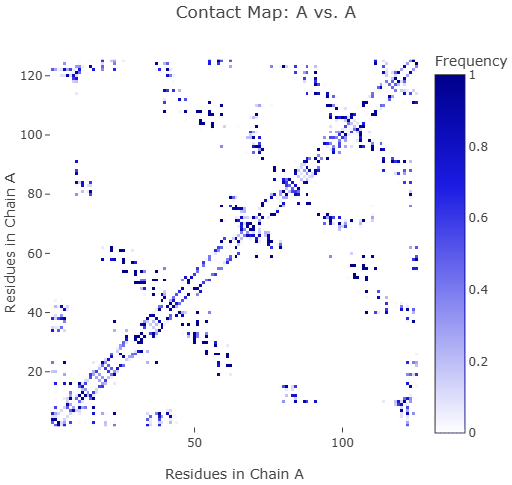
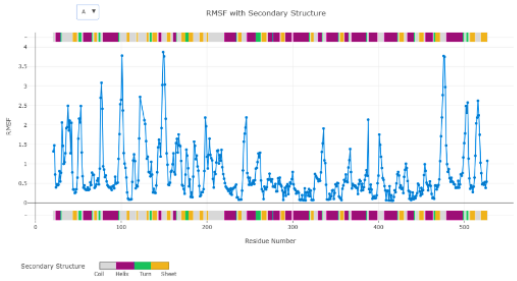
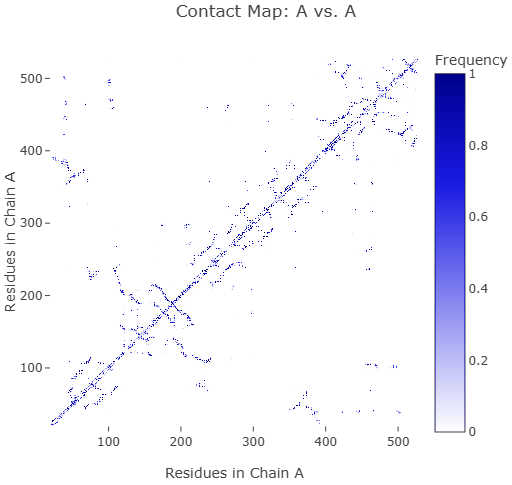


Figure 4. Visualization of docking experiment on the best binding complexes of (**a**) β-caryophyllene-odorant protein AaegOBP1 and (**b**) Chavicol-pyruvate kinase (PK1). Left: 2D visualization of complexes; Right: 3D visualization of complexes.

To evaluate conformational stability, the top-ranked complexes were subjected to MD simulations using the CABS-flex 3.0 server. The calculated RMSF and Radius of Gyration values establishing structural preservation are summarized in **Table VII**. RMSF values remaining below 3 Å confirm low atomic fluctuation and stable structural preservation of the ligand-protein complexes under simulated physiological conditions[37-39]. This structural stability supports the hypothesis that non-polar interactions maintain ligand retention within the target sites, providing a molecular basis for the extended biological activity observed in the bioassays.

These *in vivo* and *in silico* findings highlight the potential of *S. aromaticum* volatile extracts as botanical active ingredients in mosquito-control devices. Nevertheless, these results represent preliminary screening under controlled laboratory conditions using fixed concentrations. Technical challenges such as natural batch variation, susceptibility to thermal oxidation, and volatilization stability must be addressed through optimized formulation design. Future investigations should focus on histological assessments of targeted mosquito tissues, efficacy testing across broader vector species (*Culex*, *Anopheles*, and *Mansonia*), standardized chemical profiling, and safety evaluations to advance clove-oil-based vaporizer mats toward practical public health applications.

Table VII. Molecular dynamics values of top-ranked complexes.

Graphics	Molecular Dynamics Value (Å)
β-caryophyllene–odorant protein AaegOBP1	ΣRMSF = 1.535  ΣRadius of Gyration = 1.000 
Chavicol–pyruvate kinase (PK1) complexes	ΣRMSF = 1.818  ΣRadius of Gyration = 1.000 

CONCLUSION

This study demonstrated that the volatile extract of *S. aromaticum* derived from flower buds at a concentration of 1% exhibits superior mosquitocidal efficacy against *A. aegypti*, achieving the highest observed mortality of 33% under vaporizer mat exposure with a median LT_{50} of 1.468 minutes and LT_{90} of 50.543 minutes. Complementary molecular docking simulations revealed that key bioactive constituents, specifically β-caryophyllene and chavicol, exhibited the strongest binding affinities among the identified compounds, suggesting a potential contribution to sublethal neurophysiological effects by modulating olfactory signaling and glycolytic metabolic pathways. Furthermore, MD simulations confirmed the conformational stability of these target-ligand complexes, with RMSF values remaining below 3 Å. Overall, while *S. aromaticum* volatile extracts demonstrate promising yet modest insecticidal activity against *A. aegypti*, these findings highlight the viability of clove-derived flower bud extracts as botanical active candidates for household mosquito control devices. To advance these extracts toward practical application, future work should prioritize precise chromatographic quantification of active marker compounds, expanded biological dose-response evaluations, direct experimental validation of the predicted macromolecular mechanisms, and targeted formulation optimization to enhance volatilization stability and field efficacy.

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DATA AVAILABILITY

None.

CONFLICT OF INTEREST

The authors declared no conflict of interest.

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