Evaluation of the Leaf Essential Oil from *Artemisia vulgaris* and Its Larvicidal and Repellent Activity against Dengue Fever Vector *Aedes aegypti*—An Experimental and Molecular Docking Investigation

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**ABSTRACT:** *Aedes aegypti* is a mosquito vector that spreads dengue fever and yellow fever worldwide in tropical and subtropical countries. Essential oil isolated from *Artemisia vulgaris* is found to have larvicidal and repellent action against this vector. The dried leaves were subjected to hydrodistillation using a clevenger-type apparatus for 4 h. The isolated essential oil was analyzed by using gas chromatography–mass spectrometry, and the major insecticidal compounds were identified as α-humulene (0.72%), β-caryophyllene (0.81%), and caryophyllene oxide (15.87%). Larvicidal activity results revealed that the essential oil exposure for 24 h period against the third stage larvae was LC₅₀ = 6.87, LC₉₀ = 59.197 ppm and for the fourth stage larvae LC₅₀ = 4.269, LC₉₀ = 50.363 ppm. Highest mortality rates were observed at 24 h exposure period of third and fourth stages, and the exposed *A. aegypti* larvae were subjected to histo chemical studies, and the studies revealed that larvae cells got totally damaged (midgut and cortex). The repellent activity results revealed that at 50% concentration of the essential oil showed the highest repellent activity at 60 min protection time against the *A. aegypti* female mosquitoes. To gain further insights into the insecticidal activity, density functional theory and molecular docking calculations were performed with the active components of this essential oil as the ligand and NS3 protease domain (PDB ID: 2FOM) as a receptor. Molecular docking calculation results show that (E)-β-caryophyllene strongly binds with NS3 protease domain than (Z)-β-caryophyllene, α-humulene, and β-caryophyllene oxide and is the major active component for the insecticidal action. It primarily interacts with the receptor through hydrophobic and ionic forces and using water bridges between the amino acid residues in the binding pocket and (E)-β-caryophyllene.

1. **INTRODUCTION**

Dengue fever is one of the most dangerous fevers threatening mankind in the recent past. Especially in the developing countries, India is the main victim of this disease. It is reported that the Philippines with more than 169 000 cases and Malaysia with around 111 000 suspects contribute a huge population to the total dengue cases, and furthermore, Brazil alone reported having over 1.5 million cases of dengue.¹ Dengue virus belongs to the family Flaviviridae.²,³ Humans and monkeys are greatly affected by this dengue virus. Dengue fever shows symptoms in patients such as increased body fever up to 40 °C, muscle and joint pain, severe headache, facial flushing and skin rashes, and severe flu-like symptoms.⁴ The fever symptoms will not appear immediately; it will start appearing after 5 to 8 days of the infection in patients.⁴ This shows that the urgent need for treating this deadly disease. Many researchers have been toiling day and night to come up with suitable drug and medicine to treat dengue fever and to control the vector. The huge concern on public health and the limited options available in the treatment of dengue diseases have stimulated efforts to identify and characterize potential viral drug target.

One such strategy is using the medicinal plant as a source to find new medicines. In the literature, it is reported that the essential oils from plants have been used to treat various diseases. For instance, *Mentha, Melissa,* and *Salvia* plants have piperitenone oxide, which kills *Culex pipiens* larvae responsible for Japanese encephalitis.⁶ Terpenoid compounds obtained from *Pogostemon* species were found to show greater larvicidal activity against *Aedes albopictus* larvae.⁷ This indicates that essential oil from plants is important in drug designing. Essential oils are complex mixtures comprising of many single compounds, and chemically, they are derived from terpenes and their oxygenated compounds. However, more number of synthetic organic insecticides (organophosphates) have been used for controlling populations of *Aedes aegypti*. More number
Table 1. Essential Oil Constituents of *A. vulgaris* Leaf

| peak no. | chemical composition | retention time | %   |
|----------|----------------------|----------------|-----|
| 1        | tricyclo[2.2.1.0(2,6)]heptane, 1,7,7-trimethyl- | 6.91           | 0.09|
| 2        | α-pinene             | 7.25           | 1.3 |
| 3        | camphene             | 7.87           | 1.59|
| 4        | cyclohexene, 4-methylene-1-(1-methylethyl)- | 8.63           | 0.07|
| 5        | α-pinene             | 8.84           | 0.66|
| 6        | 1-octen-3-ol         | 9.29           | 1.64|
| 7        | bicyclo[4.1.0]hept-2-ene, 3,7,7-trimethyl- | 10.33          | 0.09|
| 8        | eucalyptol           | 11.08          | 5.99|
| 9        | 1,4-cyclohexadiene, 1-methyl-4-(1-methylethyl)- | 12.06          | 0.22|
| 10       | cis-α-terpinel       | 12.95          | 0.31|
| 11       | bicyclo[4.1.0]hept-2-ene, 3,7,7-trimethyl- | 3.24           | 0.04|
| 12       | 1-dodecen-3-ol       | 13.47          | 0.23|
| 13       | octen-1-ol, acetate  | 14.07          | 0.10|
| 14       | terpineol, cis-α-formula: | 14.40          | 0.63|
| 15       | campholenol, 6-     | 15.35          | 0.03|
| 16       | 2-cyclohexen-1-ol, 1-methyl-4-(1-methylethyl)-, cis- | 15.64          | 0.18|
| 17       | camphor              | 16.65          | 26.99|
| 18       | 2(10)-pinen-3-one, (δ)- | 17.06          | 0.44|
| 19       | isoborneol           | 17.35          | 2.15|
| 20       | borneol              | 17.94          | 10.79|
| 21       | naphthalene          | 18.43          | 0.825|
| 22       | p-menth-1-en-8-ol    | 18.82          | 0.465|
| 23       | bicyclo[3.1.1]hept-2-ene-2-methanol, 6,6-dimethyl- | 18.95          | 0.259|
| 24       | 2-cyclohexen-1-ol, 2-methyl-5-(1-methylethene), trans- | 20.09          | 1.81|
| 25       | 2-cyclohexen-1-ol, 2-methyl-5-(1-methylethan), cis- | 20.66          | 0.11|
| 26       | 2-cyclohexen-1-one, 2-methyl-5-(1-methylethene), (R)- | 20.88          | 0.22|
| 27       | 4-hexen-1-ol, 5-methyl-2-(1-methylethene), acetate | 22.10          | 0.29|
| 28       | bornyl acetate       | 22.26          | 1.04|
| 29       | isobornyl acetate    | 22.38          | 0.19|
| 30       | ethanone, 1-(6,6-dimethylbicyclo[3.1.0]hex-2-en-2-yl)- | 22.75          | 0.07|
| 31       | 1-cyclohexene-1-methanol, 4-(1-methylethyl)-cyclohexan-1-ol, | 23.74          | 0.28|
| 32       | 2-methyl-5-(1-2-methylethylene), acetate, cis- | 24.45          | 0.10|
| 33       | eugenol              | 26.05          | 0.40|
| 34       | ylangene             | 26.18          | 0.53|

Figure 1. GC-MS chromatogram of *A. vulgaris* essential oil.
of synthetic organic insecticides (organophosphates) have been used for controlling populations of A. aegypti. These types of insecticides are toxic to human beings and environment. Thus, this raises concern to urgently look for alternative technology for vector control management.8,9

According to this study, it has been reported that the dengue virus vector genome is 10.7–11 kb length, and virus genome is composed of single-stranded RNA. The structure of the dengue virus is generally spherical with a diameter of approximately 50 nm,10 which is proteolytically cleaved into 10 viral proteins, including three structural and seven nonstructural (NS) proteins. The order of these viral proteins is capsid, pre-membrane, envelope protein, NS1, NS2A, NS2B, NS3, NS4A, NS4B, and NS5. The structural proteins are responsible for the structure of a virus and NS proteins are necessary for the viral replication.11 NS3 is composed of a protease (NS3PRO) and a helicase (NS3HEL) domain, and the former is responsible for processing the polyprotein in specific sites.12 Thus, NS3 compound is considered as a significant target to screen and evaluate effects of different drug candidates.13 Therefore, docking studies with NS3 protease protein and the active component of the oil were performed to understand the biological activity.

The X-ray structure of the NS3 isoforms forms the DV-3 stereotype complexes with a serine protease inhibitor and its X-ray structure has been recently deposited in the Protein Data Bank (PDB code 3U1I).14–17 NS3 is a NS protein of 69 kDa and it is a multifunctional protein. It has two domains, namely N-terminal domain and C-terminal domain. The N-terminal domain is a serine protease with NS2B cofactor which is important for viral poly protein processing. The C-terminal domain is RNA helicase and a nucleoside 5’ triphosphate, and this domain is involving in the viral replication and virus particle assembly. This clearly reveals the important role of NS3 in the viral life cycle and indicates that it is the suitable target for the development of DV inhibitors.17 Molecular docking with small molecular inhibitors was done and the docking results were used in molecular dynamics (MD) simulations. Homology model was used for virtual screening, searching pharmacophore, and docking small molecule inhibitors from databases. The obtained results from MD simulations give a clear understanding of the ligand receptor interaction at a molecular level, and furthermore, they provide useful clues for the development of drugs to treat DV infections.18,19 The homology model for the DENV NS2B/NS3 protease was done first by Brinkworth and co-workers.20

The present work focuses on the extraction of the essential oil from *Artemisia vulgaris* and investigates its biological activity. It is important to note that *A. vulgaris* is a medicinal, aromatic plant and belongs to the family of Asteraceae and species of *A. vulgaris*.21 These plants are more common in Europe, Asia, Northern Africa, and Alaska and are naturalized in North America.22 Over the years, the essential oil obtained from *A. vulgaris* has been used by food and pharmaceutical industries and in folk medicine to treat gastrointestinal diseases. This essential oil contains many volatile compounds and its components are known for insecticidal properties and allelopathic effect. Moreover, the dried leaves and boiled leaves are showing greater medicinal values. It is also reported that this plant has been used in the treatment of various diseases, including anthelmintic, antiseptic, antispasmodic, carminative, cholagogue, diaphoretic, digestive, emmenagogue, expectorant, nervine, purgative, stimulant, and slightly tonic.23–25 This has

### Table 2. Larvicidal Effect of *A. vulgaris* Essential Oil against the Third Stage Larva of *A. aegypti*

| name of the mosquito species | time | concentration (ppm) | % mortality | LC_{90}(LCL−UCL)a | LC_{40}(LCL−UCL)b | χ^2 (df = 4)c |
|-----------------------------|------|---------------------|-------------|-------------------|-------------------|----------------|
| *A. aegypti* third stage     | 12 h | 5                   | 20.00 ± 0.0d| 38.31 (28.779–58.737) | 1270 (497.936–6806.185) | 3.48 |
|                             |      | 10                  | 33.50 ± 3.5f|                   |                   |                |
|                             |      | 25                  | 43.50 ± 1.5g|                   |                   |                |
|                             |      | 50                  | 54.50 ± 2.5h|                   |                   |                |
|                             |      | 100                 | 62.00 ± 2.8i|                   |                   |                |
|                             |      | 5                   | 41.25 ± 1.2j|                   |                   |                |
|                             |      | 10                 | 64.88 ± 2.4k|                   |                   |                |
|                             | 24 h | 25                  | 72.06 ± 3.0l| 6.87 (2.532–11.328) | 59.197 (33.327–219.644) | 3.14 |
|                             |      | 50                  | 86.12 ± 0.8m|                   |                   |                |
|                             |      | 100                 | 97.28 ± 0.7n|                   |                   |                |

aMeans in each column followed by different letters are significantly different (*P* < 0.05, by one-way ANOVA and Duncan’s multiple range test). LCL lower confidence level; UCL upper confidence level. b95% confidence interval. cDegrees of freedom; χ^2 chi-square value.

### Table 3. Larvicidal Effect of *A. vulgaris* Essential Oil against the Fourth Stage Larva of *A. aegypti*

| name of the mosquito species | time | concentration (ppm) | % mortality | LC_{90}(LCL−UCL)a | LC_{40}(LCL−UCL)b | χ^2 (df = 4)c |
|-----------------------------|------|---------------------|-------------|-------------------|-------------------|----------------|
| *A. aegypti* fourth stage    | 12 h | 5                   | 36.80 ± 1.6p| 135.238 (76.408–396.476) | 1.007 (355.986–8265.941) | 3.86 |
|                             |      | 10                  | 47.28 ± 1.7q|                   |                   |                |
|                             |      | 25                  | 56.36 ± 0.6r|                   |                   |                |
|                             |      | 50                  | 61.50 ± 1.5s|                   |                   |                |
|                             |      | 100                 | 74.00 ± 1.0t|                   |                   |                |
|                             |      | 5                   | 56.60 ± 1.6u|                   |                   |                |
|                             |      | 10                 | 67.35 ± 1.5v|                   |                   |                |
|                             | 24 h | 25                  | 76.26 ± 1.0w| 4.269 (0.501–8.550) | 50.363 (26.017–351.084) | 3.09 |
|                             |      | 50                  | 87.95 ± 2.0x|                   |                   |                |
|                             |      | 100                 | 98.81 ± 0.4y|                   |                   |                |

aMeans in each column followed by different letters are significantly different (*P* < 0.05, by one-way ANOVA and Duncan’s multiple range test). LCL lower confidence level; UCL upper confidence level. b95% confidence interval. cDegrees of freedom; χ^2 chi-square value.
triggered our interest toward *A. vulgaris* plant and its essential oil toward the treatment of dengue fever vector.

2. RESULTS AND DISCUSSION

2.1. Isolation of the Essential Oil. The essential oil was isolated from the leaves of *A. vulgaris* at a yield of 0.5% (v/w). The essential oil was analyzed to find out the major constituents through gas chromatography–mass spectrometry (GC–MS) analysis. This analysis shows the 63 compounds were present in the oil (Figure 1). It is important to note that the *A. vulgaris* essential oil contains the following important insecticidal terpenoid compounds; camphor (26.99%), \( \alpha \)-humulene (0.72%), \( \beta \)-caryophyllene (0.81%), and \( \beta \)-caryophyllene oxide (15.87%), respectively (Table 1). Similar compounds were reported for the *Cinnamomum zeylanicum* bark, which contains \( \alpha \)-humulene (0.28%) and \( \beta \)-caryophyllene (0.89%) respectively.\(^{26}\) Similarly, essential oil of various species of *Guarea* contains caryophyllene epoxide (40.91%) and humulene epoxide II (14.43%). Essential oil of *Guarea humaitensis* has cis-caryophyllene (33.37%) and \( \alpha \)-trans bergamotene (11.88%), respectively. Meanwhile, *Gentiana scabra* essential oil consists of caryophyllene epoxide (36.54%) and spathulenol (14.34%), respectively.\(^{27}\) The earlier study suggests that hairy root culture essential oil from *A. vulgaris* contains camphene (5.5%), camphor (20.8%), \( \alpha \)-thujone (12.3%), and \( \beta \)-caryophyllene (5.7%), respectively.\(^{28}\) This indicates that the obtained volatile compounds are in total agreement with the earlier reports.

2.2. Bioassay of Larvicidal Activity. The essential oil isolated from *A. vulgaris* leaf explant exhibits significant larvicidal activity at various concentrations (5, 10, 25, 50, and 100 ppm) against the dengue fever vector *A. aegypti* (Tables 2 and 3). In the present study, two types of the larvae are considered. It is interesting to note that 12 h exposure of the essential oil to third stage larvae leads to the highest mortality at 100 ppm (62.00 ± 2.8), \( LC_{50} \) values = 38.3 ppm, \( LC_{90} = 1270 \) ppm. On the other hand, 24 h exposure of the essential oil leads to third stage larvae produces the highest mortality at 100 ppm (97.28 ± 0.7), \( LC_{50} \) values = 6.87 ppm, \( LC_{90} = 59.197 \). In the fourth stage larvae, highest mortality is observed at 100 ppm (74.00 ± 1.0), \( LC_{50} \) values = 135.238 ppm, \( LC_{90} = 50.363 \) ppm (Tables 2 and 3). The compounds identified in *A. vulgaris* leaf essential oil such as \( \beta \)-caryophyllene, \( \alpha \)-humulene, and \( \beta \)-caryophyllene oxide have a huge impact on the larvicidal activity. It is also important to note that these compounds of essential oil interact with the cells of *A. aegypti* larvae and destroy them completely resulting in the death of cells. The results of the present study can be compared with earlier reports,\(^{29–31}\) where leaf essential oil of *Eucalyptus camaldulensis* had a very excellent activity against *A. aegypti* larvae and *A. albopictus* larvae12 and 24 exposure h with \( LC_{50} \) values of 71.8 and 192.4 \( \mu \)g/mL and \( LC_{90} \) values of 31.0 and 55.3 \( \mu \)g/mL, respectively. The activity of *Cryptomeria japonica* leaf essential oil against the fourth stage of *A. aegypti* larvae with 24 h exposure time showed \( LC_{50} = 37.6 \) \( \mu \)g/mL, \( LC_{90} = 71.9 \) \( \mu \)g/mL. *Zanthoxylum armatum* seed essential oil is found to be show greater activity against third stage mosquito larvae such as *Culex quinquefasciatus* with \( LC_{50} = 49 \) ppm, followed by *A. aegypti* \( LC_{50} = 54 \) ppm and *Anopheles stephensi* \( LC_{50} = 58 \) ppm. The above data revealed that *A. vulgaris* leaf essential oil exhibits high mortality rate against *A. aegypti* larvae compared to earlier reports.

The dead larvae have been used for histological studies. Histological images obtained for 24 h treated larvae are given in Figure 2. The figure shows the cortex (ct), midgut (mg), and determine tissue (dt) regions of the *A. aegypti* larvae with and without treatments. Figure 2a,c shows that the third and fourth stages of control larvae in which the ct, mg, and dt regions are intact, whereas in Figure 2b,d, the image of the treated larvae shows that ct, mg, and dt regions were highly affected, and there also both third and fourth stage larvae were observed to have leakage of the mg region contents. This may be due to the presence of volatile compounds such as \( \alpha \)-humulene, \( \beta \)-caryophyllene, and \( \beta \)-caryophyllene oxide in the essential oil. Literature reports suggest that compared to purified compounds, crude extracts or plant extracts are less expensive and highly effective for the control of mosquitoes.\(^{32,33}\)

2.3. Repellent Activity. Table 4 presents data pertaining to the repellent activity of the essential oil at various concentrations (2.5, 5, 15, 25, and 50%) against dengue fever female mosquito’s *A. aegypti*. This study was carried out in a laboratory screen cage

![Figure 2](https://example.com/figure2.png)

**Figure 2.** Histochemical analysis of *A. aegypti* larvae in (a) control third stage larvae, (b) third stage larvae essential oil, (c) fourth stage larvae control, and (d) fourth stage larvae essential oil.

| concentration of *A. vulgaris* essential oil (%) | percentage of repellency ± SD (min) |
|-----------------------------------------------|-------------------------------------|
| 15′                                           | 10.0 ± 2.8a/ 8.33 ± 2.8a/ 4.33 ± 1.5a |
| 30′                                           | 85.0 ± 4.3b/ 60.00 ± 10.0d/ 49.00 ± 1.7d |
| 60′                                           | 71.00 ± 1.0c/ 53.66 ± 3.2d/ 39.66 ± 2.5d |
| 5.0                                           | 39.00 ± 8.5b/ 35.33 ± 5.5c/ 30.66 ± 2.5c |
| 15                                            | 33.33 ± 2.8b/ 24.00 ± 3.6b/ 15.66 ± 4.0b |
| 25                                            | 33.15 ± 5.0c/ 9.00 ± 1.7a/ 6.66 ± 1.5a |

*Means in each column followed by different letters are significantly different (P < 0.05, by one-way ANOVA and Duncan’s multiple range test).
condition which gives protection against mosquito bites. No allergic complaints have been reported from the tested persons.

In comparison with N,N-diethyl-meta-toluamide (DEET) available in commercial antimosquito repellant compounds,
our essential oil was found to show greater activity. The essential oil showed the highest repellent percentage (6.66 ± 1.5) at 50% concentration in 60 min against A. aegypti female mosquitoes. The present study shows that increase in the concentration of the essential oil leads to increase in the protection time leading to prolonged repellent activity against A. aegypti mosquitoes. Citrus plant’s essential oil was reported to have a protection percentage from 10.0 ± 8.7 to 65.0 ± 22.9 min, biting rate ranged from 1.2 to 2.3% and protection ranged from 98.3 to 98.8%, respectively. The benzene and methanol extracts of A. vulgare were previously reported to have repellent activity against A. aegypti. Repellent activity proved that the Zingiber cassumunar essential oil has greater activity against C. quinquedactylus (165 min protection time and 0.9% biting rate) and A. aegypti (90 min protection time and 0.8% biting rate). Dry flower powder of Chrysanthemum cinerariaefolium has been used as insecticides since ancient times. Some natural products obtained from plants play a major role in the development of commercial insecticide. For instance, α-terthienyl has been known for its herbicidal activity. The skin test for evaluating the repellency of catnip oil is found to have two major active ingredients (Z, E-nepeta lactone and E, Z-nepetalactone). Earlier reports show that 3-carene, α-terpinene, limonene, γ-terpinene, terpinolene, and (−)-terpen-4-ol isolated from C. japonica essential oil are active against the A. aegypti and A. Albopictus, while confertifolin compound isolated from P. hydropiper essential oil is exhibiting a good repellent activity (100% adulticidal activity against A. albopictus). These observations indicate that our results are very promising to formulate a potent and affordable essential oil against the dengue fever vector A. aegypti.

### 2.4. Molecular Docking with NS3 Protease.

Density functional theory (DFT) calculations in conjunction with molecular docking analysis helps to understand the mechanism by which the small molecule (ligand) binds into the active site of large biomolecules. Hence, in the present work, molecular docking calculations were carried out using the DFT-optimized ligand molecules. MD simulations of the tetrapeptide (PDB code 2FP7) of this enzyme complex with an inhibitor binding were reported in important of DV-2 NS2B/NS3 protease. The NS3 protease protein cleaves the poly protein precursor into individual efficient proteins, which play an important role in the replication cycle of DV. Therefore, the identified major active components (E)-β-caryophyllene, (Z)-β-caryophyllene, α-humulene, and β-caryophyllene oxide were docked into the active site of NS3 protease to identify which one of these is the most effective compound. The flexible molecular docking was carried out by Glide XP (Extra precision) mode of docking (Glide, version 6.8, Schrodinger, LLC, New York, NY, 2015). The parameters used for docking are as follows: number of poses per ligand 500, van der Waals radii 0.80, and default parameters for strain corrections and clustering panel. The docked pose was selected based on the highest Glide score and lowest binding energy conformation, and it was used for further analysis. The docked systems show a buried beneath of protein. The crucial ligand-protein-binding free energy for (E)-β-caryophyllene, (Z)-β-caryophyllene, α-humulene, and β-caryophyllene oxide are −2.747, −2.609, −2.574, and −2.342 kcal/mol, respectively. These energy values clearly show that all the terpenoid compounds have binding energies in the same range, and (E)-β-caryophyllene, among the four, strongly binds to the NS3 protease than other three complexes. The protein–ligand interactions could be understood based on the nature of the interaction between ligand and NS3 protease. Hydrogen bond, hydrophobic and ion pair interactions, and water bridges between ligand and active site residues play a key role in the accommodation of small molecule into the catalytic domain of a protein.

### 3. CONCLUSIONS

The essential oil extracted from hydrodistillation of dried leaves of A. vulgare exhibits good insecticidal property against A. aegypti, a dengue fever vector. GC−MS analysis of the oil shows that there are 63 compounds and they have been identified with GC−MS (NIST 2005) software. Among the terpenoids, Campher (26.99%), α-humulene (0.72%), β-caryophyllene (0.8%), and β-caryophyllene oxide (15.87%) are important insecticidal compounds. Exposure of the third stage larvae for 12 and 24 h at 100 ppm showed highest mortality respectively with LC50 = 38.31 ppm and LC90 = 6.87 ppm, whereas the fourth stage larvae showed highest mortality LC50 = 135.24 ppm and LC90 = 4.26 ppm, respectively. Histological images revealed that theolatile oil compounds α-humulene, β-caryophyllene, and β-caryophyllene oxide affected ct, mg, and dt regions. A. aegypti female mosquitoes after exposure showed the highest repellent percentage (6.66%) at 50% concentration with ethyl acetate solution in 60 min. NS3 protease which plays a key role in the replication cycle of dengue virus was docked with the four active components of this essential oil (E)-β-caryophyllene, (Z)-β-caryophyllene, α-humulene, and β-caryophyllene oxide, and computational details show that (E)-β-caryophyllene binds strongly to the NS3 protease receptor and the protein ligand complex is stabilized by hydrophobic and ionic interactions and water bridges between the ligand and the amino acid residues in the receptor-binding pocket. This study concluded that β-caryophyllene has a good therapeutic potential to treat the life-threatening dengue fever.

### 4. EXPERIMENTAL SECTION

#### 4.1. Plant Material

The leaves of A. vulgare were collected from the Departmental garden from the Department of Botany, Bharathidasan University, Tiruchirappalli, Tamil Nadu, India.

#### 4.2. Isolation of the Essential Oil

The collected healthy leaves were washed thoroughly under the running tap water and air-shade-dried for 1 or 2 weeks at room temperature. Then, the dried leaves were used to get oil from hydrodistillation using Cleveky type of apparatus after 4 h. The essential oil thus obtained was stored in an amber color glass vial (sealed with parafilm) stored at 4 °C for further experiments on larvicidal and repellent activity.

#### 4.3. Identification of the Essential Oil Compound by GC−MS Analysis

The identification of the chemical constituents of the essential oil was carried out by GC−MS.
using PerkinElmer Clarus 500, Turbo mass version 5.2.0. The column type Capillary Column Elite-Sms (5% phenyl 95% dimethylpolysiloxane) was used, and the total running time is 62 min, the injector temperature is at 250 °C, and the oven initial temperature was set at 60 °C. Helium gas was used with a flow rate 1 mL/min, and the essential oil sample 0.6 μL was injected in the split mode in the ratio of 1:30; the percentage of the compound of essential oil was calculated by the GC peak areas. The mass spectrum was recorded with mass scan range 40–450 amu, electron ionization, and 70 eV. Then, transfer line and source temperature were kept at 180 and 160 °C. The identification of the spectrum components was compared with the database of the spectrum of known components provided in the GC–MS (NIST 2005) library software.

4.4. Bioassay of Larvicidal Activity. The eggs of A. aegypti were received from the Center for Research in Medical Entomology, Madurai, Tamil Nadu, India. Then, larvae eggs were maintained in the Department of Botany, Bharathidasan University. The larvae were fed on dog biscuits and yeast powder in the 1:1 ratio through the mountain at 28 ± 2 °C temperatures 70–85% relative humidity with a photoperiod of 12 h light and 12 h dark. The early A. aegypti third and fourth larvae stages were used for bioassay of larvicidal activity of A. vulgaris essential oil, and it was evaluated according to the World Health Organization (WHO) standard protocol.52 The efficacy of larvicidal activity was tested against the essential oil dissolved in 1 mL of ethyl acetate and various concentrations were prepared viz., 5, 10, 25, 50, and 100 ppm. Thirty larvae of A. vulgaris of third and fourth stages were used for the larvicidal bioassay and maintained three replicates in each concentration of essential oil was carried out and using the dead larvae after 12 and 24 h of the exposure period was used. The lethal concentrations LC50 and LC90 at which concentration (ppm) 50% of larvae showed mortality and 95% confidence limit of upper and lower confidence levels were calculated by probit analysis (SPSS, version 16.0).

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\text{Percentage of mortality} = \frac{\text{number of dead larvae}}{\text{number of larvae}} \times 100
\]

4.5. Histological Analysis. Histological test of newly ec lysed A. aegypti larvae was carried out with the highest mortality rate of third and fourth stage larvae. In this experiment, only live larvae were examined. Then, the larvae were fixed with 10% formaldehyde solution for 24 h, after which the solution was dehydrated with ethanol series and cleared xylene solutions. The larvae were embedded in melted thin paraffin wax and the section was cut with 5 μM thickness glass knives in a rotary microtome than; the larvae were stained using hematoxylin and eosin. The slide was inspected, analyzed, and photographed with a light microscope (Olympus CX31).

4.6. Repellent Activity. A. vulgaris leaf essential oil was evaluated at various concentrations (2.5, 5, 15, 25, and 50%) in ethyl acetate and control DEET against the A. aegypti mosquitoes, and biting testing time was 15, 30, and 60 min, and it was examined through laboratory screen cages (diameter 40 cm × length 30 cm) containing 50 nulliparous, nonblood fed, starved female mosquitoes.53 Human volunteer’s hands were washed and cleaned using distilled water, and both hands were covered with rubber gloves with a window (4 cm × 5 cm) on the forearm before the repellent application. The A. aegypti mosquito biting test was carried out between 8:00 a.m. and 4:00 p.m. and a total number of mosquitoes biting were recorded for 3 min of the study period. The mosquito biting percentage was calculated for each test using the formula:

\[
\% \text{ biting} = \frac{\text{no. of bites received by control arm} - \text{no. of bites received by treated arm}}{\text{no. of bites received by control arm}} \times 100
\]

4.7. Molecular Docking. 4.7.1. Computational Details. The DFT calculations were carried out with B3LYP54,55 functional using Gaussian 09.56 The ground state geometries of the (E)-β-caryophyllene, (Z)-β-caryophyllene, α-humulene, and β-caryophyllene oxide were optimized using the standard 6-31+g (d,p) basis set. The coordinates of caryophyllene complexes were taken from optimized geometries as a molfile, and they were converted into PDB format using GaussView 5.0.9 software.57 The physiological medium and ionization states were determined for all caryophyllene ligands, and the pH was set to 7.0 by the ligand preparation using “LigPrep” module, followed by minimization and conformational searched modules of the maestro. The crystal structure of both active forms with a cocrystallized substrate-based inhibitor NS53 protease (PDB IDS: 2FOM) was retrieved from the protein data bank (https://www.rcsb.org/pdb/). Proteins were prepared by the necessary key steps, followed by hydrogen atoms, missing side chains, and loops were added using a protein preparation “PRIME” module. All water molecules and heterotomons were removed from the receptor protein, followed by the receptor was refined by minimization and optimization using the OPLS 2005 force field in the protein preparation module of Schrodinger’s Maestro Molecular Modeling suit. After ligand and protein preparation, grid generation was done according to the active site of the protein. Docking calculation was performed using the energy minimized (E)-β-caryophyllene, (Z)-β-Caryophyllene, α-humulene, and β-caryophyllene oxide with NS2B/NS3 protease as the host using Glide (Glide, Version 6.8, Schrodinger, LLC, New York, NY, 2015) by means of XP precision mode and its output Glide score was calculated.58 Finally, visualization of the docked system has been analyzed using PyMOL software package.59

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S.B. and R.K.B.D. conceived of the idea of the work. S.B. and A.K.M. carried out the experiment and data interpretation. G.S., R.V.S., and P.V. performed the computations and data interpretation. All authors discussed the results and contributed to the final manuscript.

**Notes**

The authors declare no competing financial interest.
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