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Essential Oils of The Leaves of *Syzygium hemilamprum* (F. Muell.) Craven & Biffin.: Chemical Analysis, Antimicrobial, Mosquito Larvicidal, Molecular Docking, and ADMET Studies

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The chemical composition of the essential oil and *n*-hexane extract from *Syzygium hemilamprum* leaves was first performed. Gas chromatography-mass spectrometry (GC-MS) analysis revealed that the essential oil was predominantly composed of monoterpene hydrocarbons (71.5%) and oxygenated derivatives (20.2%), with β-pinene (31.5%), limonene (19.4%), α-pinene (12.3%), and α-terpineol (7.4%) being the principal constituents. The *n*-hexane extract contained monoterpene hydrocarbons (42.2%) and non-terpenic compounds (34.0%), with β-pinene (32.8%) and *n*-hexadecane (10.2%) as the major components. Antimicrobial and mosquito larvicidal assays demonstrated that both samples exhibited antimicrobial activity against *Staphylococcus aureus* (Gram-positive), *Pseudomonas aeruginosa* (Gram-negative), and *Saccharomyces cerevisiae* (yeast), with a minimum inhibitory concentration (MIC) of 128 μg/mL for all tested organisms. Both samples also showed significant mosquito larvicidal activity against *Aedes aegypti* and *Culex quinquefasciatus*, with LC_{50} and LC_{90} values below 20 μg/mL at 24 and 48 hours post-treatment. Molecular docking studies suggested that limonene and α-terpineol could serve as potent inhibitors of mosquito odorant binding proteins. Additionally, an *in silico* analysis was performed to evaluate the physicochemical and ADMET (absorption, distribution, metabolism, and toxicity) properties of the major constituents of the essential oil.

Introduction

Syzygium is one of the biggest genera of flowering plants in the Myrtaceae family, with a variety of beneficial uses, including the production of colorful, tasty, and meaty fruits.[1] The genus comprises about 1100–1200 species, which is widely distributed in Southern East Asia, the Pacific, Madagascar, and Africa.[2] The genus *Syzygium* is rich in phytochemicals type lignans, terpenoids, chalcones, flavonoids, alkyl phloroglucinols, hydrolyzable tannins, and chromones.[3] *Syzygium* plant extracts, essential oils, and isolated compounds have shown various bioactivities such as antimicrobial, anti-inflammatory, antifungal, anti-HIV, anti-diarrheal, and anthelmintic properties.[4] This genus also contained several sizable species that are widely known: *S. aqueum*, *S. guineense*, and *S. samarangense*, especially *S. cumini* and the Clove *S. aromaticum*.[1]

In another aspect, *Syzygium* species are a good reservoir of essential oils. *Syzygium* essential oils consist of several main phytochemical classes, which are monoterpene hydrocarbons, sesquiterpene hydrocarbons, and their oxygenated derivatives.[5] Besides, oxygenated diterpenes, and non-terpenic compounds were also identified.[5] Antibacterial essential oil of Egyptian *S. cumini* leaves was reported to contain α-pinene (32.32%), β-pinene (12.44%), *trans*-caryophyllene (11.19%), and

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1,3,6-octatriene (8.41 %).[6] Essential oil of *S. buxifolium* leaves, which were collected from Vietnam, showed strong mosquito larvicidal activity against *Ae. aegypti* third instar larvae with the LC_{50} value of 6.73 μg/mL, and LC_{90} value of 13.37 μg/mL for 24 h treatment.[7]

Syzygium hemilamprum, also known as the broad-leaved lilly pilly, blush satinash, cassowary gum, and Eungella gum, can be found in Southeast Asia, and Australia.[8] This rainforest plant has broadly lance-shaped to elliptical leaves, white flower panicles, and white fruits. To date, there is not yet any document to report phytochemical and biological investigations on this species. The ultimate goal of this study is to identify and compare chemical compositions in essential oils and *n*-hexane extract of the fresh leaves from this species. The obtained essential oil and extract were further subjected to antimicrobial, and mosquito larvicidal activities. Experimental outcomes were aided by *in silico* approaches.

Results and Discussion

Phytochemical Analysis

Hydro-distillation of the fresh leaves resulted in a yellow essential oil with an yield of 0.11 %, w/w. A total of 32 compounds were identified, which accounted for 99.3 % (Table 1). Monoterpene hydrocarbons (71.55 %) and their oxygenated derivatives (20.2 %) accounted for the majority of this essential oil. Sesquiterpene hydrocarbons (5.5 %), oxygenated sesquiterpenes (1.0 %), and non-terpenic chemicals (1.1 %) were the other types. The major components in this sample were β-pinene (31.5 %), limonene (19.4 %), α-pinene (12.3 %), and α-terpineol (7.4 %). Additionally, linalool (4.4 %), terpinen-4-ol (3.6 %), (*E*)-caryophyllene (2.6 %), γ-terpinene (2.4 %), *p*-cymene (2.3 %), ρ-mentha-2,4(8)-diene (1.1 %), and caryophyllene oxide (1.0 %) were among the constituents that were significant in the essential oil.

Regarding the *n*-hexane extract, 35 compounds were identified, which represented 94.6 % (Table 1). Monoterpene hydrocarbons (42.2 %) and non-terpenic compounds (34.0 %) were the principal chemical classes. In the meantime, the three remaining chemical classes ranged from 3.7–8.3 %. As can be seen, non-terpenic compounds, especially fatty acids, appeared as the main agents in the *n*-hexane extract, but they were not significant in essential oil. It can be explained by the role of extraction methods. β-Pinene (32.8 %), and α-pinene (5.2 %) were still the major compounds in the *n*-hexane extract. Three fatty acids *n*-hexadecane (10.2 %), 1-hexadecene (6.2 %), and *n*-tetradecane (6.0 %) were found to be the main agents in the extract, but they were absent in essential oil. The obtained extract was also associated with the presence of other significant compounds, such as (2*E*)-hexenal (4.3 %), (*E*)-caryophyllene (2.9 %), myrtenol (2.7 %), linalool and 1-tetradecene (2.6 %, each), (3*E*)-hexenol (2.3 %), and limonene (2.2 %).

Generally, the pinene isomers are likely to be the main compounds of essential oil and non-polar extract of Vietnamese *S. hemilampra* leaves. The results reflect the effects of time collection, geography, or extraction procedure. *Trans*-β-ocimene (31.68 %) is the primary component in the essential oil of *S. cumini* half-ripe fruit pulps using the HS-SPME/GC-MS (headspace solid-phase microextraction-gas chromatography-mass spectrometry) analytical method.[11] The two main diterpenoids, squalene, and phytol, were found to appear in the *n*-hexane extract of Malaysian *S. polyanthum* leaves by GC-MS analysis and UAE (ultrasound-assisted extraction).[12]

Biological Activities

Additional tests for antibacterial and mosquito larvicidal properties have been performed on two samples. According to Table 2, the essential oil and extract exhibited a similar minimum inhibitory concentration (MIC) of 128 μg/mL against the Gram (+) bacterium *S. aureus*, the Gram (−) bacterium *P. aeruginosa*, and the yeast *S. cerevisiae*. Additionally, essential oil exerted a MIC value of 128 μg/mL against *B. subtilis*, a Gram (+) bacteria. The Gram (+) bacterium *C. sporogenes* and the Gram (−) bacterium *E. coli* were further limited in growth by the tested materials, with MIC values ranging from 256–512 μg/mL. Nevertheless, they demonstrated little efficacy against the yeast *C. albicans* and the fungus *A. niger*, *A. brasiliensis*, and *F. oxysporum* (MIC > 512 μg/mL). *Syzygium* essential oils and extracts might be the potentials for bacterial treatments. The leaf essential oils of *S. corticosum* and *S. szemaoense* have demonstrated moderate antimicrobial activity against *Enterococcus faecalis* and *S. aureus* with the MIC value of 128.0 μg/mL, and *C. albicans* with the MIC value of 64.0 μg/mL.[13] Antimicrobial activity of *S. boisianum* leaf essential oil was also accompanied by the MIC value of 128 μg/mL against *B. cereus* and *S. aureus*.[2] The *n*-hexane extract of *S. polyanthum* leaves possessed the highest antimicrobial activity against *S. aureus* and *E. coli* with IC_{50} values of 49.25 and 27.54 μg/mL, respectively.[14]

The samples were additionally exposed to mosquito larvicidal activity targeting *Ae. aegypti* and *Cx. quinquefasciatus* larvae in their third-instar stage. It is important to highlight that four criteria are used to describe the inhibitory activity: strong ($LC_{50} \leq 50$ μg/mL), moderate ($50 < LC_{50} \leq 100$ μg/mL), weak ($100 < LC_{50} \leq 750$ μg/mL), and inactive ($LC_{50} > 750$ μg/mL).[7,13,15,16] As shown in Table 3, both essential oil and extract showed strong activity against these two larvae, as well as essential oil is always better than the *n*-hexane extract. In detail, essential oil exhibited the 24 h-LC_{50} value of 13.51 μg/mL and 24 h-LC_{90} value of 16.89 μg/mL against *Ae. aegypti* larvae, as compared with those of the *n*-hexane extract (the 24 h-LC_{50} value of 15.50 μg/mL and 24 h-LC_{90} value of 19.63 μg/mL). After 48 h, the LC_{50} and LC_{90} values decreased to about 10.75 and 14.70 μg/mL, respectively. In the same manner, essential oil was associated with the 24 h-LC_{50} value of 10.05 μg/mL and 24 h-LC_{90} value of 12.88 μg/mL against *Cx. quinquefasciatus* larvae, which were better than those of the *n*-hexane extract (the 24 h-LC_{50} value of 14.18 μg/mL and 24 h-LC_{90} value of 18.66 μg/mL). Regarding 48 h treatment, essential oil possessed the LC_{50} value of 9.86 μg/mL and LC_{90} value of 14.81 μg/mL), whereas the LC_{50}

Table 1. Chemical compositions in essential oils and *n*-hexane extract of *S. hemilampra* leaves.

No	RT	RIE	RIL	Compounds	Essential oil	*n*-Hexane extract
1	4.80	843	844	(3*E*)-Hexenol	–	2.3
2	4.87	846	846	(2*E*)-Hexenal	–	4.3
3	4.97	851	850	(3*Z*)-Hexenol	0.6	–
4	5.27	864	863	*n*-Hexanol	0.3	–
5	6.90	924	924	α-Thujene	0.4	0.2
6	7.16	932	932	α-Pinene	**12.3**	**5.2**
7	7.59	945	945	α-Fenchene	0.2	–
8	7.66	947	946	Camphene	0.7	–
9	8.03	958	952	Benzaldehyde	0.2	–
10	8.19	963	959	(4*Z*)-Hepten-1-ol	–	0.5
11	8.48	971	969	Sabinene	–	0.8
12	8.61	975	974	β-Pinene	**31.5**	**32.8**
13	9.08	989	988	β-Myrcene	0.5	0.1
14	9.63	1004	1002	α-Phellandrene	0.2	–
15	10.11	1016	1014	α-Terpinene	0.5	–
16	10.42	1023	1022	*p*-Cymene	2.3	0.3
17	10.59	1027	1024	Limonene	**19.4**	2.2
18	11.83	1057	1054	γ-Terpinene	2.4	–
19	12.45	1072	1067	*cis*-Linalool oxide (furanoid)	0.2	–
20	13.09	1088	1085	ρ-Mentha-2,4(8)-diene	1.1	0.6
21	13.58	1099	1095	Linalool	4.4	2.6
22	14.25	1115	1114	*endo*-Fenchol	1.3	0.7
23	15.33	1139	1135	*trans*-Pinocarveol	0.4	–
24	16.53	1166	1165	Borneol	2.0	–
25	17.04	1177	1174	Terpinen-4-ol	3.6	–
26	17.63	1190	1186	α-Terpineol	**7.4**	1.6
27	17.91	1197	1194	Myrtenol	0.7	2.7
28	20.51	1255	1254	Linalool acetate	0.2	–
29	22.56	1301	1298	Carvacrol	–	0.7
30	24.13	1338	1338	Bicycloelemene	–	0.4
31	25.81	1377	1374	α-Copaene	0.4	–
32	26.43	1391	1388	1-Tetradecene	–	2.6
33	26.77	1399	1400	*n*-Tetradecane	–	**6.0**
34	27.66	1420	1417	(*E*)-Caryophyllene	2.6	2.9
35	28.06	1430	1430	β-Copaene	–	0.3
36	28.48	1440	1439	Aromadendrene	0.4	–
37	29.07	1454	1454	α-Patchoulene	–	0.3
38	29.39	1462	1458	*allo*-Aromadendrene	0.4	–
39	30.02	1477	1478	γ-Muurolene	0.5	–
40	30.21	1482	1480	Germacrene D	–	0.9
41	30.88	1498	1495	2-Tridecanone	–	1.1
42	31.26	1508	1505	(*E*,*E*)-α-Farnesene	–	0.6
43	31.54	1515	1513	γ-Cadinene	0.5	0.5
44	31.90	1524	1522	δ-Cadinene	0.7	0.5
45	34.04	1579	1577	*trans*-Sesquisabinene hydrate	–	0.7
46	34.27	1585	1582	Caryophyllene oxide	1.0	1.4
47	34.52	1591	1588	1-Hexadecene	–	**6.2**
48	34.81	1598	1600	*n*-Hexadecane	–	**10.2**

Table 1. continued

No	RT	RIE	RIL	Compounds	Essential oil	*n*-Hexane extract
49	35.63	1620	1618	Isolongifolan-7-α-ol	–	0.6
50	36.97	1656	1656	Valerianol	–	0.7
51	38.53	1698	1700	*n*-Heptadecane	–	0.8
52	39.64	1729	1728	*iso*-Longifolol	–	0.3
Total					99.3	94.6
Monoterpene hydrocarbons					71.5	42.2
Oxygenated monoterpenes					20.2	8.3
Sesquiterpene hydrocarbons					5.5	6.4
Oxygenated sesquiterpenes					1.0	3.7
Non-terpenic compounds					1.1	34.0

RT: Retention time, bold: major compound, [a] Retention indices relative to n-alkanes (C7-C40) on Equity-5 column [b] Retention indices from Adams book[9] and the NIST standard database[10]

Table 2. Antimicrobial activity of essential oil and *n*-hexane extract.

Microbial strains		Minimum inhibitory concentration (MIC: µg/mL)				
		Essential oil	*n*-Hexane extract	Streptomycin	Tetracycline	Nystatin
Gram (+)	*B. subtilis*	128	256	4		
	C. sporogenes	256	512	8		
	S. aureus	128	128	8		
Gram (−)	*E. coli*	512	256		4	
	P. aeruginosa	128	128		4	
Fungi	*A. niger*	>512	>512			8
	A. brasiliensis	>512	>512			8
	F. oxysporum	>512	>512			8
Yeasts	*C. albicans*	>512	>512			4
	S. cerevisiae	128	128			8

Table 3. Mosquito larvicidal activity of essential oil and *n*-hexane extract.

Samples	LC50 (95% confidence levels)	LC90 (95% confidence levels)	χ2	*p*
	24-h treatment (*Ae. aegypti*)			
Essential oil	13.51 (12.84–14.85)	16.89 (15034–20.96)	0.073	0.995
n-Hexane extract	15.50 (14.41–17.26)	19.63 (17.73–23.19)	1.029	0.794
Permethrin (control)	0.0094 (0.0082–0.0107)	0.0211 (0.0185–0.0249)	57.6	0.000
	48-h treatment (*Ae. aegypti*)			
Essential oil	10.76 (10.06–11.44)	14.58 (13.65–15.97)	0.029	0.999
n-Hexane extract	10.75 (10.04–11.46)	14.79 (13.80–16.28)	0.044	0.998
	24-h treatment (*Cx. quinquefasciatus*)			
Essential oil	10.05 (9.36–10.68)	12.88 (12.17–13.82)	0.001	1.000
n-Hexane extract	14.18 (13.29–15.53)	18.66 (16.94–21.90)	1.102	0.777
	48-h treatment (*Cx. quinquefasciatus*)			
Essential oil	9.86 (9.06–10.66)	14.81 (13.61–16.62)	0.132	0.988
n-Hexane extract	13.59 (12.59–14.81)	19.92 (18.12–22.73)	5.309	0.151

LC_{50}: 50% Lethal concentration, LC_{90}: 90% Lethal concentration.

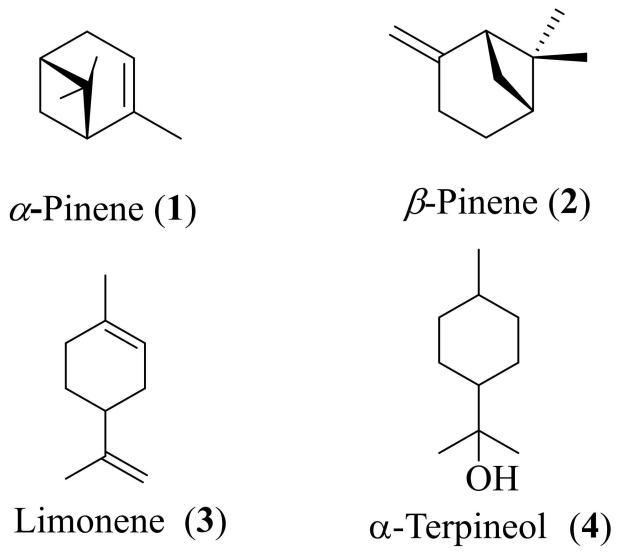

Figure 1. The major components of *S. hemilampra* leaf essential oil.

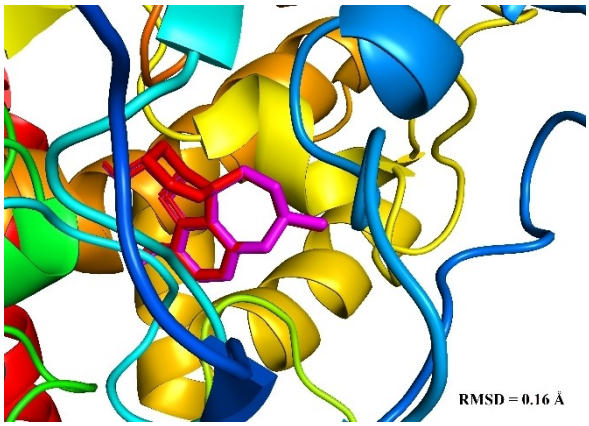

Figure 2. Superimpose docking conformation of crystallographic ligands (in magenta) with the calculated shape (in red): (**A**) permethrin docked with odorant binding protein (PDB ID: 3OGN) and (**B**) galantamine docked with acetylcholinesterase (PDB ID: 4EY6).

and LC_{90} values of the *n*-hexane extract reached 13.59 and 19.92 μg/mL, respectively.

Until now, studies using *Syzygium* essential oils on mosquito larvicidal properties have shown interests. For example, *Ae. aegypti* larvae were significantly inhibited by the leaf essential oils of *S. attopeuense* and *S. tonkinense*, with LC_{50} values of less than 30.18 μg/mL and LC_{90} values of less than 39.01 μg/mL.[7] With LC_{50} values of 92.56 and 124.42 mg/L, respectively, *S. aromaticum* bud essential oil inhibited the growth of *Ae. aegypti* and *Cx. quinquefasciatus* larvae.[7] At 200 mg/L, the aqueous-ethanolic extract (7 : 3, v/v) of *S. quineense* roots killed all of the larvae of *Ae. albopictus* in their first instar.[17] The obtained outcomes validated the effects of *Syzygium* components as insect repellents.

In Silico Studies

It is assumed that mosquito larvicidal activity of the studied essential oil is due to the actions of the main compounds α-pinene (**1**), β-pinene (**2**), limonene (**3**), and α-terpineol (**4**) (Figure 1). The odorant binding protein (OBP) and acetylcholinesterase (AChE) were selected as the protein targets. Traditionally, the OBP has been a potential insecticidal drug target.[18] This protein plays a key role in host-seeking activities by carrying smells to olfactory receptors. However, the AChE, an essential enzyme for biological nerve transmission, can break down acetylcholine and stop the nerve impulse at cholinergic synapses.[19] The AChE is a target enzyme of several pesticides used worldwide for the aforementioned reason.

The docking was validated with the root mean square deviation (RMSD) of the dock pose of the co-crystallized ligand being less than 2.0 Å.[20] In the current research, the docking approach was validated by obtaining the docking conformation of co-crystallized ligands (Figure 2).

Given the beneficial mosquito larvicidal properties of the examined essential oils, AutoDock4 has been widely used for predicting binding free energy and conformation.[21] According to the ranking criteria of the AutoDock, the more negative value of the docking score, the better the binding affinity of the compound towards the targeted receptor.[22] Docking results are presented in Table 4.

Two reference inhibitors, permethrin and galantamine, yielded docking scores of −9.97 kcal/mol for 3OGN and −12.73 kcal/mol for 4EY6, respectively. Any compound that has docking energies near this threshold would therefore be regarded as a potential inhibitor of the targeted proteins. Table 1 also showed that compounds **1**–**4** had binding affinities

Table 4. Docking results of studied compounds and their interacting residues.

Compounds	Biding affinity (kcal/mol)		Interacting residues with 3OGN
	3OGN	4EY6	
1	−6.58	−5.94	Leu73, Leu76, His77, Ala88, and Trp114
2	−6.59	−5.74	Leu73, Leu76, His77, Ala88, and Trp114
3	−8.23	−5.71	Leu76, Ala88, Met91, and Trp114
4	−9.18	−5.97	Leu80, Met84, Ile87, Ala88, Met91, and Phe123
Permethrin	−9.97	–	Leu15, Leu19, Leu73, Leu76, His77, Ala88, Met91, Gly92, His111, Trp114, Phe123, and Leu124
Glantamine	–	−12.73	–

to acetylcholinesterase that ranged from −5.71 kcal/mol–−5.97 kcal/mol, which was significantly lower than galantamine. Therefore, it is reasonable to exclude the hypothesis that these compounds exhibited pesticidal activities via AChE functional inhibition. Compounds **3**–**4** showed the best binding affinities for the mosquito odorant binding protein, at −8.23 kcal/mol and −9.18 kcal/mol, respectively. These values were comparable to those of permethrin.

The binding conformation of inhibitors in the mosquito odorant protein active sites, as predicted by docking simulation, is shown in Figure 3. The mosquito odorant binding protein active site is comprised of up of the significant residues Tyr10, Leu15, Leu19, Leu73, Leu80, Met84, Ile87, Ala88, Met91, His111, Trp114, His121, and Phe123, as was previously described.[23] The residues Leu15, Leu19, Leu73, Leu76, His77, Ala88, Met91, Gly92, His111, Trp114, Phe123, and Leu124 participated in hydrophobic interactions, according to a binding conformation analysis of permethrin. Compound **3** docked within the 3OGN active site with a docking score of −8.23 kcal/mol. Binding orientation analysis exhibited Leu76, Ala88, Met91, and Trp114 initiating the hydrophobic interaction with the potent inhibitor. Compound **4** has a docking energy of −9.18 kcal/mol. Binding mode analysis with targeted protein revealed that Leu80, Met84, Ile87, Ala88, and Met91 were the key residues that participate in hydrophobic interactions and Phe123 formed a hydrogen bond with this compound.

Besides good activities, a compound becomes a potential insecticide since its physicochemical and ADMET (absorption, distribution, metabolism, and toxicity) profiles are acceptable.[24] All studied compounds **1**–**4** meet the criteria of Lipinski's Rule of Five, indicating that these compounds have a high potential bioavailability. It should be mentioned that the hydrogen bond donor (HBD) and acceptor (HBA) were associated with oxygenated compound **4**, whereas they were absent in monoterpene hydrocarbons **1**–**3**. The octanol/water partition coefficient (LogP) was valued between 2.50 and 3.30 (Table 5), suggesting advantageous conditions for penetration, and reaching the target sites in living organisms. Except for compound **4**, which has a blood-brain barrier (BBB) value of 0.956, the remaining compounds may easily penetrate the brain cell membrane and interact with the central nervous system (CNS). The high human intestinal absorption (HIA) values of compounds **1**–**4** ranged from 94.18–95.43, indicating that they could be absorbed through the intestine, reached the bloodstream, and transported to the intended molecular target. All studied compounds **1**–**4** also showed acceptable metabolic stability with no inhibition against Cytochrome P450 (CYP450), expecting a good metabolic drug aspect. Finally, none of the studied compounds showed toxicity to the liver.

Conclusions

Phytochemicals from *S. hemilamprum* leaf essential oil and *n*-hexane extract were detected for the first time. Monoterpene hydrocarbons and their oxygenated derivatives were the main constituents of essential oil. Monoterpene hydrocarbons and non-terpenic compounds were found in the *n*-hexane extract. Overall, the two samples exhibited antibacterial activity with MIC values ranging from 128–512 μg/mL against the tested Gr (±) bacteria. Notably, after 24 and 48 hours of treatments, they demonstrated robust mosquito larvicidal action against the third star of *Ae. aegypti* and *Cx. quinquefasciatus* larvae, with LC_{50} and L_{90} values of less than 20 μg/mL. Docking and ADMET profiling analyses suggested that α-terpineol and limonene were highly penetrant molecules towards the cell brain membrane, and acted in the CNS of larvae. These compounds also exhibited mosquito larvicidal activity as potential odorant-binding protein inhibitors.

Experimental Section

Materials

The fresh leaves were collected from Hue province, Vietnam at 16°27′48.0"N and 107°34′35.0"E on 12 December 2023. Botanical identification was confirmed by Dr. Hoang Xuan Thao, Hue University. A voucher number SH-2023 has been also deposited in Hue University.

Microbial strains were supplied from American Type Culture Collection, USA, including three Gram (+) bacteria *Bacillus subtilis* ATCC 19659, *Clostridium sporogenes* ATCC 19404, and *Staphylococcus aureus* ATCC 6538, two Gram (−) bacteria *Escherichia coli* ATCC 8379 and *Pseudomonas aeruginosa* ATCC 9027, three fungi *Aspergillus niger* ATCC 16404, *A. brasiliensis* ATCC 9642, and *Fusarium oxysporum* ATCC 62506, and two yeasts *Candida albicans* ATCC 2091 and *Saccharomyces cerevisiae* ATCC 2601. They were cultured on Muller Hilton Agar (MHA, Merck) plates for 24 h at 37 °C.

Eggs of *Aedes aegypti* and *Culex quinquefasciatus* third larvae were purchased from the Institute of Biotechnology, VAST, and maintained at the Laboratory of the Department of Pharmacy of Duy Tan University, Da Nang, Vietnam. The standards streptomycin,

Table 5. The physicochemical and ADMET characteristics of the studied compounds.

Compounds	MW	HBD	HBA	BBB	HIA	Hepatoxicity	CYP450 inhibition	LogP
1	136.24	0	0	0.779	95.06	No	No	2.99
2	136.24	0	0	0.817	95.43	No	No	2.99
3	136.24	0	0	0.728	95.40	No	No	3.30
4	154.25	1	1	0.956	94.18	No	No	2.50

MW: Molecular weight, HBD: Hydrogen bond donor, HBA: Hydrogen bond acceptor, BBB: blood-brain barrier value, and HIA: Human intestinal absorption.

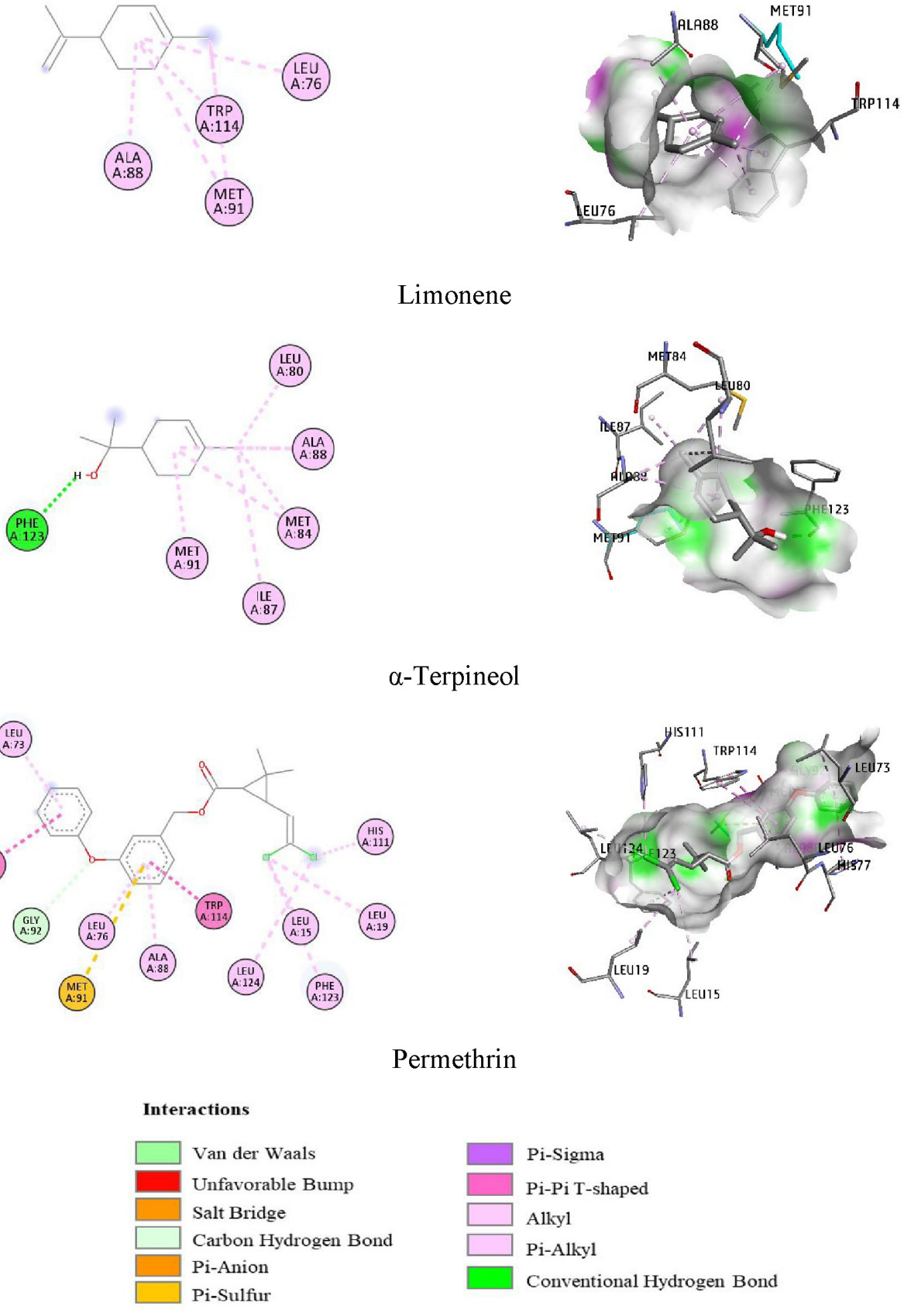

Figure 3. Docking conformation of potential compounds against mosquito odorant binding protein.

tetracycline, nystatin, permethrin, and other chemicals were purchased from Sigma, Aldrich.

Hydro-Distilled Procedure

The fresh leaf powder (1.0 kg) was combined with 2.0 L of deionized water, and the mixture was then cooked three times for 2.2 h at 100 °C for in a Clevenger-type apparatus. Essential oil was extracted four times from the aqueous layer using *n*-hexane solvent. After being dehydrated over anhydrous Na_2SO_4, this was filtered and evaporated to remove *n*-hexane. The resultant oil was stored at 0 °C for further analysis. In the second procedure, 1.0 kg of the leaf powder was also immersed in *n*-hexane (three times) without heating. The *n*-hexane extract (4.5 g) was obtained by evaporating to discard the solvent. The yield was denoted as the weight of the product divided by the weight of the fresh leaf powder, including 0.11 %, w/w for essential oil, and 0.45 % w/w for *n*-hexane extract.

GC-MS Analysis

Shimadzu Technologies GCMS-QP2010 Plus (Shimadzu, Kyoto, Japan) coupled with a fused silica Equity-5 capillary column (30 m, 0.25 mm, film thickness 0.25 μm, Supelco, USA) was used to analyze chemical compositions.[15,25,26] The condition settings have included carrier helium (1.5 mL/min), the injector and interface temperature at 270 °C, and the ramp temperature from 50 °C (1.5 min hold) to 250 °C (9 min hold) at 4 °C/min and to 270 °C at 5 °C/min for the column (9 min hold). A split ratio of 9:1 applied to inject the samples, whereas the inlet pressure and the injection volume were was 93.2 kPa and 1.0 μL, respectively. Ionization voltage at 70 eV, detector voltage at 0.82 kV, and acquisition scan mass from 40–500 amu at a sampling rate of 0.5 scan/s were the MS establishments. The RI (retention index) was determined by comparing the obtained data to a homologous sequence of *n*-alkanes (C_7-C_{40}). The process of chemical identification involved comparing the RI values of the samples to published data.[9,10] Using the NIST 11 and WILEY 7 libraries, the MS fragmentations were corrected in comparison to those of other essential oils with known compositions. Based on the relative area of the volatile compounds' total ion chromatogram (TIC) peaks, each volatile chemical was quantified.[15,25,26]

Antimicrobial and Mosquito Larvicidal Assays

The detailed assays were identical to our previous publications and available in supplementary material.[13,16]

Molecular Docking Study

The 3D structures of compounds **1**–**4** were prepared using MarvinSketch 19.27.0 and PyMOL version 1.3r1.[27] The MM2 force field was used to optimize the ligands' energy, and Gaussian 09 was used to perform quantum chemical calculations at the B3LYP/6-31g (d,p) level using.[28] The Protein Data Bank provided the X-ray crystal structures of the mosquito odorant binding protein (PDB ID: 3OGN) and the acetylcholinesterase receptor (PDB ID: 4EY6).[29,30] The co-crystallized ligand was redocked to confirm that the binding interactions matched those in the original structure, thereby validating the docking technique. The reference compounds were galantamine and permethrin, which are known to inhibit acetylcholinesterase and the mosquito odorant binding protein, respectively. To attain the proper tautomeric states and ionization of the amino acid residues, the protein structures were created. Polar hydrogen atoms were supplied after the water molecules were eliminated. After that, solvation parameters and Kollman united atom partial charges were determined. The outcome of this preparation procedure was a PDBQT file with the protein's atomic coordinates in the format needed for AutoGrid and AutoDock.[31]

The location and dimensions of the grid box for each protein were selected to encompass the amino acid domain involved in binding with the reference compound. This was enclosed in a box with grid points in the X×Y×Z directions and a grid spacing of 0.375 Å. Specifically, the grid box parameters were 70×60×60 for 3OGN and 66×60×66 for 4EY6. A molecular docking study was carried out using AutoDock 4.2. The docking parameters were configured: 50 runs, 1 elitism, 0.02 mutation rate, 300 population sizes, 0.80 crossover rate, 27,000 generations, 10,000,000 energy evaluations, and 2.0 Å root-mean-square cluster tolerance for each run. From the most favor cluster, the ligand conformation with the lowest binding free energy was selected for additional examination. The PyMOL and Discovery Studio Visualizer were used to assess the AutoDock modeling studies' findings.

ADMET Study

The ADMET properties of the studied compounds were predicted using the online bioactivity prediction tools Molin-spiration (https://www.molinspiration.com/cgi-bin/properties) and pkCSM-ADMET (https://biosig.lab.uq.edu.au/pkcsm). Parameters related to the ADMET, including drug-likeness, permeability, intestinal absorption, liver toxicity, and CYP450 inhibition, were investigated.

Author Contributions

T. V. P.: Sample collection, formal analysis, and experiments, N. D. L., N. H. H., V. T. H., T. T. T. T., and N. T. C.: Experiments, P. M. Q.: Computational study, N. T. S. wrote the manuscript, N. N. L.: Supervision. All authors have read and approved the finalized manuscript.

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Conflict of Interests

The authors declare no conflict of interest.

Data Availability Statement

The data that support the findings of this study are available in the supplementary material of this article.

Keywords: Antimicrobial · Essential oil · Mosquito larvicidal · *n*-Hexane · *Syzygium hemilamprum*

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