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Article

Chemical Compositions, Mosquito Larvicidal and Antimicrobial Activities of Essential Oils of *Hedychium stenopetalum* Lodd. and *Hedychium villosum* Wall. of Zingiberaceae from Vietnam

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Abstract: The chemical constituents, larvicidal and antimicrobial activities of hydrodistilled essential oils from the leaves and rhizomes of *Hedychium stenopetalum* Lodd. and *Hedychium villosum* Wall. from Vietnam are reported. The main constituents of both essential oils were α-pinene (8.5%-21.6%) and β-pinene (32.2%-52.2%). Linalool (28.5%) and 1,8-cineole (10.7%) were identified in the rhizomes of *H. stenopetalum* and *H. villosum,* respectively. Essential oils from leaf and rhizome of *H. stenopetalum* exhibited larvicidal activity against *Aedes aegypti* with median lethal concentrations (LC_{50}) values of 16.33 µg/mL and 19.37 µg/mL, at 24 h, respectively. The lethal concentration required to kill 90% of population exposed (LC_{90}) values at the same test period were 49.76 µg/mL and 26.19 µg/mL, respectively. On the other hand, *H. villosum* oils were the most active against *Culex quiquefasciatus* with LC_{50} values of 19.58 µg/mL (LC_{90} value of 26.67 µg/mL) and 19.78 µg/mL (LC_{90} value of 27.13 µg/mL), respectively. *Hedychium villosum* oils showed antimicrobial activity against *Staphylococcus aureus* ATCC25923, *Bacillus cereus* ATCC14579 and *Candida albicans* ATCC10231 with minimum inhibitory concentrations (MIC) values between 7.66 µg/mL and 8.57 µg/mL. *H. stenopetalum* leaf oil displayed antimicrobial activity towards *Enterococcus faecalis* ATCC299212 (MIC 7.88 µg/mL). The results may provide basis for further exploitation of *H. stenopetalum* and *H. villosum* as larvicidal and antimicrobial agents.

Keywords: *Hedychium stenopetalum*; *Hedychium villosum;* Monoterpenes; *Aedes aegypti*; *Culex quiquefasciatus*; *Staphylococcus aureus*; *Bacillus cereus*; *Candida albicans*; *Enterococcus faecalis.*

Introduction

In Vietnam, there are many plant species mainly used as food and for medicinal purposes which are yet to be investigated upon for their biologically

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active substances. Hence, information on the potential of majority of these species is scanty. *Hedychium stenopetalum* Lodd. is a wide spread species growing up to 3 to 4 m tall. It is a perennial herb with big and thick leafy shoots over 2 m. The flowers are white with a faint greenish-yellow tint to the base of the labellum and prominent white or off-white stamens [1]. *H. stenopetalum* has a large natural population range from north-eastern India to Guangxi province in China and down into northern Vietnam and Laos [2]. A glycoside, syringetin 3-rhamnoside was previously isolated from *H. stenopetalum* [3]. The main chemical composition of essential oil from the rhizome of *H. stenopetalum* comprised of linalool (50.5%), β-pinene (12.4%), nerolidol (8.6%) and α-pinene (5.6%) [4]. Also, α-pinene (52.5%) and β-pinene (31.8%) were previously identified as the main components of the leaf oil, while linalool (45.2%), (*E*)-nerolidol (8.7%) and α-pinene (5.0%) were the major constituents in the root oil [5]. The essential oil of the rhizome of *H. stenopetalum* exhibited antimicrobial activity against *Staphylococcus aureus* and *Bacillus subtilis* [4].

Hedychium villosum Wall., is an epiphytic perennial herbs with erect and slender leafy shoots of 60-90 cm high [6]. The pale yellow flowers are 4.7-5.2 cm long, highly fragrant with many open at a time [7]. The plant is often confused with *H. tenuiflorum*, although the two species are also completely reproductively isolated [8]. The phytochemical compounds of *H. villosum* include villosin, coronarin E and β-sitosterol [9]. A compound, (11*S*,15*R*)-11-hydroxy-8(17),12(*E*)-labdadien-15,16-dial-11,15-hemiacetal, with strong cytotoxic activity against HL-60 and SMMC-7721 cells was also characterized from the plant [10]. The principal component of the oil from the cell wall of *H. villosum* was cineole [11].

Vietnam is a country prone to Dengue fever epidemics [12,13] and recent reports has shown the occurrence of chikungunya and Zika virus (ZIKV) infections [12,14], especially in southern Vietnam [12]. By the year 2016 and 2017, dengue fever was transmitted through large population (80%) of all ages [14]. Therefore, a concerted approach is necessary to control vector-causing disease in order to reduce the spread of the arboviral infections. Several researches have shown that the use of insecticides derived from plant products including essential oils have potentials for mosquito control because of their being environmentally friendly than synthetic pesticides [15,16].

Considering the potential of *Hedychium* species as sources of biologically active products, this paper reports the chemical compositions, larvicidal potential and antimicrobial activity of essential oils from *H. stenopetalum* and *H. villosum*. Previously, the chemical constituents and biological activities of essential oils from some Vietnamese plants have been reported [17-24].

Material and methods

The leaves and rhizomes of H. stenopetalum and H. villosum

The leaves and rhizomes of *H. stenopetalum* were collected (2.3 kg) in July 2019 from plants wild-growing in Vũ Quang National Park (GPS 18°17′15″ N, 105°21′39″ E), north-central Vietnam, at an elevation of 124 m, while *H. villosum* were obtained from Pù Hoạt Nature Reserve (GPS 19°42′18″ N, 104°49′42″ E, elevation of 648 m), in November 2018. The plants were identified by Dr. Dai, DN and voucher specimens DND 793 and DND 831, respectively, were deposited at the plant specimen room, Faculty of Agriculture, Forestry and Fishery, NgheAn College of Economics, Vietnam.

Hydrodistillation of essential oils from the leaves and rhizomes of H. stenopetalum and H. villosum

The amount of each sample of *H. stenopetalum* and *H. villosum* used for the isolation of essential oils was 2 kg. In accordance with laboratory practice [17-24], hydrodistillation was performed three times for each of the plant sample. For each experiment, the sample was poured inside a 5 L-distillation flask, distilled water was then added to submerge the sample. The set-up was made into completion by connecting the hydrodistiller unit (Clevenger-type) with the flask and heat was subsequently applied. Essential oils

were made to distill over distilled water for 3 h at normal pressure, according to the established procedure [21-24]. Each of the distilled essential oils was recovered separately into weighed sample bottles. The essential oils were kept under refrigeration (4°C) before the instrumental and biological analyses. The essential oil yield (%) was calculated by mass (g) of the essential oil divided by the mass (g) of the plant.

Analysis of the essential oils from the leaves and rhizomes of H. stenopetalum and H. villosum

The instrumental analysis was done in order to identify the components of the essential oils. This involves two stages. The first of the stages is the analysis involving the use of Gas chromatography (GC) which was conducted using HP 7890A Plus Gas chromatograph (Agilent Technologies) supported with a flame ionisation detector (FID). The column used in the GC chromatograph was HP-5ms of dimension 30 m x 0.25 mm, and film thickness of 0.25 µm. Following the practice adopted in our laboratory described previously [17-24], essential oil samples (1.0 µL, diluted in 10% hexane) were injected into the GC by a split mode with a split ratio of 10:1 and at the Inlet pressure of 6.1 kPa. The GC was temperature programmed at 60°C and held 2 min hold to rise 220°C, with another 10 min hold, at 4°C/min. During the process, H_2 was used as the carrier gas at a flow rate of 1 mL/min. In addition, the injector temperature and detector temperature were maintained at 250°C and 260°C, respectively. Quantification was done by external standard method using calibration curves generated by running GC analysis of representative compounds.

The second step involves the use of gas chromatography-coupled with mass spectrometry. The GC was coupled to Mass spectrometer HP 5973 MSD as described previously [17-24]. The GC column conditions and analytical procedures were similar to those reported for the GC analysis. However, He was used as the carrier gas at a flow rate of (1 mL/min). The operating conditions under the Mass spectrometer were: ionization voltage of 70 eV, emission current of 40 mA, acquisitions scan mass range of 45-350 amu and sampling rate of 1.0 scan/s as described previously [17-24].

Identification of the constituents of the essential oils

The individual constituents present in the studied essential oils of *Hedychium* species were identified by comparison of the Mass spectral data with MS fragmentation patterns of known compounds in literature [25] as described recently [17-24]. The retention indices (RI Exp.) of each compounds was also compared with reference to a homologous series of n-alkanes (C_8-C_{40}), run under identical experimental GC conditions as with samples. Also, co-injection with known compounds under the same GC conditions was also used to identify some compounds [17-24].

Test for biological studies of Hedychium essential oils

The assays of mosquito larvicidal action

The adults of *Culex quinquefasciatus* and *Aedes aegypti* used in the study of larvicidal activities of the essential oils were collected from Hoa Khanh Nam ward, Lien Chieu District, Da Nang city (16°03'14.9" N, 108°09'31.2" E), Vietnam. The tests were conducted at the Center for Entomology and Parasitology Research, Duy Tan University. The mosquito vectors were maintained under identical conditions as described previously [17-21]. Briefly, *C. quinquefasciatus* and *A. aegypti* were kept in cages (40 X 40 X 40 cm). The adults mosquito vectors sustained on 10% sucrose solution were fed with blood of mice. Tap water was used to induce hatching of eggs. The resulting larvae were rare in plastic trays (24 X 35 X 5 cm) built for this purpose with temperature condition sustained at 25 ± 2°C and relative humidity of 65-75%. There are equal 12:12 h light:dark cycle through the duration of the study. Feeds for the larvae include dog biscuits and yeast powder in the ration of 3:1 as reported earlier [17-21].

The larvicidal activities of the essential oils from *Hedychium* plants were evaluated as previously described [17-21]. Four different concentrations of the essential oils (12.5, 25, 50,

and 100 µg/mL) were used in the experiment. With ethanol (EtOH) used as a negative control, permethrin, a larvicidal drug, was used as a positive control. Prior to analysis, 200 mg of each of the essential oils was dissolved in 20 mL of ethanol which was transferred into different beakers stocked with 20 fourth instar larvae of *C. quinquefasciatus* and *A. aegypti*. The mortality of larvae of *C. quinquefasciatus* and *A. aegypti* was recorded after 24 h and 48 h of exposure to the different concentrations of the essential oils and repeated four times.

The mortality rate of *C. quinquefasciatus* and *A. aegypti* was calculated according to the formula described previously [17-21];

$$MC = \frac{Mo - Mt}{100 - Mt} \times 100$$

Mo = mortality in the treated groups, Mt = mortality in the control group and Mc = calculated mortality

Measurement and evaluatiion of the anti-microbial activity

The anallytical procedures previously developed were used to measure and evaluate the antimicrobial activitties of the essential oils. These methods were described earlier [17,20-22,24]. The evaluation of the minimum inhibitory concentration (MIC) and median inhibitory concentration (IC_{50}) was done in accordance with the method of microdilution broth susceptibility. The Gram-positive, Gram-negative and yeast microbes used for the study are shown in Tables 4 and 5. In addition, the concentration of essential oils used for the study were within the speciifc range used in previous studies [17,20-24,26]. To achieve the antimicrobial study, the stock solutions of the studied essential oils of Hedychium plants were dissolved in dimethylsulfoxide (1%) as described in previous studies [17,20-24,26]. By using a two-fold dilution range in sterile distilled water in micro-test tubes, several concentrations of the solutions were prepared within the range of 16,384-2 µg/mL. After the solutions were placed in 96-well microtiter plates, bacteria (5×10^5 CFU/mL) fungi (1×10^3 CFU/mL) were added separately. The serial dilutions of the essential oils placed in the last row of the micro-test tubes to serves as a negative control. The standard drugs namely streptomycin nystatin and cycloheximide were used as positive controls during th experiment [17,20-24,26]. After incubation at 37°C for 24 h, the MIC values were determined to be the lowest concentration of essential oils which completely inhibited the growth of microorganisms as described previously [17,20-24,26], while the IC_{50} values were determined by the percentage of microorganisms that inhibited growth based on the turbidity measurement data of EPOCH2C spectrophotometer and calculated accordingly using the equation;

$$\%\ inhibition = \frac{\text{ODcontrol(+)} - \text{ODtest agent}}{\text{ODcontrol(+)} - \text{ODcontrol (−)}} \times 100$$

$$IC_{50} = High_{conc} - \frac{(\text{Highinh\%} - 50\%) \times (\text{HighConc} - \text{Low Conc})}{(\text{Highinh\%} - \text{Lowinh\%})}$$

OD = the optical density, control (+) is the cells in medium without the antimicrobial agent, test agent corresponds to a known concentration of the antimicrobial agent; control (-) = the culture medium without essential oils; High Conc/Low Conc = the concentration of test agent at high concentration/low concentration, and High Inh%/Low Inh% = the % inhibition at high concentration/% inhibition at low concentration.

Statistical analysis

The LC_{50} values, LC_{90} values, and 95% confidence limits were determined by log-probit analysis using Minitab® 19 (Minitab, LLC, State College, PA, USA). Statistical analysis (ANOVA) of the differences between mean values obtained for experimental groups were calculated as a mean of standard deviation (SD, ±) of three (chemical constituents and antimicrobial analysis) and four independent measurements for the larvicidal test, using Microsoft excel program 2003.

Results and discussion

Obtained yields of the studied essential oils

The essential oils from the leaves and rhizomes of *H. stenopetalum* were obtained in average yields of the essential oils were 0.15% and 0.35%, respectively. However, *H. villosum* gave essential oils in yields of 0.14% (leaves) and 0.22% (rhizomes).

Analysis of chemical constituents of the studied essential oils

A cursory look at Table 1 gave insight on the chemical compounds present in the leaf and rhizome essential oils of *H. stenopetalum* and *H. villosum*. The retention indices on HP-5MS column and percentage compositions of each of the constituents were also given.

A total of 29 compounds accounting for 94.4% of the total oil content were identified in the essential oil from the leaves of *H. stenopetalum*. The components were represented by monoterpene hydrocarbons (76.8%), sesquiterpene hydrocarbons (9.7%) and oxygenated sesquiterpenes (5.0%). The main constituents of the essential oil were β-pinene (52.5%), α-pinene (11.3%),

Table 1. Chemical compositions of the leaf and rhizome essential oils of *H. stenopetalum* and *H. villosum* collected in Vietnam

S. No	Rt (min)	Compounds [a]	RI[b]	RI[c]	Percentage composition [d]			
					*Hs*L	*Hs*R	*Hv*L	*Hv*R
1	10.01	α-Thujene	930	926	0.2	0.4	0.2	0.4
2	10.28	α-Pinene	939	932	11.3	8.5	21.6	14.1
3	10.76	Camphene	955	946	0.7	1.9	0.2	0.4
4	11.43	Sabinene	978	972	2.1	0.7	1.1	0.6
5	11.57	β-Pinene	984	982	52.2	32.2	46.8	36.1
6	11.82	Myrcene	998	988	1.0	1.3	0.9	1.2
7	12.50	α-Phelandrene	1010	1004	0.9	4.6	1.5	3.6
8	12.72	d-3-Carene	1016	1014	0.4	0.8	0.3	0.5
9	12.91	α-Terpinene	1022	1018	0.1	0.8	0.3	0.7
10	13.01	o-Cymenne	1030	1028	5.1	5.5	5.0	6.9
11	13.11	Limonene	1035	1032	1.9	1.8	1.5	2.1
12	13.27	β-Phellandrene	1036	1034	0.3	0.2	0.2	-
13	13.32	1,8-Cineole	1038	1036	0.3	1.8	2.6	10.7
14	13.47	(*E*)-β-Ocimene	1049	1042	-	-	3.0	5.7
15	14.02	γ-Terpinene	1063	1060	-	7.5	0.2	0.4
16	14.12	2-Nonanone	1093	1087	-	-	-	0.1
17	15.04	Terpinolene	1094	1092	0.3	0.5	0.2	0.4
18	15.73	Linalool	1105	1102	0.5	28.5	0.9	9.0
19	16.21	*cis*-Sabinol	1148	1146	0.3	-	-	-
20	17.31	Camphor	1156	1154	-	0.2	-	-
21	17.48	Pinocarvone	1172	1170	0.3	-	-	0.2
22	18.32	Borneol	1178	1178	0.1	0.7	-	0.5
23	18.68	Terpinene-4-ol	1187	1187	0.4	1.2	-	0.2
24	19.12	α-Terpineol	1200	1198	0.2	0.4	0.3	-
25	19.88	Myrtenol	1204	1202	0.3	-	0.3	-
26	20.13	Myrtenal	1206	1206	0.5	-	-	-
27	22.34	Bornyl acetate	1294	1294	-	-	-	0.1
28	24.35	d-Elemene	1348	1348	-	-	0.1	0.2
29	26.23	β-Caryophyllene	1437	1437	8.4	-	2.6	0.3
30	27.81	Aromadendrene	1457	1457	-	-	-	0.1
31	28.15	(*Z*)-β-Farnesene	1460	1460	0.3	-	-	-
32	28.31	α-Humulene	1471	1471	0.6	-	-	0.3
33	28.95	β-Selinene	1505	1505	-	-	0.1	0.1

table 1. (continued).

S. No	Rt (min)	Compounds [a]	RI[b]	RI[c]	Percentage composition [d]			
					*Hs*L	*Hs*R	*Hv*L	*Hv*R
34	29.01	(*E,E*)-α-Farnesene	1512	1510	0.4	-	-	-
35	29.34	Bicyclogermacrene	1513	1515	-	-	0.5	-
36	30.93	(*E*)-Nerolidol	1570	1572	1.1	0.5	4.0	0.2
37	31.76	Spathulenol	1598	1598	-	-	0.3	-
38	32.15	Caryophyllene oxide	1605	1602	3.7	-	2.0	0.7
39	33.19	6-*epi*-Cubenol	1630	1628	-	-	-	0.2
40	33.23	Humulene oxide	1632	1632	0.2	-	-	-
41	34.21	Alismol	1648	1650	-	-	-	0.6
42	34.63	γ-Bicyclofarnesal	1699	1700	-	-	-	0.1
43	38.08	γ-Bicyclohomofarnesal	1829	1830	-	-	-	0.2
Total					94.4	100.0	97.0	96.5
Monoterpene hydrocarbons (S. No. 1-12, 14, 15, 17)					76.8	66.7	83.0	73.1
Oxygenated monoterpenes (S. No. 13, 18-27)					2.9	32.8	4.1	20.6
Sesquiterpene hydrocarbons (S. No. 28-35)					9.7	-	3.6	0.7
Oxygenated sesquiterpenes (S. No. 36-43)					5.0	0.5	6.3	2.0
Aliphatic ketone (S. No. 16)					-	-	-	0.1

[a] Elution order on HP-5MS column; [b] Experimental retention indices; [c] Literature retention indices; [d] means of three values, SD (±) omitted to avoid congestion; S. No, serial number; *Hs*, *H. stenopetalum*; *Hv*, *H. villosum*; L, leaf; R, rhizome; - not identified

β-caryophyllene (8.4%) and o-cymene (5.1%). Table 1 also showed that twenty-one constituents making up 100.0% of the essential oil contents were identified in the rhizomes of *H. stenopetalum*. These comprised of monoterpene hydrocarbons (66.7%), oxygenated monoterpenes (32.8%) and oxygenated sesquiterpene (0.5%). No sesquiterpene hydrocarbons was found in the essential oil. The compounds occurring in higher amount in the essential oi comprised of β-pinene (32.2%), linalool (28.5%), α-pinene (8.5%), g-terpinene (7.5%) and o-cymene (5.5%). The high contents of α-pinene, β-pinene and linalool identified in the essential oils of *H. stenopetalum* confers similarity with data previously reported for samples of the rhizome oils analysed in Thailand [4], as well as leaves and roots oils analysed in Vietnam [5]. However, the 1,8-cineole [4] and (*E*)-nerolidol contents of the essential oils of *H. stenopetalum* under investigation were lower than reported for previous analysed oil samples [4,5].

Monoterpene hydrocarbons (83.0%) was the main class of compounds present in the leaves oil of *H. villosum*. There are sizeable quantity of oxygenated sesquiterpenes (6.3%), oxygenated monoterpenes (4.1%) and sesquiterepene hydrocarbons (3.6%). A total of 27 compounds accounting for 97.0% of the oil content could be identified in the essential oil. The main constituents of the essential oil includes β-pinene (46.8%), α-pinene (21.6%), and o-cymene (5.0%). The 31 compounds of the rhizomes essential oil consist mainly of monoterpene hydrocarbons (73.1%) and oxygenated monoterpenes (20.7%). The composition of the essential oil was dominated by β-pinene (36.1%), α-pinene (14.1%), 1,8-cineole (10.7%), linalool (9.0%), o-cymene (6.9%), and (*E*)-β-ocimene (5.7%). The authors are aware of only one report on the essential oil component of *H. villosum* where cineole was identified as the principal component of the cell wall [11]. In the present study, 1,8-cineole was also identified in a sizeable proportion in the rhizome of *H. villosum* essential oil. It is interesting to note that α-pinene, β-pinene and linalool have featured prominently in the essential oils of some *Hedychium* species analysed previously from Vietnam [5] and other parts of the world [4,11].

Results of mortality tests

The essential oils from the leaves and rhizomes of the studied *H. stenopetalum* exhibited 100% mortality against *A. aegypti* and *C. quinquefasciatus* at tested concentration of 100 µg/mL (Table 2). At the concentration of 50 µg/mL, the leaf and rhizome oils of *H. stenopetalum* also exhibited mortality of 100% against *A. aegypti*. However, only the leaf oil showed 100% mortality towards *C. quinquefasciatus* at 24 h, while the rhizome oil achieved 96.3% and 100% mortality at 24 h and 48 h, respectively (Table 2). At concentration of 25 µg/mL, *H. stenopetalum* leaf and rhizome oils displayed mortality towards

Table 2. Mortality (%) and larvicidal action (µg/mL) of *H. stenopetalum* leaf and rhizome essential oils

Mortality (%)[a,b]	**Concentration (µg/mL)**			
	12.5	**25.0**	**50.0**	**100.0**
A. aegypti				
Leaf				
24 h	27.5 ± .002	55.0 ± .291	100.0 ± .000	100.0 ± .000
48 h	53.7 ± .816	80.0 ± .500	100.0 ± .000	100.0 ± .000
Rhizomes				
24 h	2.5 ± .000	68.7 ± .582	100.0 ± .000	100.0 ± .001
48 h	6.2 ± .000	72.5 ± 1.160	100.0 ± .001	100.0 ± .000
C. quinquefasciatus				
Leaf				
24 h	5.0 ± .000	27.5 ± .001	100.0 ± .000	100.0 ± .001
48 h	31.2 ± .001	72.5 ± .001	100.0 ± .000	100.0 ± .001
Rhizomes				
24 h	25.0 ± .500	66.3 ± 1.016	96.3 ± .000	100.0 ± .005
48 h	36.3 ± 1.000	77.5 ± 1.644	100.0 ± .005	100.0 ± .000

Minimum lethal concentration (µg/mL)					
	LC_{50}	**LC_{90}**	**Regression equation**	***X^2***	***P***
A. aegypti					
Leaf					
24 h	16.33 (9.347-83.186)	49.76 (23.508-13849.378)	y = -3.214+2.649x	5.431	0.000
48 h	7.77 (3.094-24.016)	17.72 (10.196-857.220)	y = -3.189+3.580x	8.660	0.000
Rhizomes					
24 h	19.37 (17.996-20.739)	26.19 (24.254-28.996)	y = -12.586+9.778x	.001	0.000
48 h	17.89 (16.658-18.605)	24.76 (22.750-27.692)	y = -11.394+9.905x	.001	0.000
C. quinquefasciatus					
Leaf					
24 h	31.31 (26.355-42.213)	65.71 (46.454-137.744)	y = -5.956+3.982x	0.242	0.000
48 h	19.63 (11.941-119.128)	56.21 (26.990-17918.903)	y = -3.628+2.806x	4.744	0.000
Rhizomes					
24 h	20.67 (17.908-24.903)	53.21 (40.246-82.693)	y = -4.106 +3.121x	3.108	0.000
48 h	14.97 (13.247-17.159)	36.55 (29.633-49.354)	y = -3.886 +3.306x	2.418	0.000

[a] n =4; [b]no mortality in the EtOH used as negative control; [c] Permethrin, the standard drug used as positive control displayed larvicidal activity against *C. quinquefasciatus* and *A. aegypti* with LC_{50} values in the range of 2.19 - 3.43 µg/mL

A. aegypti in the range 55.0%-80.0% (Table 2). The leaf and rhizome oils exhibited mortalities of 72.5% (48 h) and 66.3% (24 h) against *C. quinquefasciatus*. Moreover, only the leaf oil of *H. stenopetalum* exhibited mortality of 53.7% against *A. aegypti* at the lowest concentration of 12.5 µg/mL and at 48 h test period.

From Table 3, *H. villosum* essential oils achieved mortality of 100% towards *A. aegypti* and *C. quinquefasciatus* at tested concentration of 100 µg/mL (Table 3). At 50 µg/mL, the leaf and rhizome oils displayed 100% mortality against *A. aegypti* while mortality in the range of 96.3%-98.7% were exhibited against *C.*

Table 3. Mortality (%) and larvicidal action (µg/mL) of *H. villosum* leaf and rhizome essential oils

Mortality (%) [a,b]	**Concentration (µg/mL)**			
	12.5	**25.0**	**50.0**	**100.0**
A. aegypti				
Leaf				
24 h	3.7 ± .002	10.0 ± .000	100.0 ± .000	100.0 ± .000
48 h	7.5 ± .000	18.7 ± .005	100.0 ± .000	100.0 ± .000
Rhizomes				
24 h	2.5 ± .000	10.0 ± .582	100.0 ± .000	100.0 ± .001
48 h	7.5 ± .000	15.0 ± 1.160	100.0 ± .001	100.0 ± .000
C. quinquefasciatus				
Leaf				
24 h	2.5 ± .000	66.3 ± .001	96.3 ± .000	100.0 ± .001
48 h	13.7 ± .001	78.7 ± .001	98.7 ± .000	100.0 ± .001
Rhizomes				
24 h	2.5 ± .500	66.3 ± .1016	96.3 ± .001	100.0 ± .005
48 h	15.0 ± .100	77.5 ± 1.646	97.5 ± .005	100.0 ± .000

Minimum lethal concentration (µg/mL)					
	LC_{50}	**LC_{90}**	**Regression equation**	***X^2***	***P***
A. aegypti					
Leaf					
24 h	63.82 (39.130-452.751)	184.88 (76.857-7334.645)	y = -5.008+2.774x	0.339	0.002
48 h	43.64 (31.956-87.488)	127.03 (69.506-540.254)	y = -4529+2.762x	1.180	0.000
Rhizomes					
24 h	61.98 (38.589-435.822)	173.14 (73.780-6801.722)	y = -5.149+2.873x	0.258	0.000
48 h	53.86 (35.967-147.995)	181.19 (84.564-1354.587)	y = -4.211+2.432x	2.558	0.000
C. quinquefasciatus					
Leaf					
24 h	19.58 (18.187-20.968)	26.67 (24.656-29.614)	y = -12.340+9.552x	0.000	0.000
48 h	15.49 (14.506-16.736)	20.63 (18.770-23.790)	y = -12.257+10.298x	0.009	0.000
Rhizomes					
24 h	19.78 (18.371-21.194)	27.13 (25.045-30.243)	y = -12.108+9.340x	0.000	0.000
48 h	15.63 (14.593-16.844)	21.58 (19.664-24.667)	y = -10.928+9.151x	0.006	0.000

[a] n =4; [b]no mortality in the EtOH used as negative control; [c] Permethrin, the standard drug used as positive control displayed larvicidal activity against *C. quinquefasciatus* and *A. aegypti* with LC_{50} values in the range of 2.19 - 3.43 µg/mL

quinquefasciatus at tested periods (Table 3). At the concentration of 25 µg/mL, the rhizome oil of *H. villosum* displayed mortality in the range of 66.3%-78.7% towards only *C. quinquefasciatus* at tested periods (Table 3). However, at the lowest concentration of 12.5 µg/mL, *H. villosum* oils achieved mortality of less than 20% against the insect larvae (Table 3).

Results of larvicidal tests

From Table 2, *H. stenopetalum* leaf and rhizome essential oils exhibited larvcidal activity towards *C. quinquefasciatus* with LC_{50} values of 31.31 µg/mL ($LC_{90,}$ 65.71 µg/mL) and 20.67 µg/mL ($LC_{90,}$ 53.21 µg/mL) at 24 h, respectively. The LC_{50} values at 48 h were 19.63 µg/mL ($LC_{90,}$ 56.21 µg/mL) and 14.97 µg/mL ($LC_{90,}$ 36.55 µg/mL), respectively. On the other hand, *H. villosum* oils displayed larvcidal activity with LC_{50} values of 19.58 µg/mL (leaf, $LC_{90,}$ 26.67 µg/mL) and 19.78 µg/mL (rhizome, $LC_{90,}$ 27.13 µg/mL) at 24 h, while at 48 h, the LC_{50} values were 15.49 µg/mL ($LC_{90\ ,}$ 20.63 µg/mL) and 15.63 µg/mL ($LC_{90,}$ 21.58 µg/mL), respectively (Table 3). Also, the leaf and rhizome oils of *H. stenopetalum* showed larvicidal action against *A. aegypti* with the lowest LC_{50} values of 16.33 µg/mL ($LC_{90,}$ 49.76 µg/mL) and 19.37 µg/mL ($LC_{90,}$ 26.19 µg/mL) at 24 h, respectively. At 48 h, both oils displayed lower LC_{50} values of 7.77 µg/mL ($LC_{90\ ,}$ 17.72 µg/mL) and 17.89 µg/mL ($LC_{90,}$ 24.76 µg/mL), respectively (Table 2). However, *H. villosum* essential oils exhibited lower larvicidal action against *A. aegypti* (Table 3) depicted by higher LC_{50} values of 63.82 µg/mL (leaf, $LC_{90,}$ 184.88 µg/mL) and 61.98 µg/mL (rhizome, $LC_{90,}$ 173.14 µg/mL) at 24 h, when compared with *H. stenoepatlum* oils. The LC_{50} values of 43.64 µg/mL ($LC_{90,}$ 127.03 µg/mL) and 53.86 µg/mL ($LC_{90,}$ 181.19 µg/mL) were obtained at 48 h, respectively, for the leaf and rhizome oils. Permethrin, the standard drug used as control displayed larvicidal activity against *C. quinquefasciatus* and *A. aegypti* with LC_{50} values in the range 2.19-3.43 µg/mL.

In an effort to identify novel classes of plant natural products with activity against adult larvae of *C. quinquefasciatus* and *A. aegypti*, a high-throughput larval screening method was performed on the studied *Hedychium* essential oils. The essential oils of *H. stenopetalum* displayed mortality at lower concentrations against *A. aegypti,* while *H. villosum* oils exhibited stronger mortality against *C. quinquefasciatus.* These results showed that mortality increases as concentration increases. Overall, the studied essential oils displayed potential mortality against both *A. aegypti* and *C. quinquefasciatus.* Although there is no report on the mortality of *H. stenopetalum* and *H. villosum* towards larvae of mosquitoes, the rhizome oil of *H. coronarium* displayed mortality, oviposition deterrent and repellent activity against fourth instar larvae of *A. aegypti* and *C. quinquefasciatus* [27,28]. Thus the essential oils of *H. stenopetalum* and *H. villosum* showed mortality against *A. aegypti* (100% mortality at 100 µg/mL and 50 µg/mL, respectively) when compared with previous reports on *H. coccineium* which gave 100% mortality at 125 mg/L, as well as *H. forrestii*, *H. elatum*, *H. bousigonianum*, *H. flavum*, *H. thyrsiforme* and *H. flavesens* that produced mortality of 100% at 500 mg/L [4]. From the results of Tables 2 and 3, it can be concluded that *H. villosum* essential oils exhibited activity towards *C. quinquefasciatus*, while *H. stenopetalum* essential oils was active against *A. aegypti*.

The essential oils of *H. stenopetalum* and *H. villosum* in this present study will be categorized according to previous procedures [17-21] where the substances with LC_{50} > 100 µg/mL were considered not active, substances with LC_{50} between 100 µg/mL-50 µg/mL were considered active and those with LC_{50} < 50 µg/mL were considered highly active. Overall the results of this study showed that essential oils of *H. stenopetalum* and *H. villosum* may be considered to exhibit moderate level of activity against *A. aegypti* and *C. quinquefasciatus*.

The most recent dengue outbreak in Vietnam was more severe than previous ones because it covers more locations (84.0%) and affected a large part of the population with attendance increase in the number of patients (57.3%) [14]. The resultant effect shows that patients with plasma leakage accompanied by dengue shock

were 8.1%, those patients having severe organ impairment accounted 2.5%, while patients diagnosed with severe bleeding was 0.75%. An increase of 0.8% mortality was also observed [14]. The obtained essential oils fraction from *H. stenopetalum* and *H. villosum* and their major compounds were thought to display larvicidal activity against *C. quinquefasciatus* and *A. aegypti*.

Information is scanty on the larvicidal actions of *Hedychium* essential oils. The studied essential oils of *H. stenopetalum* and *H. villosum* were considered highly active against *A. aegypti* than the leaves and rhizomes of *H. coronarium* [29]. The leaf oil of *H. coronarium* exhibited mosquito larvicidal activity with LC_{50} values of 111 ppm (2 h) and 90 ppm (24 h) while the rhizome oil showed LC_{50} values of 86 ppm (2 h) and 47 ppm (24 h) [29]. In addition, fractions of *H. spicatum* displayed anti-feedant activity against the larvae of *Spilosoma obliqua* Walker [30]. The observed larvicidal activities of *H. stenopetalum* and *H. villosum* may be attributed to some compounds such as α-pinene, β-pinene and 1,8-cineole identified in the essential oils. Previously linalool, α-pinene, β-pinene and 1,8-cineole were reported to displayed larvicidal effects against *A. aegypti* larvae with LC_{50} values of 96.60, 50.92, 22.39 and 74.91 ppm, respectively [31]. Linalool was also reported as a moderate larvicide with LC_{50} value of 275.2 mg/mL [32]. β-Pinene was thought to displayed larvicidal action against *A. aegypti* among others, with LC_{50} of 35.9 ppm [33] and 21.1 ppm [34]. In addition, β-pinene has demonstrated strong larvicidal potential against *C. quinquefasciatus* larvae with LC_{50} of 32.23 ppm [35] and 19.6 ppm [36]. The LC_{50} value of the larvicidal activity of linalool towards *A. aegypti* larvae was reported to be 70.56 mg/L [37]. Moreover, essential oil isolated from the fruits of *Callistemon citrinus* fruit growing in Vietnam, whose major compounds were α-pinene and 1,8-cineole also exhibited good larvicidal activity against both *A. aegypti* and *C. quinquefasciatus* with LC_{50} of 17.3 µg/mL at 24 h [38]. This showed that the major components present in the studied essential oils possessed different grades of activity against the mosquito larvae. Several other minor compounds present in the essential oils were known for their larvicidal activity [17-21].

The antimicrobial data

The results of the antimicrobial data of the essential oils are presented in Table 4 (*H. stenopetalum*) and Table 5 (*H. villosum*). Both essential oils displayed different patterns of antimicrobial activities. The leaf essential oil of *H. stenopetalum* displayed activity towards *Enterococcus faecalis* ATCC299212 (MIC 7.88 µg/mL) than all other tested essential oils (Table 4). The leaf essential oil of *H. villosum* showed antimicrobial activity against *S. aureus* ATCC25923, *B. cereus* ATCC14579 and *C. albicans* ATCC10231 with MIC values of 7.89 µg/mL, 7.66 µg/mL and 8.33 µg/mL, respectively (Table 5). The MIC values of 8.54 µg/mL, 8.57 µg/mL and 8.67 µg/mL, respectively, were obtained by the rhizome oil. The leaf oil of *H.*

Table 4. Antimicrobial activity of essential oils of *H. stenopetalum*

Microorganisms	MIC (µg/mL) [a]						IC_{50} (µg/mL) [a]	
	L	Strp	Nyst	R	Str	Nyst	L	R
E. faecalis	7.88	0.50	nt	16.23	1.07	nt	32.0	64.0
S. aureus	56.89	0.50	nt	50.78	0.50	nt	128.0	128.0
B. cereus	18.98	0.87	nt	89.78	1.07	nt	128.0	16.0
P. aeruginosa	-	-	-	-	-	-	-	
C. albicans	5.67	nt	2.80	126.67	nt	2.80	128.0	256.0
E. coli	-	-	-	-			-	-
S. enterica	-	-	-	-			-	-

[a] n = Mean value of three replicate assays, SD (±) are insignificant; -: No activity; nt: not tested; L: leaf; R: rhizome; Strep: Streptomycin; Nyst: Nystatine

Table 5. Antimicrobial activity of essential oils of *H. villosum*

Microorganisms	MIC (µg/mL) [a]							IC_{50} (µg/mL) [a]	
	L	Strp	Nyst	R	Strp	Nyst	Cyc	L	R
E. faecalis	16.44	0.87	nt	16.33	1.07	nt	nt	32.0	32.0
S. aureus	7.89	0.50	nt	8.54	1.07	nt	nt	16.0	16.0
B. cereus	7.66	0.50	nt	8.57	0.50	nt	nt	16.0	16.0
P. aeruginosa	15.67	-	-	65.44	-	-	1.2	32.0	128.0[h]
C. albicans	8.33	nt	4.20	8.67	nt	2.8	nt	16.0	16.0
E. coli	-	-	-	-	-	-	-	-	-
S. enterica	-	-	-	-	-	-	-	-	-

[a] n = Mean value of three replicate assays, SD (±) are insignificant; -: No activity; nt: not tested; L: leaf; R: rhizome; Strep: Streptomycin; Cyc: Cycloheximide; Nyst: Nystatine

villosum exhibited antimicrobial activity against *P. aeruginosa* ATCC27853 with MIC of 15.67 µg/mL. The essential oils of *H. stenopetalum* did not showed antimicrobial action against *P. aeruginosa* ATCC27853. Also, both essential oils of *H. stenopetalum* and *H. villosum* did not displayed antimicrobial activity towards *E. coli* ATCC25922 and *S. enterica* ATCC13076 (Tables 4 and 5). The studied *Hedychium* essential oils possessed pronounced activity against the Gram-positive than Gram-negative bacteria.

The essential oils of *H. stenopetalum* and *H. villosum* displayed broad spectrum of activity against the tested microorganisms. The results of the antimicrobial data are in agreement with previous study which showed that the rhizome oils of *H. stenopetalum, H. coronarium, H. neocarneum, H. flavescens* and *H. speciosum* displayed antimicrobial action against strains of *S. aureus* and *B. cereus,* but not *P. aeruginosa* [4]. Thus, essential oils from leaves and rhizomes of *H. stenopetalum* and *H. villosum* can be adjudged to be of good antimicrobial activity with MIC < 100 µg/mL, in accordance with standard procedure [39].

The observed antimicrobial activities could be ascribed to the monoterpenes present as the main components of the essential oils, especially the oxygenated monoterpenes, 1,8-cineole and linalool, which were previously observed to be compounds with high antibacterial properties [4,17,21,40]. Previously, essential oil with high contents of α-pinene, linalool and 1,8-cineole was reported displayed good antibacterial activity against *E. faecalis* with MIC value of 16 µg/mL [38]. In addition, α-and β-pinene, linalool and 1,8-cineole are ubiquitous monoterpenoids in conifer and other aromatic plants, and each compound was widely tested against many organisms, and cancer cell lines [41]. The present data of antimicrobial activity were also in agreement with several reports about the relationship between monoterpene content and the antimicrobial property of essential oils [17,21,24,42], especially against *S. aureus*.

It could be observed that studied essential oils only displayed antimicrobial activity towards the Gram-positive pathogens and yeast. Hence it may be concluded that the essential oils of *H. stenopetalum* and *H. villosum* are Gram-positive specific. The results of MIC obtained in this study confirmed known observations of Gram-positive bacteria being more susceptible to growth inhibition by plant essential oils than Gram-negative bacteria. These differences could be attributed in part to the great complexity of the double membrane-containing cell envelope in Gram-negative bacteria compared to the single membrane structure of the Gram-positive ones [43]. These differences may also be attributed to the presence of the lipopolysaccharides in the outer membrane of the Gram-negative bacteria, which provides a hydrophilic surface and functions as a permeability barrier for many plant extracts, antibiotics, detergents, and lipophilic compounds [44]. However, the ability of essential oil to disrupt the permeability barrier of cell membrane structures and the accompanying

loss of chemiosmotic control is the most likely reason for its lethal action [45].

The larvicidal and antimicrobial activities of the essential oils were tested along with the standard drugs. Permethrin, the standard drug used as control displayed larvicidal activity against *C. quinquefasciatus* and *A. aegypti* with LC_{50} values much lower than the essential oils. In addition, the reference compounds used for the antimicrobial study, namely streptomycin, cycloheximide and nystatin, displayed higher activity than the essential oils of *H. stenopetalum* and *H. villosum*. Essential oils are known to consist of several constituents of diversed structural patterns. These differences in the observed larvicidal and antimicrobial activities are expected considering the pure nature of the various standard drugs. These differences may be explained in parts by the synergistic or otherwise effects of the different constituents of the essential oils [17,20,22,24,46]. Nevertheless, the essential oils exhibited moderate activities based on the mentioned criteria. However, further research activities are on-going to determine the compounds that will promote the observed larvicidal and antimicrobial activities of the essential oils of *H. stenopetalum* and *H. villosum*.

Conclusions

The result indicates that main compounds of the essential oils of *H. stenopetalum* and *H. villosum* were α-pinene (8.5%-21.6%) and β-pinene (32.2%-52.2%). In addition, linalool (28.5%) and 1,8-cineole (10.7%) were identified in the rhizomes of *H. stenopetalum* and *H. villosum,* respectively. Both essential oils displayed mortality and larvicidal effects on the defense of *A. aegypti* and *C. quinquefasciatus* mosquito larvae. In addition, the oil samples also displayed antimicrobial activity against *S. aureus* ATCC25923, *B. cereus* ATCC14579 and *E. faecalis* ATCC299212, and showed anti-candidal potential towards *C. albicans* ATCC10231. The leaf oil of *H. villosum* exhibited good activity against *P. aeruginosa* with MIC value of 15.67 µg/mL. Overall, the studied essential oils of *Hedychium* plants possessed moderate larvicidal and antibacterial properties.

Competing interests

The authors declare that no competing interest exists.

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References

1. **Sakhanokho, H.F., Islam-Faridi, M.N., Rajasekaran, K. and Pounders, C.T. (2018).** Diversity in nuclear DNA content and ploidy level of *Hedychium* species and hybrids. J. Crop. Improvm. 32: 431-435.
2. **Supriyo, B., Ramesha, A.M., Vigya, K., Ajay, P., Sudip, M. and Latha, R. (2014).** Genetic diversity and relationship of Hedychium from Northeast India as dissected using PCA analysis and hierarchical clustering. Meta Gene. 2: 459-468.
3. **Williams, C.A. and Harborne, J.B. (1977).** The leaf flavonoids of the Zingiberales. Biochem. Syst. Ecol. 5: 221-229.
4. **Suksathana, R., Siriwoot, S., Somboon, A. and Sunee, C. (2013).** Chemical composition and antibacterial activity of rhizome oils from Five *Hedychium* Species. Nat. Prod. Commun. 8: 519-522.
5. **Thanh, B.V., Dai, D.N., Thang, T.D., Binh, N.Q., Anh, L.D.N. and Ogunwande, I.A. (2014).** Composition of essential oils of four *Hedychium* species from Vietnam. Chem. Cent. J. 8: 54-61.
6. **Sanoj, E., Sabu, M. and Pradeep, A.K. (2013).** Circumscription and lectotypification of *Hedychium villosum* and its variety *H. villosum* var. *tenuiflorum* (Zingiberaceae**).** PhytoKeys. 25: 75-85.
7. **Wu, T.L. and Larsen, K. (2000).** Zingiberaceae. In: Wu ZY, Raven PH. (Eds) Flora of China. Science Press, Beijing & Missouri Botanical Garden Press, St. Louis, Vol. 24, p. 322.
8. **Gao, J.Y., Liu, Q. and Li, Q.J. (2014).** The comparative reproductive biology of a tetraploid species, *Hedychium villosum*,

and its diploid progenitor *H. tenuiflorum* (Zingiberaceae). Plant Biol. 16: 683-689.

9. **Xiao, P., Sun, C., Muhammad, Z., Omar, I. and Pan, Y. (2001).** New diterpene from *Hedychium villosum*. Fitoter. 72: 837-838.
10. **Zhao, Q., Zou, C., Yu, Q., Zao, S.-D., He, H.-P. and Hao, X.-J. (2012).** Cytotoxic labdane-type diterpenes and diterpene derivative from *Hedychium villosum*. Chem. J. Chinese Univ. 33: 1220-1225.
11. **Si, M., Li, L., Zhang, C., Zhang, D. and Li, J. (2007).** In situ research on *Hedychium villosum* wall oil cell with Raman spectroscopy. Acta Laser Biol. Sin. 4: 210-224.
12. **Quyen, L.D., Le, N.T., Anh, V.C.T., Nguyen, N.B., Hoang, V.D., Montgomery, J.L., Kutcher, S.C., Le, N.H., Hien, N.T., Kien, D.T.H., Rabaa, M., O'Neill, S.L., Smmons, C.P., Anh, D.C. and Anders, K.L. (2018).** Epidemiological, serological, and virological features of dengue in Nha Trang City, Vietnam. Am. J. Trop. Med. Hyg. 98: 402-409.
13. **Quyen, N.T.H., Kien, D.T.H., Rabaa, M., Tuan, N.M., Vi, T.T., Tan, L.V., Hung, N.T., Tuan, H.M., Tram, T.V., Ha, N.L.D., Quang, H.K., Doanh, N.Q., Chau, N.V.V., Wills, B. and Simmons, C.P. (2017).** Chikungunya and Zika virus cases detected against a backdrop of endemic dengue transmission in Vietnam. Am. J. Trop. Med. Hyg. 97: 146-150.
14. **Huy, B.V., Hoa, L.N.M., Thuy, D.T., Kinh, N.V., Ngan, T.T.D., Duyet, L.V., Hung, N.T., Minh, N.N.Q., Truong, N.T. and Chau, N.V.V. (2019).** Epidemiological and clinical features of dengue infection in adults in the 2017 outbreak in Vietnam. Biomed. Res. Int. 2019: 1-13.
15. **Masetti, A. (2016).** The potential use of essential oils against mosquito larvae: A short review. Bull. Insectol. 69: 307-310.
16. **Benelli, G. (2015).** Research in mosquito control: Current challenges for a brighter future. Parasitol. Res. 114: 2801-2805.
17. **Chau, D.T.M., Chung, N.T., Huong, L.T., Hung, N.H., Ogunwande, I.A., Dai, N.D. and Setzer, W.N. (2020).** Chemical compositions, mosquito larvicidal and antimicrobial activities of leaf essential oils of eleven species of Lauraceae from Vietnam. Plants. 9: 606-640.
18. **Dai, D., Hung, N.D., Chung, N.T., Huong, L.T., Hung, N.H. and Ogunwande, I.A. (2020).** Chemical constituents of the essential oils from the leaves of *Litsea umbellata* and *Litsea iteodaphne* and their mosquito larvicidal activity. J. Essent. Oil Bearing Plants. 23(6): 1334-1344.
19. **Huong, L.T., Huong, T.T., Huong, N.T.T., Hung, N.H., Dat, P.T.T., Luong, N.X. and Ogunwande, I.A. (2020).** Chemical composition and larvicidal activity of essential oils from *Zingiber montanum* against three mosquito vectors. Bol.Latinoam. Caribe Plant Med. Arom. 19: 569-579.
20. **Dai, D.N., Chung, N.T., Huong, L.T., Hung, N.H., Chau, D.T.M., Yen, N.T. and Setzer, W.N. (2020).** Chemical compositions, mosquito larvicidal and antimicrobial activities of essential oils from five species of *Cinnamomum* growing wild in north central Vietnam. Molecules. 23: 1123-1136.
21. **Le, N.V., Sam, L.N., Huong, L.T. and Ogunwande, I.A. (2022).** Chemical compositions of essential oils and antimicrobial activity of *Piper albispicum* C. DC. from Vietnam. J. Essent. Oil Bearing Plants. 25(1): 82-92.
22. **Chau, D.T.M., An, N.T.G., Huong, L.T. and Ogunwande, I.A. (2022).** Essential Oils of Lauraceae: Chemical compositions and antimicrobial activity of essential oils from the leaves of *Beilschmiedia fordii* Dunn. and *Lindera glauca* (Siebold & Zucc.) Blume from Vietnam. J. Essent. Oil Bearing Plants. 25(1): 93-102.
23. **Huong, L.T., Sam, L.N., Chau, D.T.M., Dai, D.N. and Ogunwande, I.A. (2021).** Chemical compositions of essential oils and antimicrobial activity of the leaves and rhizomes of *Zingiber manang* and *Zingiber tamii* from Vietnam. J. Essent. Oil Bearing Plants. 24(5): 1087-1096.

24. **Nhan, N.T., Lan, C.T., Linh, L.D., Huong, L.T., Dai, D.N. and Ogunwande. I.A. (2021).** Chemical compositions of essential oils and antimicrobial activity of *Alpinia Kwangsiensis* from Vietnam. J. Essent. Oil Bearing Plants. 24(4): 714-723.
25. **NIST. (2018).** National Institute of Standard and Technology. Chemistry Web Book Data. Data from NIST Standard Reference Database 69.
26. **Ha, C.T.T., Diep, L.N., Thuy, D.T.T., Bon, T.N., Cham, L.T.T. and Setzer, W.N. (2022).** Composition and antimicrobial activity of essential oils from leaves, twigs and ripe fruits of *Magnolia grandis*. Rec. Nat. Prod. 16: 503-508.
27. **Tawatsin, A., Preecha, A., Usavadee, T., Prapai, W., Jaree, B., Thidarat, B., Chavalittumrong, P., Soonthornchareonnon, N., Komalamisra, N. and Mulla, M.S. (2006).** Repellency of essential oils extracted from plants in Thailand against four mosquito vectors (Diptera: Culicidae) and oviposition deterrent agains*t Aedes aegypti* (Diptera: Culicidae). Southeast Asian J. Trop. Med. Public Health. 37: 915-931.
28. **Phukerd, U. and Soonwera, M. (2013).** Larvicidal and pupicidal activities of essential oils from Zingiberaceae plants against *Aedes aegypti* (Linn.) and *Culex quinquefasciatus* Say mosquitoes. Southeast Asian J. Trop. Med. Public Health. 44: 761-771.
29. **Ho, J.C. (2011).** Antimicrobial, mosquito larvicidal and antioxidant properties of the leaf and rhizome of *Hedychium coronarium.* J. Chin. Chem. Soc. 58: 563-567.
30. **Rawat, A., Payal, T., Prakash, O., Ravendra, K., Pant, A.K., Srivastava, R.M. and Rawat, D.S. (2019).** Chemical composition, herbicidal, antifeedant and cytotoxic activity of *Hedychium spicatum* Sm.: A Zingiberaceous herb. Trends Phytochem. Res. 3: 123-126.
31. **Perumalsamy, H., Kim, N.-J. and Ahn, Y.-J. (2009).** Larvicidal activity of compounds isolated from *Asarum heterotropoides* against *Culex pipiens pallens, Aedes aegypti*, and *Ochlerotatus togoi* (Diptera: Culicidae). J. Med. Entomol. 46: 1420-1423.
32. **Fujiwara, G.M., Vinícius, A., de Oliveira, C.F., Lara, R.A., Gabriel, M.M., Betim, F.C.M., Nadal, J.M., Farago, P.V., Dias, J.F.G., Miguel, O.G., Miguel, M.D., Matques, F.A. and Zanin, S.M.W. (2017).** Evaluation of larvicidal activity and ecotoxicity of linalool, methyl cinnamate and methyl cinnamate/linalool in combination against *Aedes aegypti*. Ecotoxicol. Environ. Safety. 139: 238-244.
33. **Ali, A., Tabanca, N., Kurkcuoglu, M., Duran, A., Blythe, E.K. and Khan, I.A. (2014).** Chemical composition, larvicidal, and biting deterrent activity of essential oils of two subspecies of *Tanacetum argenteum* (Asterales: Asteraceae) and individual constituents against *Aedes aegypti* (Diptera: Culicidae). J. Med. Entomol. 51: 824-830.
34. **Lucia, A., Audino, G.A., Seccacini, E., Licastro, S., Zerba, E. and Masuh, H. (2020).** Larvicidal effect of *Eucalyptus grandis* essential oil and turpentine and their major components on *Aedes aegypti* larvae. J. Am. Mosq. Cont. Assoc. 23: 299-303.
35. **Govindarajan, M. (2010).** Chemical composition and larvicidal activity of leaf essential oil from *Clausena anisata* (Willd.) Hook. f. ex Benth (Rutaceae) against three mosquito species. Asian Pac. J. Trop. Med. 3: 874-877.
36. **Huang, Y., Lin, M., Jia, M., Hu, J. and Zhu, L. (2019).** Chemical composition and larvicidal activity against *Aedes* mosquitoes of essential oils from *Arisaema fargesii*. Pest Managem. Sci. 76: 534-542.
37. **Andrade, S., Sanchez-Aldana, D., Chacon-Vargas, K.F., Rivera-Chavira, B.E., Sanches-Torres, L.E., Camacho A.D., Nogueda-Torres, B. and Navarez-Moorillon, G.V. (2018).** Oviposition deterrent and larvicidal and pupaecidal activity of seven essential oils and their major components against *Culex quinquefasciatus* Say (Diptera; Culicidae): synergism-anatgonism effects. Insects. 9: 24-40.
38. **An, N.T.G., Huong, L.T., Prabodh, S.,**

Thieu, A.T., Dai, D.N., Hung, N.H., Ngoc, N.T.B. and Setzer, W.N. (2020). Mosquito larvicidal activity, antimicrobial activity and chemical compositions of essential oils from four species of Myrtaceae from Central Vietnam. Plants. 9: 544-562.

39. **Holetz, F.B., Pessini, G.L., Sanches, N.R., Cortez, D.A.G., Nakamura, C.V. and D Dias-Filho, B.P. (2002).** Screening of some plants used in the Brazilian folk medicine for the treatment of infectious diseases. Mem. Inst. Oswaldo Cruz. 97: 1027-1031.
40. **Silveira, S.M., Júnior, A.C., Scheuermann, G.N., Secchi, F.L. and Vieira, C.R.W. (2012).** Chemical composition and antimicrobial activity of essential oils from selected herbs cultivated in the South of Brazil against food spoilage and food borne pathogens. Ciência Rural. 42: 1300-1306.
41. **Asakawa, Y. (2021).** Dietary Mono-terpenoids. In. Handbook of Dietray Phytochemicals. (Xiao, J.; Sarker, S. D.; Asakawa, Y. eds.). Springer, Singapore, vol. 2: 607-622.
42. **Sabulal, B., George, V., Dan, M. and Pradeep, N.S. (2007).** Chemical composition and antimicrobial activities of the essential oils from the rhizomes of four *Hedychium* species from South India. J. Essent. Oil Res. 19: 93-97.
43. **El-Abed, N., Guesmi, F., Mejri, M., Marzouki, M.N. and Ben Hadj, S. (2014).** Phytochemical screening and assessment of antioxidant, antibacterial and cytotoxicity activities of five Tunisian medicinal plants. Int. J. Pharm. Res. Biosci. 3: 770-789.
44. **Musicha, P., Cornick, J.E., Bar-Zeev, N., French, N., Maseas, C., Denis, B., Kennedy, N., Mallewa, J., Gordon, M.A., Msefula, C.L., Heyderman, R.S., Everett, B.D. and Feasey, N.A. (2017).** Trends in antimicrobial resistance in bloodstream infection isolates at a large urban hospital in Malawi (1998-2016): a surveillance study. The Lancet Infect Dis. 17: 1042-1052.
45. **Ismail, M.H., El-Bessoumy, A., Al-Bataineh, E., Joseph, M.R.P., Prasanna, R., Chandramoorthy, H.C. and Hadj Ahmed, S.M. (2019).** Antimicrobial efficiency of essential oils from traditional medicinal plants of Asir Region, Saudi Arabia, over drug resistant isolates. BioMed. Res. Int. 2019: 1-9
46. **Soković. M., Glamočlija, J., Marin, P.D., Brkić, D. and Griensven, L.J.L.D. (2010).** Antibacterial effects of the essential oils of commonly consumed medicinal herbs using an *in vitro* model. Molecules. 15: 7532-7546.