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Chemical Compositions, and Antimicrobial and Mosquito Larvicidal Activities of Essential Oils from Four *Syzygium* Species *Syzygium formosum* (Wall.) Masam., *S. syzygioides* (Miq.) Merr. & L.M. Perry, *S. megacarpum* (Craib) Rathakr. & N.C. Nair, and *S. chantaranothaianum* W.K. Soh & J. Parn

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ABSTRACT

Chemical compositions in the leaf essential oils of four *Syzygium* species were first identified by the GC-FID/MS analysis. Monoterpene hydrocarbons (39.6-51.1%), and sesquiterpene hydrocarbons (27.0-37.8%) were the main chemical classes in *S. formosum* and *S. syzygioides* leaf oils, whereas sesquiterpene hydrocarbons (83.0-83.8%) were predominant in *S. Megacarpum* and *S. chantaranothaianum* leaf essential oils. Bicyclogermacrene (9.1-37.0%) was the principal compounds in these essential oils. All the tested essential oils with the MIC values of 16-128 µg/mL were comparable to the positive control streptomycin against Gram-positive bacteria *Staphylococcus aureus* and *Bacillus cereus*, and Gram-negative bacterium *Pseudomonas aeruginosa*. The leaf essential oils of *S. formosum*, *S. syzygioides*, and *S. chantaranothaianum* with the MIC values of 16-128 µg/mL were comparable to the positive control cycloheximide against the yeast *Candida albicans*. Four samples also exhibited good larvicidal activity against the mosquito *Aedes aegypti* with the 24-h and 28-h LC₅₀ values of 25-40 µg/mL

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Syzygium; essential oils; antimicrobial; mosquito larvicidal

1. Introduction

Numerous factors can influence the synthesis, yield, and composition of chemical components in essential oils, as well as their existence from inside the plants until their final isolation. By mean of this, the influential aspects have been researched, especially for economically significant crops, in an effort to maximize the growing conditions, and harvest timing while achieving increased yields of premium essential oils that meet market demands (1–3). Therefore, it is crucial to understand the variables that affect species' chemical variability and production. Physiological changes, environmental influences, geographic variances, genetic factors, plant material/space requirements, and the necessity for time collection are a few of these (4,5).

Syzygium is one of the largest genera in the family Myrtaceae with ca. 1200 evergreen trees and shrubs (6). The plants of this genus were recorded to distribute from Africa through Asia, Malesia and Australia to the Pacifica islands (6). The Clove (*S. aromaticum*) is a well-known economically crucial species, in which its unopened

flower buds are used as a precious spice (7). Medicinally, Syzygium plant extracts and isolated compounds have also a broad panel of pharmacological activities, such as antimicrobial, antidiabetic, antiinflammatory, and nephroprotective activities (7,8). It is also recognized that Syzygium plants are rich in essential oils containing terpenoid compounds. As an example, the antibacterial essential oil of S. cumini leaves was characterized by the presence of major components pinocarveol (15.1%), and α -terpeneol (8.9%) (9). Among 49 species recorded in Vietnam, some of them have been objects in phytochemical studies to identify chemical profiles in their essential oils. β -Caryophyllene (42.53–64.53%) can be seen as the main compound in the leaf essential oils of two Vietnamese Syzygium species S. caryophyllatum and S. lineatum, whereas the leaf essential oil of S. hancei was represented by γ -guaiene (11.07%) (10).

Regarding biological activities, *Syzygium* species showed potentials in antimicrobial and mosquito larvicidal treatments. The leaf essential oil of *S. grande*

inhibited the growth of *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus vulgaris*, *P. aeruginosa*, *S. aureus*, and *B. subtilis* with the MIC values of 0.25–0.75 mg/mL (11). The Clove essential oil controlled wound infections in mice caused by methicillin resistant *S. aureus* via its interaction with imipenem (12). *S. zeylanicum* leaf essential oil exhibited remarkably effective and ecofriendly larvicides against *Anopheles subpictus*, *An. albopictus*, and *Culex tritaeniorhynchus* larvae (13). The Clove essential oil was also reported to protect against *An. stephensi* larvae with the LC₅₀ and LC₉₀ values of 57.49 and 93.14 ppm, respectively (14).

The current study aims to report the chemical identification of essential oils of four wild *Syzygium* species, collected from the North Central Coast region of Vietnam, including *S. formosum* (Wall.) Masam. (local name: Trâm lá chụm ba), *Syzygium syzygioides* (Miq.) Merr. & Perry (Trâm kiền kiền), *S. megacarpum* (Craib) Rathakr. & N.C.Nair (Trâm lá lớn), and *Syzygium chantaranothaianum* W. K. Soh & J. Parn. (Trâm chan lốt), as well as their antimicrobial, and mosquito larvicidal activities.

2. Materials and methods

2.1. Plant materials

The fresh leaves of four studied species (7-year-old plants) were collected from Pu Hoat Natural Reserve, Nghean, Vietnam in 04/2022. The Latin names were identified by the co-author Le Thi Huong. The geographic coordinates included S. formosum (19°40'3"N and 104°55'30"), S. syzygioides (19°44'36" and 104°48'2"), S. megacarpum (19°48'31" and 105°5'47"), and S. chantaranothaianum (19°42'21" and 104°50'2"). The voucher specimens, including SL-01 (S. formosum leaves), SL-02 (S. syzygioides leaves), SL-03 (S. megacarpum leaves), and SL-04 (S. chantaranothaianum leaves), have been deposited in Faculty of Biology, College of Education, Vinh University. The obtained samples (2.5 kg each) were immediately cut into pieces, and immersed in distilled water at a ratio of 1:1, w/v. They were then hydro-distilled using a Clevenger apparatus for 3.5 h to give the yellow essential oils. The yields of extraction, which were calculated following dried materials, reached a range of 0.15–0.25%.

2.2. GC-FID/MS analysis

Gas chromatography with flame ionization detection (GC-FID) was carried out following the conditions (15–18): Agilent Technologies HP-5 MS column (30 m x 0.25 mm, film thickness 0.25μ m), Helium carrier gas (1.1 mL/min), injector temperature of 260°C, detector

temperature of 270°C, column temperature program: 65°C (3 min hold), increase to 230°C (4°C/min), 230°C (10 min hold), inlet pressure of 6.0 kPa, split mode injection (split ratio, 10:1), $1.1 \,\mu$ L injection volume.

Gas chromatography-mass spectrometry (GC-MS) was performed in the same manner: Agilent Technologies HP 7890A Plus Chromatograph (Santa Clara, CA, USA), HP-5 MS (30 m × 0.25 mm, film thickness 0.25 µm) column, HP 5973 MSD mass detector, Helium carrier gas (1.1 mL/min), MS ionization voltage of 70 eV, emission current of 40 mA, acquisitions range of 40-400 amu, a sampling rate of 1.0 scan/s. The GC was operated under the same circumstances as GC-FID. The retention indices (RI) based on a series of n-alkanes, co-injection with pure compounds (Sigma-Aldrich, St. Louis, MO, USA) or identified essential oil components, MS search (NIST 17 and Wiley 10th Version libraries), and comparison with the literature MS fragmentation were used to identify the chemical components of the essential oils (15-18). It was mainly based on the GC peak area (FID response) and without the use of correction factors, the relative concentrations (%) of the constituents were computed. The measurements were made three times.

2.3. Antimicrobial assay

Antimicrobial effect of six leaf essential oils was performed using the broth dilution method (19). Seven pathogenic bacterial strains have been used, including three Gram-positive bacterial strains *Enterococcus faecalis* ATCC299212, *Staphylococcus aureus* ATCC25923, and *Bacillus cereus* ATCC14579, three strains of Gramnegative bacterial strains *Escherichia coli* ATCC 25,922, *Pseudomonas aeruginosa* ATCC27853, and *Salmonella enterica* ATCC13076, and one yeast strain *Candida albicans* ATCC 10,231.

The selection of investigated concentrations was based on our previous publication (19), in which the tested essential oil was active with the specific concentration ranges. Stock solution of essential oil was prepared by DMSO (1%). Dilution series (2-fold) were prepared from 512 to $16 \,\mu\text{g/mL}$ by distilled water. They were then transferred to 96-well plates. Bacteria grown in double-strength Mueller-Hinton broth were standardized to 5×10^5 CFU/mL. The last row of well plates containing only the serial dilutions of samples without microorganisms was used as a positive control (no growth). Distilled water and medium served as a negative control (no antimicrobial agent). Streptomycin and nystatin were used as standards for antibacterial and anti-yeast activities, respectively. Experiments were repeated in triplicate. The results

were displayed by the MIC values (the lowest dose at which bacterial growth is totally inhibited).

2.4. Mosquito larvicidal assay

Eggs of Ae. aegypti were purchased from Institute of Biotechnology, VAST, and maintained at the Laboratory of Department of Pharmacy of Duy Tan University, Da Nang, Vietnam. For the assay (20,21), aliquots of Syzygium essential oils, dissolved by DMSO (1% stock solution), were placed in a 300-mL beaker and added to distilled water, containing 20 larvae (3th and early 4th instars). In each experiment, a set of controls using DMSO was also run for comparison. Mortality was calculated after 24 h and 48 h of exposure during which no nutritional supplement was added. The experiments have been carried out at room temperature. Each test was carried out with 3 replicates with several concentrations (100, 50, 25, 12.5, 6.0, 3.0, 1.5, and 0.75 µg/mL). Permethrin with the same tested concentrations was used as a positive control. The acute larvicidal effects on Ae. aegypti were recorded for 24 h and 48 h treatments. The data obtained were subjected to logprobit analysis to obtain LC₅₀ values, LC₉₀ values, and 95% confidence limits using XLSTAT v. 2018.5 (Addinsoft, Paris, France).

3. Results and discussion

A total of 46 compounds were identified in the leaf essential oil of S. formosum, which accounted for 94.1% (Table 1). This leaf essential oil was dominated by monoterpene hydrocarbons (51.1%), sesquiterpene hydrocarbons (27.0%), and oxygenated sesquiterpenes (15.2%). Oxygenated monoterpenes presented in a trace amount of 0.8%. The principal compounds were monoterpene hydrocarbons (E)- β -ocimene (18.0%) and β pinene (16.8%), and sesquiterpene hydrocarbon bicyclogermacrene (9.1%). There were lesser percentages of *cis*- β -elemene (7.6%), spathulenol (6.6%), α -pinene (6.3%), (*Z*)-β-ocimene (3.8%), β-caryophyllene (3.2%), myrcene (2.4%), viridiflorol (2.1%), alismol (1.8%), germacrene D (1.6%), limonene (1.4%), and cubeban-11-ol (1.1%). The remaining compounds were observed with amounts of less than 1.0%.

Similar to *S. formosum* leaf essential oil, the extraction of *S. syzygioides* leaves gave a yellow essential oil with 47 identified compounds (94.9%), which was predominated by monoterpene hydrocarbons (39.6%) and sesquiterpene hydrocarbons (37.8%). Oxygenated sesquiterpenes were also significant with 16.6%, whereas oxygenated monoterpenes was present in a trace mount of 0.9%. α -Pinene (15.2%), (*Z*)- β -ocimene (10.3%), and (*E*)- β -ocimene (7.1%) can be seen as characteristic monoterpene hydrocarbons. The principal sesquiterpene hydrocarbon, once again, was bicyclogermacrene (16.5%). Spathulenol (7.4%) was the most abundant compound in the group of oxygenated sesquiterpenes. Obviously, the amounts of α -pinene, bicyclogermacrene, and (*Z*)- β -ocimene in *S. syzygioides* leaf essential oil were higher than those in *S. formosum* leaf essential oil by 8.9, 7.4, and 6.5%, respectively. However, in contrast to *S. formosum* leaf essential oil, β -pinene (0.9%) and *cis*- β -elemene (1.1%) have been identified as minor compounds in *S. syzygioides* leaf essential oil. Likewise, the highest amount compound, (*E*)- β -ocimene in *S. formosum* leaf oil was associated with only 7.1% in *S. syzygioides* leaf essential oil.

The yellow essential oil of S. megacarpum leaf was obtained with 31 identified compounds (94.2%) that was almost entirely sesquiterpene hydrocarbons (83.8%) in character. In the meantime, their oxygenated derivatives reached 9.9%. Monoterpene hydrocarbons were not prevalent with 0.3%, while phenyl ethyl hexanoate (0.2%) represented as a non-terpenic compound only. The percentage of major compound bicyclogermacrene (37.0%) was recorded to outnumber the ones in the leaf essential oils of S. formosum and S. syzygioides by 27.9 and 20.5%, respectively. The percentages of β caryophyllene and germacrene D in the leaf essential oils of S. formosum and S. syzygioides are not remarkable, but they achieved significant amounts of 9.4 and 16.4% in the leaf essential oils of S. megacarpum, respectively. Likewise, with 11.2%, *cis*- β -elemene in S. megacarpum leaf essential oil was found to outstrip that in the two first essential oils. S. megacarpum leaf oil also contained lower levels of spathulenol (3.6%), caryophyllene oxide (2.3%), δ -elemene (2.0%), α -humulene (1.9%), and δ -cadinene (1.0%).

Considering S. chantaranothaianum species, 36 compounds were identified in its leaf essential oil, which represented 96.2%. In the same manner with S. megacarpum leaf essential oil, sesquiterpene hydrocarbons (83.0%) were predominant in this oil sample, followed by oxygenated sesquiterpenes (12.2%), and monoterpene hydrocarbons (1.0%). Resembling S. megacarpum leaf essential oil, oxygenated monoterpenes were completely absent. The leaf essential oil of S. chantaranothaianum was associated with the appearance of two major components β -caryophyllene (19.4%) and bicyclogermacrene (24.7%). In addition, there have been accompanied by smaller percentages, such as germacrene D (7.9%), δ -elemene (7.5%), *cis*- β -elemene (5.4%), germacrene B (4.3%), and *y*-elemene (3.1%), δ -selinene (1.6%), 9-epi-(E)-caryophyllene (1.3%), and aromadendrene and α -humulene (1.1%).

1 100 10	No.	RI _E	RI _L	Constituents	S. formosum	S. syzygioides	S. megacarpum	S. chantaranothaianum
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39.14971484Germacrene D1.61.116.47.940.15031489 β -Selinene0.61.60.80.341.15051488 δ -Selinene0.41.00.142.15111498 δ -Selinene0.41.00.143.15131500Bicyclogermacrene9.116.537.024.744.15211511 δ -Amorphene0.20.40.30.345.15281513 y -Cadinene0.20.40.30.346.15351522 δ -Cadinene0.20.40.30.346.15591545Selina-3.7 (11)-diene0.20.60.50.34.350.15761566Germacrene B0.40.50.34.30.351.15861575Globulol0.40.50.34.351.15861575Globulol0.40.50.70.753.15871576Palustrol0.40.50.70.754.15961580Spathulenol6.67.43.61.155.16031589Viridiflorol2.12.92.657.16101591Guaiol0.40.50.50.758.16221602Ledol0.40.50.50.759.16201600Rosifoliol0.40.5 <td></td> <td></td> <td></td> <td></td> <td></td> <td></td> <td></td> <td></td>								
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	70.	1674	1658	neo-Intermedeol	0.7	0.5	0.5	0.6 (Continued)

Table 1. The Identified Compounds (%) in the Essential Oils of Four Syzygium Leaves.

(Continued)

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Table 1. (Continued).

No.	RI _E	RI_L	Constituents	S. formosum	S. syzygioides	S. megacarpum	S. chantaranothaianum
			Total	94.1	94.9	94.2	96.2
			Monoterpene hydrocarbons	51.1	39.6	0.3	1.0
			Oxygenated monoterpenes	0.8	0.9		
			Sesquiterpene hydrocarbons	27.0	37.8	83.8	83.0
			Oxygenated sesquiterpenes	15.2	16.6	9.9	12.2
			Non-tepernic compounds			0.2	

 RI_E : Retention indices relative to *n*-alkanes (C₇-C₃₀) on HP-5 MS column.

 $\mathsf{RI}_\mathsf{L}:\mathsf{Retention}$ indices from NIST standard database.

Bold: Compounds with greater than 5%.

To date, there were several GC-MS analytical studies on Vietnamese Syzygium species. (Z)- β -Ocimene (20.3%) and caryophyllene oxide (13.2%) were characteristic compounds of *S. nervosum* leaf oil from Southern Vietnam (22). Another example is that β caryophyllene (23.40%), bicyclogermacrene (21.23%), and (Z)- β -ocimene (10.61%) established as the main compounds in the leaf essential oil of *S. tsoongii*, also collected from North Central Coast region of Vietnam (23). Thereby, it might be possibly concluded that monoterpene hydrocarbons and sesquiterpene hydrocarbons seem to be characters of the essential oils of Vietnamese Syzygium plants.

The obtained oils have been subjected to antimicrobial activity against three Gram-positive bacteria *E. faecalis*, *S. aureus*, and *B. cereus*, three Gramnegative bacteria *E. coli*, *P. aeruginosa*, and *S. enterica*, and one yeast *C. albicans*. As shown in Table 2, four samples showed powerful antimicrobial activity against Gram-positive bacteria *S. aureus* and *B. cereus* since these leaf oils possessed the MIC values better than those of the positive control streptomycin. In another case, the leaf essential oils of *S. megacarpum* (MIC = 16 μ g/mL) and *S. syzygioides* (MIC = 32 μ g/mL) were comparable with streptomycin (MIC = 32 μ g/mL) against Gram-positive bacterium *E. faecalis*, while two remaining essential oils exerted the same MIC value of 64 μ g/mL.

Regarding the Gram-negative bacteria, the MIC values assigning to four essential oils against *P. aeruginosa* orderly run as *S. megacarpum* and *S. chantaranothaianum* (MIC = $32 \mu g/mL$) >

S. syzygioides (MIC = 64 μ g/mL) > S. formosum and streptomycin (MIC = $128 \mu g/mL$). However, all samples failed to inhibit E. coli and S. enterica. Of the yeast C. albicans, the leaf oils of S. formosum and S. syzygioides were associated with the MIC value of 16 µg/mL, as compared with that of the standard cycloheximide (MIC = 32 μ g/mL). The leaf essential oil of S. chantaranothaianum induced an equivalent MIC value of 32 µg/mL to that of cycloheximide, whereas the leaf essential oil of S. megacarpum was strongly active against C. albicans with the same MIC value of 64 µg/mL. As can be seen, the leaf essential oils of S. formosum and S. syzygioides are better than two remaining essential oils in antimicrobial assay against C. albicans. It may be due to the abundance of monoterpene hydrocarbons in these two samples.

This is the first time that Vietnamese *Syzygium* plants have been subjected to antimicrobial assays. The obtained results match well with previous reports. For instance, the Gram-positive bacterium *Bacillus subtilis* was susceptible to the leaf essential oils of *S. aromaticum* and *S. polyanthum* (MIC = 31.25 µg/mL), but these oils were inactive to *E. coli* (23). Essential oil of clove *S. aromaticum* showed remarkable antimicrobial activity against 20 different isolates of *Listeria monocytogenes* at both concentrations of 1 and 10 µg/mL growth medium, and inhibited *Streptococcus mutans* with the MIC value of 30 µg/mL (24,25).

The 24-h and 48-h larvicidal activity of four *Syzygium* leaf essential oils against mosquito *Aedes aegypti* is shown in Table 3. It noted that essential oils have been considered actively larvicidal action if the

		Gram (+)			Gram (-)		Yeast
Samples	E. faecalis	S. aureus	B. cereus	E. coli	P. aeruginosa	S. enterica	C. albicans
S. formosum leaf oil	64	64	32	_	128	_	16
S. syzygioides leaf oil	32	64	32	-	64	-	16
S. megacarpum leaf oil	16	64	32	-	32	-	64
S. chantaranothaianum leaf oil	64	32	32	-	32	-	32
Streptomycin	32	128	256	256	128	256	-
Cycloheximide	-	_	_	-	_	-	32

"-": inactive.

Table 3. Mosquito Larvicidal Activity of Syzygium Leaf Oils Against Ae. Aegypti

Samples	LC ₅₀ (95% confidence levels)	LC ₉₀ (95% confidence levels)	χ ²	р
24-h treatment				
S. formosum leaf oil	30.1829 (28.1738-33.4702)	38.8101 (34.6692–47.9616)	0.0405804	0.980
S. syzygioides leaf oil	40.0409 (35.9258-43.1261)	50.6895 (47.2371-55.5091)	0.0178952	0.999
S. megacarpum leaf oil	28.3124 (26.4661–30.5315)	39.0577 (35.3101–46.0244)	0.0512473	0.975
S. chantaranothaianum leaf oil	27.5695 (25.4163–30.0059)	43.0427 (38.3092–51.1411)	6.11088	0.106
Permethrin (control)	0.0094 (0.0082-0.0107)	0.0211 (0.0185-0.0249)	57.6	0.000
48-h treatment				
S. formosum leaf oil	25.5460 (24.0321-27.6987)	33.0007 (30.1325-38.8792)	0.0010657	0.999
S. syzygioides leaf oil	39.2594 (35.1459–42.4376)	49.2314 (45.7001–53.9207)	0.0105410	1.000
S. megacarpum leaf oil	26.7038 (24.9437-29.2504)	35.7358 (32.3128-42.5290)	0.440285	0.802
S. chantaranothaianum leaf oil	26.6563 (24.6262-28.9373)	40.9687 (36.5517-48.6822)	6.15491	0.104

 LC_{50} value is less than 100 µg/mL (20). Based on this criterion, all studied samples exhibited good larvicidal activity with the 24-h and 48-h LC50 values of around $25-40 \,\mu\text{g/mL}$, as well as the 24-h and 48-h LC₉₀ values of around 33-50 µg/mL. In detail, the leaf essential oils of S. megacarpum and S. chantaranothaianum having the respective 24-h LC50 values of 27 and 28 µg/mL are better than those of S. formosum leaf oil (LC₅₀ = 30 μ g/mL) and S. syzygioides leaf oil (LC₅₀ = 40 μ g/mL). This can be explained by the high concentration of sesquiterpene hydrocarbons, especially in terms of bicyclogermacrene. However, S. formosum leaf essential oil with the highest percentages of β -pinene (16.8%), (*E*)- β ocimene (18.0%), and *cis*- β -elemene (7.6%) induced the lowest 48-h LC₅₀ value of 25 μ g/mL, and the lowest 24-h and 48-h LC₉₀ values of 33-38 µg/mL. Significantly, our current results are superior to previous analogs. The leaf essential oils of Indian S. lanceolatum and Brazilian S. jambolana generated the LC₅₀ values of 55.11 and 433 μ g/mL, respectively (26,27). The leaf essential oil of another Brazilian S. aromaticum caused the LC₅₀ value of > 90 μ g/mL against A. aegypti (28). Thereby, it is expected that Vietnamese Syzygium essential oils are now promising agents for antimicrobial and mosquito repellency drug developments.

4. Conclusion

The current results exhibited a great variation in chemical compositions of four *Syzygium* leaf essential oils. Monoterpene hydrocarbons (39.6–51.1%) and sesquiterpene hydrocarbons (27.0–37.8%) represented the leaf essential oils of *S. formosum* and *S. syzygioides*, whereas the leaf essential oils of *S. megacarpum* and *S. chantaranothaianum* were dominated by sesquiterpene hydrocarbons (83.0– 83.8%). Bicyclogermacrene (9.1–37.0%) was detected in four studied essential oils. β -Pinene (16.8%) and (*E*)- β -ocimene (18.0%) were also characteristics of *S. formosum* leaf oil, *S. syzygioides* leaf essential oil also contained α -pinene (15.2%), (Z)- β -ocimene

(10.3%), spathulenol (7.4%), and (*E*)- β -ocimene (7.1%). cis- β -Elemene (5.4–11.2%), β -caryophyllene (9.4-19.4%), and germacrene D (7.9-16.4%) were found in the leaf essential oils of S. megacarpum and S. chantaranothaianum. All the tested oils with the MIC values of 16-128 µg/mL were comparable or better than the positive control streptomycin against Gram-positive bacteria S. aureus and B. cereus, and Gram-negative bacterium P. aeruginosa. In the same manner, the leaf essential oils of S. formosum, S. syzygioides, and S. chantaranothaianum with the MIC values of 16-128 µg/mL were comparable or better than the positive control cycloheximide against the yeast C. albicans. Four oil samples have so far shown good larvicidal activity against mosquito Ae. aegypti with the 24-h and 28-h LC₅₀ values of 25-40 μ g/mL, and the 24-h and 28-h LC₉₀ values of 33–50 $\mu g/mL$.

Disclosure statement

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