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Original Article

Chemical composition, antimicrobial and larvicidal activities of essential oils of two *Syzygium* species from Vietnam

Composição química, atividades antimicrobiana e larvicida de óleos essenciais de duas espécies de *Syzygium* do Vietnã

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Abstract

The present study is the first to investigate the chemical composition, antimicrobial and larvicidal activities of the essential oils from the leaves of *Syzygium attopeuense* (Gagnep.) Merr. & L.M.Perry and *Syzygium tonkinense* (Gagnep.) Merr. & L.M.Perry collected in Vietnam. The essential oils were extracted by hydrodistillation and analyzed by GC and GC–MS. The study indicated the presence of a high percentage of sesquiterpenes in both investigated essential oils. The major components of *S. attopeuense* essential oil were bicyclogermacrene (24.26%), (*E*)-caryophyllene (11.72%), and (*E*)-β-ocimene (6.75%), whereas *S. tonkinense* essential oil was dominated by (*E*)-caryophyllene (80.80%). The antimicrobial activity of essential oils was evaluated by broth microdilution assay to determine the minimum inhibitory concentration (MIC) and median inhibitory concentration (IC_{50}). Both essential oils exhibited remarkable inhibitory activity against all tested Gram-positive bacteria and yeast than Gram-negative bacteria. Among them, essential oils of *S. attopeuense* and *S. tonkinense* possessed the strongest activity against *Enterococcus faecalis* (MIC = 4.00 µg/mL; IC_{50} = 1.69 µg/mL) and *Candida albicans* (MIC = 16.00 µg/mL; IC_{50} = 8.67 µg/mL), respectively. Furthermore, the larvicidal activity of essential oils was tested using fourth-instar larvae of *Aedes aegypti*. Results from the larvicidal test revealed that both essential oils had an excellent inhibitory effect against *A. aegypti* larvae with LC_{50} values from 25.55 to 30.18 µg/mL and LC_{90} values from 33.00 to 39.01 µg/mL. Our findings demonstrate that the essential oil extracted from *S. attopeuense* and *S. tonkinense* are potential sources of natural antimicrobials and can act as inexpensive mosquito larvicidal agents.

Keywords: *Syzygium attopeuense*, *Syzygium tonkinense*, essential oil, bacteria, *Aedes aegypti*.

Resumo

O presente estudo é o primeiro a investigar a composição química, as atividades antimicrobiana e larvicida dos óleos essenciais das folhas de *Syzygium attopeuense* (Gagnep.) Merr. & L.M.Perry e *Syzygium tonkinense* (Gagnep.) Merr. & L.M.Perry coletadas no Vietnã. Os óleos essenciais foram extraídos por hidrodestilação e analisados por GC e GC–MS. O estudo indicou a presença de alta porcentagem de sesquiterpenos em ambos os óleos essenciais investigados. Os principais componentes do óleo essencial de *S. attopeuense* foram biciclogermacreno (24,26%), (*E*)-cariofileno (11,72%) e (*E*)-β-ocimeno (6,75%), enquanto o óleo essencial de *S. tonkinense* foi dominado por (*E*)-cariofileno (80,80%). A atividade antimicrobiana dos óleos essenciais foi avaliada pelo ensaio de microdiluição em caldo para determinar a concentração inibitória mínima (CIM) e a concentração inibitória mediana (IC_{50}). Ambos os óleos essenciais exibiram notável atividade inibitória contra todas as bactérias Gram-positivas e leveduras testadas do que bactérias Gram-negativas. Entre eles, os óleos essenciais de *S. attopeuense* e *S. tonkinense* possuíam a atividade mais forte contra *Enterococcus faecalis* (CIM = 4,00 µg/mL; IC_{50} = 1,69 µg/mL) e *Candida albicans* (CIM = 16,00 µg/ml; IC_{50} = 8,67 µg/ml), respectivamente. Além disso, a atividade larvicida de óleos essenciais foi testada usando larvas de quarto instar de *Aedes aegypti*. Os resultados do teste larvicida revelaram que ambos os óleos essenciais tiveram um excelente efeito inibitório contra larvas de *A. aegypti* com valores de CL_{50} de 25,55 a 30,18 µg/ml e valores de CL_{90} de 33,00 a 39,01 µg/ml. Nossos achados demonstram que o óleo essencial extraído de *S. attopeuense* e *S. tonkinense* são fontes potenciais de antimicrobianos naturais e podem atuar como agentes larvicidas baratos para mosquitos.

Palavras-chave: *Syzygium attopeuense*, *Syzygium tonkinense*, óleo essencial, bactérias, *Aedes aegypti*.

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1. Introduction

The Myrtaceae consists of around 132 genera and 5,950 species (Christenhusz and Byng, 2016). One important member of this family is *Syzygium*, which is one of the large genera with around 1200–1800 species (Soh, 2017; Ranghoo-Sanmukhiya et al., 2019). They are widely distributed in the tropical and sub-tropical regions of Africa, Madagascar, Asia, and throughout Oceania and the Pacific, with the highest diversity found in Australia and Southeast Asia (Craven and Biffin, 2010; Nigam et al., 2012; Tuiwawa et al., 2013). Some *Syzygium* species have been used as a traditional medicine to treat diabetes, opium poisoning, liver disorders, centipede bites, renal problems, dysentery, inflammation, leucorrhoea, stomachache, fever, constipation, vomiting, dermopathy, bleeding disorders, and metrorrhagia (Cock and Cheesman, 2018; Uddin et al., 2022; Kadir et al., 2022). Previous phytochemical studies on *Syzygium* species have revealed the presence of secondary metabolites such as terpenoids, lignans, chalcones, flavonoids, tannins, alkyl phloroglucinols, and chromone derivatives (Aung et al., 2020; Uddin et al., 2022). Modern pharmacological studies have shown the bioactivities of these metabolites, such as antioxidant, antibacterial, anticancer, anti-inflammatory, hepatoprotective, and antidiarrheal activities (Aung et al., 2020; Uddin et al., 2022).

Syzygium attopeuense (Gagnep.) Merr. & L.M.Perry and *Syzygium tonkinense* (Gagnep.) Merr. & L.M.Perry are two species of the genus *Syzygium*, which grow in secondary or primary forests, and along rivers on rocky terrain at an altitude of 300 to 1500m (Soh and Parnell, 2015). While *S. tonkinense* is an endemic species to Vietnam, *S. attopeuense* is found in Thailand, Laos, and Vietnam (Pham, 1999; Soh and Parnell, 2015). The fruits of *S. attopeuense* are edible and its roots are used medicinally after soaking (Soh and Parnell, 2015). To date, present knowledge about these two species of *Syzygium* is still limited with respect to their phytochemistry and biological activities.

Essential oils are volatile and aromatic liquids derived from various parts of plants such as flowers, leaves, stems, roots, and seeds (Bakkali et al., 2008; Thinh et al., 2021). These oils contain a complex mixture of chemical compounds, including terpenes, esters, alcohols, and phenols, which give the oils their characteristic fragrance and medicinal properties (Bakkali et al., 2008). Currently, essential oils are of growing interest both in the industry and scientific research because of their various biological activities such as antimicrobial, antioxidant, antiviral, and larvicidal (Bakkali et al., 2008; Mutlu-Ingok et al., 2020; Thin et al., 2022). Indeed, there is ample evidence that essential oils have been suggested as alternative sources of synthetic larvicides for insect control as repellents, insecticides, or larvicides because they offer advantages such as biodegradability and negligible effects on non-target species and the environment (Pavela, 2015; Osanloo et al., 2018; Esmaili et al., 2021). In addition, with the increase in bacterial resistance to antibiotics, there is also considerable interest in using essential oils as safe and natural antimicrobial agents for infection control or food preservation (Bassolé and Juliani, 2012).

Although the compositions of essential oils and biological activities of other *Syzygium* species are well-known (Kadir et al., 2022). Furthermore, several essential oils of *Syzygium* have been tried as mosquito larvicides as well as antimicrobials (Sarvesan et al., 2015; Siddique et al., 2015; Govindarajan and Benelli, 2016; Hamad et al., 2017; Benelli et al., 2018; Huong et al., 2022; Fernandes et al., 2022). However, to our best knowledge, there are no published reports on the chemical composition, antimicrobial and larvicidal activities of the essential oils of *S. attopeuense* and *S. tonkinense*. Therefore, the present study aimed to (1) analyze the chemical composition of the essential oils from the leaves of *S. attopeuense* and *S. tonkinense* collected in Vietnam, (2) evaluate their antimicrobial activity effect against bacteria and fungi by broth microdilution assay, and (3) determine their larvicidal activity against fourth-instar larvae of *Aedes aegypti*.

2. Materials and Methods

2.1. Plant material

The fresh leaves of *S. attopeuense* and *S. tonkinense* were collected from their wild-growing populations from Nghe An province, Vietnam (Table 1 and Figure 1). The plant samples were identified by Assoc. Prof. Dr. Le Thi Huong (Vinh University, Vietnam) based on morphological characteristics. The voucher specimens were deposited in the herbarium of Vinh University, Vietnam.

2.2. Essential oils isolation procedure

The fresh leaves of both *Syzygium* species were cut into small pieces and separately subjected to hydrodistillation using a Clevenger-type apparatus for 4 h (Hung et al., 2020; Thin et al., 2021). The obtained essential oils were dried over anhydrous sodium sulfate and stored in amber vials at 4 °C before analysis. The yield of essential oil was calculated according to Equation 1:

$$Y = \frac{V}{W} \times 100 \quad (1)$$

Where Y is the extraction yield (%), V is the volume of extracted essential oil (mL), and W is the weight of the sample (gram).

2.3. Essential oil analysis

The essential oils were analysed by gas chromatography (GC) and gas chromatography–mass spectrophotometry (GC–MS) as previously described (Thinh et al., 2022; Chac et al., 2022). GC analyses were performed using an Agilent Technologies HP 7890A Plus gas chromatograph equipped with a flame ionization detector (FID) and fitted with HP-5MS column (30 m × 0.25 mm i.d., film thickness 0.25 µm, Agilent Technologies, Santa Clara, CA, USA). The oven temperature was held at 60 °C for 2 min and then programmed to 220 °C at a rate of 4 °C/min. The injector and detector temperatures were 250 °C and 260 °C, respectively. Helium was used as the carrier gas at a flow rate of 1 mL/min.

Table 1. Collection details for *Syzygium attopeuense* and *Syzygium tonkinense* in Nghe An province, Vietnam.

Voucher number	Science name	Vietnamese name	Collection month/year	Collection location
885	*Syzygium attopeuense* (Gagnep.) Merr. & L.M.Perry	Trâm attopeuen	3/2022	Pu Hoat Natural Reserve; 19°48'30.0"N, 105°05'44.0"E; elev. 274 m
946	*Syzygium tonkinense* (Gagnep.) Merr. & L.M.Perry	Trâm bắc	6/2022	Pu Huong Natural Reserve; 19°14'41.0"N, 104°55'51.0"E; elev. 274 m

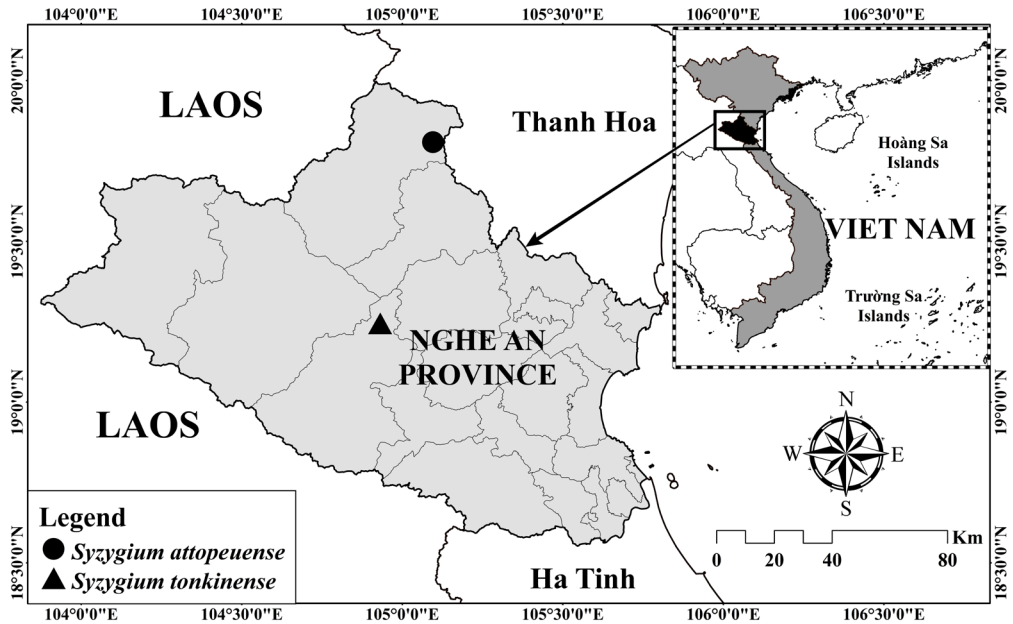

Figure 1. Location map of *Syzygium attopeuense* (●) and *Syzygium tonkinense* (▲) collection in Nghe An province, Vietnam.

GC–MS analyses were carried out in an Agilent Technologies HP 7890A Plus Chromatograph (Santa Clara, CA, USA) fitted with an HP-5MS fused silica column (30 m × 0.25 mm i.d., film thickness 0.25 µm) and interfaced with a mass spectrometer HP 5973 MSD. The oven temperature was 60 °C – 220 °C at a rate of 4 °C/min, transfer line temperature 260 °C, carrier gas helium with a flow rate of 1 mL/min, split ratio 10:1, ionization energy 70 eV, emission current 40 mA, sampling rate of 1.0 scan/s and mass range of 35–350 amu. The components of essential oil were identified by their GC retention time relative to known compounds and by comparison of their mass spectra with those present in the computer data bank (NIST, 2018) and published spectra (Adams, 2007). Determination of the percentage composition was based on peak area normalisation without using correction factors.

2.4. Antimicrobial assay

Three strains of Gram-positive bacteria (*Enterococcus faecalis* ATCC 299212, *Staphylococcus aureus* ATCC 25923, and *Bacillus cereus* ATCC 14579), three strains of Gram-negative bacteria (*Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, and *Salmonella enterica* ATCC 13076), and one strain of yeast (*Candida albicans* ATCC 10231) were used to evaluate the antimicrobial activity of essential oils. All strains were obtained from the laboratory stock of the Institute of Marine Biochemistry, Vietnam Academy of Science and Technology, Hanoi, Vietnam.

Determination of minimum inhibitory concentration (MIC) and median inhibitory concentration (IC_{50}) was carried out using the broth microdilution susceptibility method as described previously (Dai et al., 2020). The bacteria were grown in the Mueller-Hinton broth (MHB) and *C. albicans* in the Sabouraud broth (SB). The investigated oils were dissolved in 1% dimethylsulfoxide (DMSO) and then diluted to the highest concentration. Serial doubling dilutions of oils were prepared in a 96-well microtiter plate over the range of 2–16,384 µg/mL. Overnight broth cultures of each strain were prepared and the final concentration in each well was adjusted to 5×10^5 CFU/mL for bacteria and to 1×10^3 CFU/mL for *C. albicans*. Positive controls, Streptomycin for bacteria and Nystatin for *C. albicans*, and a negative control of the vehicle (DMSO), were prepared under the same experimental conditions. The bacteria and *C. albicans* were incubated for 24 h, at 37 °C and at 30 °C, respectively. The MIC values were defined as the lowest concentration of the essential oil at which the microorganism does not demonstrate visible growth.

The IC_{50} values were determined by the percentage of microorganisms that inhibited growth based on the turbidity measurement data of EPOCH2C spectrophotometer (BioTeK Instruments, Inc Highland Park Winooski, VT, USA) and

RawData computer software (Brussels, Belgium) according to the following Equations 2 and 3:

$$\% \text{ inhibition} = \frac{OD_{control(-)} - OD_{test\ agent}}{OD_{control(-)} - OD_{control(+)}} \times 100 \quad (2)$$

$$IC_{50} = High_{conc} - \frac{(High_{inh\%} - 50\%) \times (High_{conc} - Low_{conc})}{(High_{inh\%} - Low_{inh\%})} \quad (3)$$

Where OD is 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 (+) is the culture medium without essential oils, $High_{conc}$/Low_{conc} is the concentration of test agent at high concentration/low concentration, and $High_{inh\%}$/$Low_{inh\%}$ is the % inhibition at high concentration/% inhibition at low concentration.

2.5. Larvicidal assay

The method of Hung et al. (2020) was employed to conduct the larvicidal activity test. Eggs of *A. aegypti* were obtained from a mosquito colony maintained at the Laboratory of the Department of Pharmacy of Duy Tan University, Da Nang, Vietnam. 20 fourth-instar larvae were collected by direct pipetting from an egg incubation flask and transferred to 250 mL beakers that contained the essential oils dissolved in EtOH (1% stock solution) and 150 mL of water. Each test comprised mainly three replicates with five concentrations (100, 50, 25, 12.5, and 6 µg/mL). Control tests were carried out in parallel, using EtOH (negative control) and permethrin (positive control) for comparison. The evaluation of the mortality rate was performed at 24 h and 48 h after the beginning of the experiment, verifying the number of dead larvae. Larvae were considered dead if they did not move when they were slightly stimulated with a Pasteur pipette. The room temperature was observed during the experiment, with variations between 25 °C and 28 °C.

2.6. Statistical analysis

All experiments were conducted in triplicates. Data from larvicidal assay were evaluated through log-probit analysis using Minitab® 19 (Minitab, LLC, State College, PA, USA) to determine the LC_{50} and LC_{90}, representing the concentrations in µg/mL that caused 50% and 90% mortality along with 95% confidence intervals.

3. Results and Discussion

3.1. Yields and chemical constituents of essential oils

The essential oils isolated by hydrodistillation from the leaves of *S. attopeuense* and *S. tonkinense* were found to be yellow liquids and obtained in yields of 0.22% and 0.30% (v/w), respectively. The chemical composition of essential oils of two *Syzygium* species was investigated using GC and GC/MS techniques. The percentages and the retention indices of the identified oil components were listed in Table 2 and 3 in the order of their elution on the HP-5MS column.

Table 2. Chemical composition of *Syzygium attopeuense* essential oil.

RT[a]	Compound[b]	RI[c]	RI[d]	Area (%)
10.02	α-Pinene	938	932	2.01
11.37	β-Pinene	984	974	2.96
11.60	Myrcene	991	988	0.99
12.98	Limonene	1033	1024	0.24
13.12	(*Z*)-β-Ocimene	1037	1044	0.44
13.51	(*E*)-β-Ocimene	1049	1052	6.75
23.74	δ-Elemene	1347	1335	0.79
24.14	α-Cubebene	1360	1345	0.11
24.99	Isoledene	1385	1370	0.19
25.09	α-Copaene	1388	1374	0.24
25.55	*cis*-β-Elemene	1402	1389	1.32
26.44	α-Santalene	1431	1415	2.06
26.62	(*E*)-Caryophyllene (= β-Caryophyllene)	1436	1417	11.72
26.89	*trans*-α-Bergamotene	1445	1432	3.00
27.10	α-Maalinene	1452	1435	0.26
27.22	Aromadendrene	1456	1439	2.54
27.35	(*Z*)-β-Farnesene	1460	1440	0.24
27.65	(*E*)-β-Farnesene	1469	1443	2.69
27.69	α-Humulene	1471	1452	0.84
27.91	9-*epi*-(*E*)-Caryophyllene	1478	1464	1.49
28.23	γ-Curcumene	1488	1470	0.35
28.27	γ-Muurolene	1489	1476	0.60
28.51	Germacrene D	1497	1484	3.15
28.70	β-Selinene	1503	1489	0.85
28.76	δ-Selinene	1505	1492	0.50
29.03	Bicyclogermacrene	1514	1500	24.26
29.12	β-Bisabolene	1517	1507	1.22
29.20	β-Curcumene	1520	1514	0.35
29.30	Aromadendra-1(10),4(15)-diene	1523	1516	0.12
29.33	Sesquicineol	1524	1517	0.43
29.46	γ-Cadinene	1529	1519	0.30
29.62	β-Sesquiphellandrene	1534	1526	0.37
29.67	δ-Cadinene	1536	1528	1.30
29.99	*trans*-Cadina-1,4-diene	1546	1533	0.24
30.10	(*E*)-α-Bisabolene	1550	1535	0.40
30.15	α-Cadinene	1552	1537	0.46
30.66	(*E*)-Nerolidol	1569	1561	0.78
30.88	Germacrene B	1576	1562	1.02
31.20	Palustrol	1587	1567	0.66
31.46	Spathulenol	1595	1577	3.40
31.67	Viridiflorol	1603	1592	2.46
31.94	Cubeban-11-ol	1612	1595	1.90
32.16	Rosifoliol	1620	1600	0.54
32.25	Ledol	1624	1602	0.40
32.75	5-Guaiene-11-ol	1641	1637	0.51
33.19	*epi*-α-Cadinol (= τ-Cadinol)	1657	1638	0.22
33.22	*epi*-α-Muurolol (= T-Muurolol)	1658	1640	0.23
33.59	α-Cadinol	1671	1652	0.73
34.25	*epi*-α-Bisabolol	1694	1670	0.42
34.30	Unidentified	1696	-	1.51
34.65	(*Z*)-α-*trans*-Bergamotol	1709	1706	1.00
	Monoterpene hydrocarbons			13.39
	Sesquiterpene hydrocarbons			62.98
	Oxygenated sesquiterpenes			13.68
	Total identified			90.05

Note: [a]RT – Retention time (min); [b]Elution order on HP-5MS column; [c]Retention indices on HP-5MS column; [d]Literature retention indices.

Table 3. Chemical composition of *Syzygium tonkinense* essential oil.

RT[a]	Compound[b]	RI[c]	RI[d]	Area (%)
13.63	(*Z*)-β-Ocimene	1037	1044	0.24
25.70	α-Copaene	1389	1374	2.42
27.34	(*E*)-Caryophyllene (= β-Caryophyllene)	1441	1417	80.80
27.50	*trans*-α-Bergamotene	1446	1432	1.31
27.68	α-Guaiene	1452	1437	2.54
28.31	α-Humulene	1472	1452	4.79
28.88	β-Chamigrene	1490	1478	0.12
29.33	β-Selinene	1504	1489	1.08
29.57	α-Selinene	1513	1498	1.39
29.82	α-Bulnesene (= δ-Guaiene)	1521	1512	0.81
30.28	δ-Cadinene	1537	1528	0.95
30.30	7-*epi*-α-Selinene	1537	1531	0.61
32.30	Caryophyllene oxide	1605	1582	0.96
	Monoterpene hydrocarbons			0.24
	Sesquiterpene hydrocarbons			96.82
	Oxygenated sesquiterpenes			0.96
	Total identified			98.02

Note: [a]RT – Retention time (min); [b]Elution order on HP-5MS column; [c]Retention indices on HP-5MS column; [d]Literature retention indices.

A total of 50 constituents amounting to 90.05% in the *S. attopeuense* essential oil were identified (Table 2). Among these 62.98% were sesquiterpene hydrocarbons, 13.68% were oxygenated sesquiterpenes, and it also contained 13.39% monoterpene hydrocarbons. The major constituents in the *S. attopeuense* essential oil were bicyclogermacrene (24.26%), (*E*)-caryophyllene (11.72%), and (*E*)-β-ocimene (6.75%). In the essential oil extracted from *S. tonkinense*, 13 constituents were identified, corresponding to 98.02% of the total oil (Table 3). Essential oil of *S. tonkinense* was characterized by a very high percentage of sesquiterpene hydrocarbons (96.82%) with (*E*)-caryophyllene (80.80%) found to be the most abundant constituent. To the best of our knowledge, the essential oil composition of *S. attopeuense* and *S. tonkinense* has not been reported before and therefore our results could be considered the first report about the composition of the essential oil of these plants.

The composition analysis of essential oils from the leaves of *S. attopeuense* and *S. tonkinense* revealed that sesquiterpenes predominated. Of which, *S. tonkinense* essential oil had greater amounts of sesquiterpenes than *S. attopeuense* essential oil. These results are consistent with previous studies, which found greater amounts of sesquiterpene compounds in essential oils of *Syzygium* species (Rameshkumar et al., 2015; Sarvesan et al., 2015; Huong et al., 2017; Khanh and Ban, 2020). Furthermore, the high content of (*E*)-caryophyllene in the essential oils of the two studied *Syzygium* species is noteworthy, especially *S. tonkinense*. It was also noted previously that the volatile constituents of most *Syzygium* species reported from Vietnam contained high amounts of (*E*)-caryophyllene (Huong et al., 2017; Khanh and Ban, 2020; Tran et al., 2021; Huong et al., 2022). However, several other species of *Syzygium* with either a low content or total absence of (*E*)-caryophyllene have been reported from other countries (Chalannavar et al., 2011; Saroj et al., 2015; Petrachaianan et al., 2019; Jugreet and Mahomoodally, 2020; Jena et al., 2021). This difference may be attributed to different factors such as the nature and age of the plants, geographical areas, time of collection, as well as the differences in the parts of the plants used for analysis (Thinh et al., 2022).

3.2. Antimicrobial activity of essential oils

The essential oils of *S. attopeuense* and *S. tonkinense* were examined for their antimicrobial activity potential against a panel of microorganisms including Gram-positive bacteria (*E. faecalis*, *S. aureus*, and *B. cereus*), Gram-negative bacteria (*E. coli*, *P. aeruginosa*, and *S. enterica*), and the yeast (*C. albicans*). The MIC and IC_{50} values of the essential oils are presented in Table 4.

As can be seen in Table 4, essential oils of *S. attopeuense* and *S. tonkinense* showed varying degrees of antimicrobial activity against strains tested. Both essential oils exhibited activity against all tested Gram-positive bacteria. Among Gram-positive bacteria, the most susceptible bacterium for *S. attopeuense* and *S. tonkinense* essential oils was *E. faecalis* with MIC values of 4 µg/mL and 32 µg/mL, respectively, while IC_{50} values of 1.69 µg/mL and 17.33 µg/mL, respectively. Meanwhile, each essential oil only showed activity against a Gram-negative bacterial strain with *S. attopeuense* for *P. aeruginosa* (MIC = 64 µg/mL; IC_{50} = 19.67 µg/mL) and *S. tonkinense* for *E. coli* (MIC = 256 µg/mL; IC_{50} = 128.46 µg/mL). Furthermore, both essential oils exhibited remarkable inhibitory activity against the yeast *C. albicans*. These results are consistent with data obtained for the antimicrobial activities of essential oils of some *Syzygium* plants (Shafi et al., 2002; Nadarajan and Pujari, 2014; Sarvesan et al., 2015; Siddique et al., 2015; Hamad et al., 2017; An et al., 2020; Fernandes et al., 2022).

The present investigation revealed that Gram-positive bacteria tested were more sensitive to two studied essential oils than Gram-negative bacteria. It has frequently been reported that Gram-negative bacteria were resistant to the inhibitory effects of essential oil and their components (Thinh et al., 2022). This resistance has been attributed to the presence of cell wall lipopolysaccharides that can screen out the essential oil (Ouattara et al., 1997). Such a tendency is also observed in other studies (Zomorodian et al., 2018; Ghavam et al., 2020). Compared with the essential oil of *S. tonkinense*, the oil isolated from *S. attopeuense* exhibited greater antimicrobial potential due to the much lower concentration of MICs. The varying antimicrobial activities of the essential oils may be attributed to their predominant constituents such as bicyclogermacrene, (*E*)-caryophyllene, and (*E*)-β-ocimene. Althought, several investigations have shown (*E*)-caryophyllene to be broadly antimicrobial (Sabulal et al., 2006; Dahham et al., 2015). However, essential oils rich in both bicyclogermacrene and (*E*)-β-ocimene have been reported to show significant antimicrobial activity (Costa et al., 2009). On the other hand, minor components may also contribute to the antimicrobial activity of essential oil in addition to the major components (Zouari et al., 2011). In fact, some studies have shown that whole essential oils have greater antimicrobial activity than the major components mixed

Table 4. Antimicrobial activity of essential oils of *Syzygium attopeuense* and *Syzygium tonkinense*.

Microorganisms	Essential oils			
	Syzygium attopeuense		*Syzygium tonkinense*	
	MIC[a]	IC_{50}[b]	MIC	IC_{50}
Bacteria (Gram-positive)				
Enterococcus faecalis	4	1.69	32	17.33
Staphylococcus aureus	64	19.45	128	65.33
Bacillus cereus	16	6.78	256	128.67
Bacteria (Gram-negative)				
Escherichia coli	NA[c]	NA	256	128.46
Pseudomonas aeruginosa	64	19.67	NA	NA
Salmonella enterica	NA	NA	NA	NA
Yeast				
Candida albicans	32	15.67	16	8.67

Note: [a]MIC – minimum inhibitory concentration (μg/mL); [b]IC_{50} – 50% inhibition concentration (μg/mL); [c]NA – not active.

(Gill et al., 2002). Although the mechanisms associated with the antimicrobial activities of essential oils are not fully understood. However, in general, this may be due to synergistic interactions between major and minor components that result in different antimicrobial activities of the whole essential oil (Sharma et al., 2020).

3.3. Larvicidal activity of essential oils

Larvicidal tests of essential oils of *S. attopeuense* and *S. tonkinense* against fourth-instar *A. aegypti* larvae were conducted. Table 5 summarizes the LC_{50} and LC_{90} values after 24 h and 48 h for these essential oils. After 24-hour exposure, essential oils of *S. attopeuense* and *S. tonkinense* exhibited activity against *A. aegypti* with LC_{50} values of 30.18 μg/mL and 28.31 μg/mL, respectively, while LC_{90} values of 38.81 μg/mL and 39.01 μg/mL, respectively. After 48-hour exposure, the LC_{50} value of *S. attopeuense* essential oil was 25.55 μg/mL and its LC_{90} value was 33.00 μg/mL, whereas the corresponding values for *S. tonkinense* essential oil were 26.70 μg/mL and 35.74 μg/mL, respectively. Several studies have reported that essential oil and plant extracts are considered larvicidal against *A. aegypti* if the LC_{50} values are less than 100 μg/mL (Dias and Moraes, 2014; Ramos et al., 2017). Based on these guidelines, it can be concluded that both essential oils showed good larvicidal activity against the fourth-instar larvae of *A. aegypti*. Similar results were reported in the earlier works done in several species of the genus *Syzygium* (Govindarajan and Benelli, 2016; Benelli et al., 2018, An et al., 2020). The good larvicidal activity of *S. attopeuense* and *S. tonkinense* essential oils may be related to their chemical constituents. Indeed, bicyclogermacrene, (*E*)-β-ocimene and (*E*)-caryophyllene are the main components in essential oils of *S. attopeuense* and *S. tonkinense* that have been shown to have larvicidal activity (Chau et al., 2020; Nogueira Sobrinho et al., 2021). In addition, the components in lower amounts could also contribute to the larvicidal activity of the essential oil by acting in a synergistic manner. This has been confirmed in previous studies (Bassolé et al., 2003; Senthilkumar and Venkatesalu, 2010; Chau et al., 2020).

Table 5. Larvicidal activity of essential oils of *Syzygium attopeuense* and *Syzygium tonkinense* against *Aedes aegypti* after 24 h and 48 h of exposure.

Essential oils	LC_{50} (μg/mL) 95% CI[a]	LC_{90} (μg/mL) 95% CI	χ^2	*p*
Syzygium attopeuense				
At 24 h	30.18 (28.17–33.47)	38.81 (34.67–47.96)	0.0405804	0.980
At 48 h	25.55 (24.03–27.70)	33.00 (30.13–38.87)	0.0010657	0.999
Syzygium tonkinense				
At 24 h	28.31 (26.47–30.53)	39.01 (35.31–46.02)	0.0512473	0.975
At 48 h	26.70 (24.94–29.25)	35.74 (32.31–42.53)	0.4402850	0.802

Note: [a]Confidence interval at 95%.

4. Conclusion

In summary, this is the first study to investigate the chemical composition, antimicrobial and larvicidal activities of essential oils from the leaves of *S. attopeuense* and *S. tonkinense* collected in Vietnam. According to GC and GC-MS analyses, the major components of *S. attopeuense* essential oil were bicyclogermacrene (24.26%), (*E*)-caryophyllene (11.72%), and (*E*)-β-ocimene (6.75%), whereas those of *S. tonkinense* essential oil was (*E*)-caryophyllene (80.80%). Both essential oils showed promising antimicrobial efficacy against all tested Gram-positive bacteria and yeast, especially *S. attopeuense* oil. In addition, using the leaf essential oil from *S. attopeuense* and *S. tonkinense* had excellent inhibitory effects against *A. aegypti* larvae. The present findings would substantiate the further utilization of these plants as potential sources of natural antimicrobials and also as a larvicide in mosquito control programs.

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