Journal of Essential Oil Bearing Plants
https://www.tandfonline.com/journals/teop
DOI: 10.1080/0972060X.2023.2192370

Taylor & Francis
Taylor & Francis Group
ISSN Print: 0972-060X; ISSN Online: 0976-5026

Research Article

Antimicrobial activity and constituents of the leaf essential oil of *Syzygium petelotii* Merr. & Perry and *Syzygium syzygioides* (Miq.) Merr. & Perry from Vietnam

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Received 05 July 2022
Revised 13 March 2023
Accepted 13 March 2023

Abstract
Syzygium plants are known as sources of biologically active substances. The essential oils constituents and antimicrobial activity of *Syzygium petelotii* Merr. & Perry and *Syzygium syzygioides* (Miq.) Merr. & Perry from Vietnam are being reported. Essential oils were obtained by hydrodistillation of the leaves of *S. petelotii* and *S. syzygioides* collected from Pù Hoạt Nature Reserve, Vietnam. The constituents of the essential oils were determined by using gas chromatography (GC) and gas chromatography-mass (GC/MS) spectrometry methods. The antimicrobial activity was evaluated by microdilution broth susceptibility assay. The results indicate that γ-guaiene (24.1%), β-caryphyllene (9.3%), α-pinene (7.8%), and β-pinene (6.1%) were the major compounds of *S. petelotii*. The main constituents of *S. syzygioides* were caryophyllene oxide (14.8%), α-pinene (10.6%), β-pinene (6.6%) and spathulenol (6.4%). The essential oil of *S. petelotii* showed pronounced antimicrobial activity against *Pseudomonas aeruginosa* ATCC27853 and *Enterococcus faecalis* ATCC299212, with minimum inhibitory concentration (MIC) values of 16.0 µg/mL and 64.0 µg/mL, respectively. The essential oil of *S. syzygioides* only displayed activity towards *Pseudomonas aeruginosa* ATCC27853 with MIC value of 32.0 µg/mL. Both essential oils exhibited antimicrobial activity towards *Bacillus cereus* ATCC14579 (MIC value, 128.0 µg/mL), and anti-candidal action against *Candida albicans* ATCC10231 with MIC value of 64.0 µg/mL. The chemical compositions and antimicrobial activity of essential oils of *S. petelotii* and *S. syzygioides* are being reported for the first time.

Keywords
Anti-candidal activity, essential oil, gram-positive bacteria, gram-negative bacteria, GC/MS, *Syzygium*

Introduction

Syzygium is a large genus of flowering plants, of the Myrtaceae family with several species used as important resources in the food and pharmaceutical industries[1]. In our continuing search for biologically active agents from plants growing in Vietnam[2], the chemical constituents and antimicrobial activity of essential oils from the leaves of *Syzygium petelotii* Merr. & Perry and *Syzygium syzygioides* (Miq.) Merr. & Perry were studied and reported. *Syzygium syzygioides* (syn *Eugenia syzygioides* (Miq.) M.R.Hend.) is known in Vietnamese as Trâm kiền kiền.

The plant is an evergreen tree of about 30 m height. It has opposite leaves of different shapes mostly oval. The bark varies from dark-greyish to reddish-brown colour. The white flowers are bisexual and exist in clusters near the axils of its leaves. *S. syzygioides* has round to flattened fleshy fruits. The fruits have dark red to purplish-black when ripe. The seeds are either oblong or round in shape. Mostly, the fruits are eaten by birds[3]. *Syzygium petelotii* (Vietnamese name: Trâm petelot) is a tree of about 20 m tall. The bark is brownish and glabrous. The opposite leaves are dark greenish to brownish. The whitish flowers are 0.5-1 mm while the fruits are dark purplish to blackish. The plant resembles *S. mekogense* with short inflorescences[4].

The authors are not aware of any literature information on the essential oil constituents and antimicrobial activity of any parts of *S. petelotii* and *S. syzygioides* grown in Vietnam or any parts of the world. However, information abounds on the compositional patterns of essential oils from some other *Syzygium* plants. The main compounds identified in the leaf and stem of *S. grande* were β-caryophyllene (25.6% and 29.3%), sabinene (16.8% and 10.2%) and (*E*)-β-ocimene (11.9% and 9.5%) respectively[5]. On the other hand, α-pinene (35.4%) and (*E*)-nerolidol (30.4%) were the major compounds present in the leaf of *S. sterrophyllum*[5]. The main constituents of the leaf of *S. hancei* were γ-guaiene (11.07%), β-caryophyllene (9.11%), and *cis-c*alamenene (7.46%), with β-caryophyllene (42.53%) and (*E*)-β-ocimene (19.38%) present in the leaf of *S. caryophyllatum,* while β-caryophyllene (64.53%), and α-pinene (6.14%) constitute the bulk of the constituents of the leaf of *S. lineatum*[6]. Previously, (*Z*)-β-ocimene (20.3%), caryophyllene oxide (13.2%) and (*E*)-caryophyllene (12.1%) were identified in *S. nervosum*[7]. It could be observed that *Syzygium* plants produced essential oils with varying compositional patterns.

Essential oils from *Syzygium* species have demonstrated some biological activities. Essential oils from *S. nervosum* from Vietnam displayed larvicidal actions against *Aedes aegypti* with median lethal concentration (LC_{50}) of 28.63 μg/mL and *Culex quiquinfasciatus* (LC_{50} 46.09 μg/mL), in addition to antimicrobial activity towards *Enterococcus faecalis* with minimum inhibitory concentration (MIC) value of 32 μg/mL; and anti- candidal property towards *Candida albicans* (MIC 128 μg/mL)[7]. The antibacterial, antioxidant, and anti-trypanosomal activity of essential oil from *S. aromaticum* of Brazilian origin has been reported[8]. Likewise, essential oil from clove (*S. aromaticum*) buds grown in Vietnam showed antibacterial property against *Bacillus cereus*, *Staphylococcus aureus*, *Escherichia coli*, *Salmonella typhimurium* and *Pseudomonas aeruginosa*[9]. The leaf oils of *S. aromaticum, S. malaccense* and *S. samaragense* from Nigeria showed antioxidant and phytotoxic activities, as well as antibacterial potentials, but not *Kiebsiella* spp., *Proteus* spp., *Pseudomonas* spp. and *Mucormucelo*[10]. Essential oil from the wax of *S. samarangense* also exhibited potential antibacterial activity towards *B. cereus, E. coli, S. enteritidis* and *S. aureus*[11].

Vietnam is located in the tropical monsoon region where natural conditions are favourable for the formation and development of plants, especially high-value essential oil-bearing plants[12]. The studies of the beneficial effects of essential oils have been documented. Essential oils have been used in medicinal and agricultural activities. They are known to possess antimicrobial and anti-inflammatory activities among others. Moreover, essential oils have been used in killings of insect pests. This research is unending and continues with new findings year-round. The present study center on the determination of the chemical constituents and effect of essential oils from the leaves of *S. petelotii* and *S. syzygioides* on some of the most pathogenic bacteria, which were proven to be involved in acquired infections.

Materials and methods

The experimental methods and analytical procedures employed in this study follows the patterns used in our previous reports[2]. The designated analyses were highlighted under different headings.

Place of collection and identification of S. petelotii and S. syzygioides leaves

The leaves of *S. petelotii* and *S. syzygioides* were obtained from Pù Hoạt Nature Reserve (GPS: 19°48′36″N, 105°5′35″E) in January 2022. The plants were identified by Assoc. Prof. Dr. Le Thi Huong, Faculty of Biology, College of Education, Vinh University, Vietnam. A total of over 2.0 kg of each sample were collected. On identification, the plants were preserved with specimen numbers LTH 918 and LTH 903, respectively.

Isolation of essential oils

For the leaves of *S. petelotii* and *S. syzygioides*, fresh leaves (2.0 kg each) were subjected to hydrodistillation using a Clevenger-type apparatus. The procedures were described in earlier studies[2]. The pulverised sample of each plant was divided into three equal parts to enable the distillation of essential oils three times. Each of the samples was packed separately inside a 5-L flask. The samples were submerged completely inside the flask with enough quantity of distilled water (3.5-L), which was connected to the Clevenger-type apparatus. The essential oils were allowed to distill for 3 h after the whole apparatus was connected to a heating mantle maintained at normal pressure according to Vietnamese Pharmacopoeia[2]. On completion, the hydrodistilled essential oils were collected separately into clean and previously weighed sample bottles.

Following the normal laboratory procedures[2-7], the essential oils were refrigerated at 4°C till moment of analysis. The yield (%) of the essential oils was calculated separately by dividing the mass (g) of the essential oil over the mass (g) of the pulverized leaves of *S. petelotii* and S. *syzygioides,* as described previously[2].

Analysis of the constituents of the essential oils

The constituents present in the essential oils and the individual percentages were determined using the techniques of gas chromatography (GC) and gas chromatography-mass spectrometry (GC/MS). The procedures and instrumental conditions follow the pattern described in previous studies[2].

The analysis of essential oils by Gas chromatography (GC) method

In the GC analysis of the essential oils, the Gas chromatograph used was HP 7890A Plus. Among the GC components include HP-5MS column which has dimension of 30 m x 0.25 mm and a flame ionization detector (FID). The GC column has a film thickness of 0.25 µm. The GC analytical conditions include the injector and detector temperatures set and maintained at 250°C and 260°C, respectively. To commence the analysis, 1.0 µL of diluted essential oil (1% n-hexane) was injected into the GC column which was done by using the split ratio of 10:1 and the inlet pressure of 6.1 kPa. Helium under the flow rate of 1 mL/min, was used as the carrier gas. The GC analysis was performed under normal pressure. The temperature programmed condition commences from 40°C (held isothermally for 2 min) and rises to 220°C (with 10 min hold). The GC analysis was conducted in triplicate. The individual constituents of each essential oil were quantified using the calibration curves generated from the analyses of representative standard compounds from each class, as reported previously[2].

The analysis of essential oils using Gas chromatography-mass spectrometry (GC/MS)

The experimental procedure in GC/MS analysis involves a coupling of GC chromatograph described above with a HP 5973 MSD Mass spectrometer. All the analytical conditions described for GC above were also employed for the GC/MS experiment. The MS spectral data in the GC/MS procedure was obtained with the set conditions of ionization voltage (70 eV), emission current (40 mA), acquisitions scan mass range (45-350 amu) and sampling rate (1.0 scan/s) as reported previously[2].

Identification of terpenes and non-terpene compounds of the essential oils

The last stage of the analysis was the identification of the individual constituents present in Mass spectral of each essential oil generated from GC/MS. This was accomplished by comparison of the retention indices (RI) of each compound of the essential oils with a homologous series

of n-alkanes (C_6-C_{40}). Also, the Mass spectral data of each essential oil was compared with MS fragmentation patterns of compounds documented in literature[13]. Moreover, the use of co-injection with known compounds under the same GC conditions was also used to identify some compounds[2].

The procedure of antimicrobial activity analysis

The Gram-positive bacteria, Gram-negative bacteria and fungus were all obtained from the Institute of Marine Biochemistry, Vietnam Academy of Science and Technology, Hanoi, Vietnam. The microbes were *Enterococcus faecalis* ATCC 29212, *Staphylococcus aureus* ATCC 25923, *Bacillus cereus* ATCC 14579, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Salmonella enterica* ATCC 13076 and *Candida albicans* ATCC 10231. The testing medium for the bacteria was double-strength Mueller-Hinton broth, while double-strength Sabouraud dextrose broth was used for the fungus. The procedures employed were described previously in our various reports[14,15]. The concentrations of each essential oil of *S. petelotii* and *S. syzygioides* used for the antimicrobial study were determined according to reports of similar studies[14,15]. Essential oils were reported to have displayed antimicrobial activity within the already determined concentration range. The antimicrobial level of the essential oils was evaluated by the microdilution broth susceptibility assay accompanied by the measurement of the minimum inhibitory concentration (MIC) and median inhibitory concentration (IC_{50}) values.

The bacteria were standardized to 5×10^5 CFU/mL while the fungi strength was 1×10^3 CFU/mL, and each grown in specific media. The stock solutions of each essential oils of *S. petelotii* and *S. syzygioides* were prepared in 1% dimethylsulfoxide from 1.6384×10^4 mg/mL to 2^1 mg/mL. The dilute solutions of essential oils and microorganisms were separately placed in 96-well microtiter plates and incubated for 24 h (37°C). The last row of each plates which has only the serial dilutions of the essential oils was used as the negative (no growth) control. The positive controls are streptomycin for antibacterial and nystatin for anticandidal.

After 24 h, the MIC values were evaluated from the wells where the lowest concentration of each of the essential oil completely inhibited the growth of microorganisms, while the IC_{50} values were determined by the percentage of microorganisms that inhibited the microbial growth from the turbidity data of EPOCH2C spectrophotometer (BioTeK Instruments, Inc Highland Park Winooski, VT, USA) and Rawdata computer software (Brussels, Belgium) from the equation:

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

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

where OD is the optical density, control(-) are the cells with medium but without antimicrobial agent, test agent corresponds to a known concentration of antimicrobial agent, control(+) is the culture medium without cells, Highconc/Lowconc is the concentration of test agent at high concentration/low concentration, and Highinh%/Lowinh% is the % inhibition at high concentration/% inhibition at low concentration).

Statistical analysis

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), using Microsoft Excel program 2003.

Results and discussion

The yield of the essential oil from the leaves of *S. petelotii* was 0.16% (w/w). The essential oil of *S. petelotii* was light-yellow coloured. The main classes of compounds present in the leaf essential oil of *S. petelotii* were monoterpene hydrocarbons (16.2%) and sesquiterpene hydrocarbons (71.6%). The oxygenated counterparts were identified in relatively lower amount of 0.1% and 2.8%, respectively. γ-Guaiene (24.1%) was the most abundant compound of the leaf essential oil, along with β-caryophyllene (9.3%), α-pinene (7.8%), β-pinene (6.1%), and

α-zingiberene (5.3%). β-bisabolene (4.0%), *cis*-muurola-3,5-diene (3.0%) and α-selinene (3.0%) were also present in sizeable proportion. The yield of the leaf essential oil of *S. syzygioides* was 0.21% (w/w). The representative classes of compounds of the essential oil are monoterpene hydrocarbons (28.5%), oxygenated monoterpenes (7.1%), sesquiterpene hydrocarbons (15.0%) and oxygenated sesquiterpenes (40.6%). The compounds occurring in higher quantity in the essential oil include caryophyllene oxide (14.8%), α-pinene (10.6%), spathulenol (6.4%), β-pinene (6.6%), o-cymene (4.7%), and humulene epoxide II (4.6%). The chemical constituents of essential oils of *S. petelotii* and *S. syzygioides* were being reported for the first time (Table 1).

It has been postulated that *Syzygium* plants produced essential oils with varying compositional pattern. This is evident from the comparative analysis of the constituents of previously reported essential oils from *Syzygium* plants and present investigated samples. The major constituent of the leaf essential oil of *S. bullockii* was (*E*)-caryophyllene (49.65%), while (*E*)-caryophyllene (23.40%), bicyclogermacrene (21.23%), (Z)-β-ocimene (10.61%), α-humulene (6.33%), were present in *S. tsoongii*[16]. Eugenol was the main compound of *S. aromaticum* from Brazil (52.5%)[8], Vietnam (76.54%)[9] and Nigeria (75.0%)[10]. Additionally, *S. malaccense* from Nigeria consists mainly of limonene (48.8%) and γ-terpinene (26.2%), while α-cadinol (12.7%), juniper camphor (8.2%) and δ-cadinene (5.7%) occurred in *S. samarangense*[10]. The essential oil of *S. cordatum* had a compositional pattern dominated by hexaydrofarnesyl acetate (14.4%), and a non-terpene, 2,3-butanediol diacetate (13.3%)[17]. The composition of essential oil of *S. cumini* from India was found to be dominated by β-caryophyllene (37.65%) and α-humulene (18.37%)[18]. While *S. malaccense* consists of aristolochene (20.06%), γ-himachalene (16.5(%), α-amoprhene (11.79%), myltayl-4(12)-ene (11.43%), viridiflorol (10.67%) and (2*E*,6*E*)-farnesyl acetate (10.10%); β-caryophyllene (17.67%), (*E*, *E*)-α-farnesene (16.10%), caryophyllene alcohol (11.45%), and α-humulene (10.66%) were found in *S. jambos*[19]. The wax of *S. samarangense* from Vietnam produced essential oil whose major constituents were α-pinene (14.02%), ocymene (13.47%), α-cubebene (21.49%), epizonarene (13.10%), β-gurjunene (10.73%), α-selinene (20.11%) and caryophyllene oxide (15.02%) across all samples[11]. The major compounds obtained from the leaf essential oil of *S. benthamianum* grown in India were sitosteryl acetate (11.83%), stigmastan-3,5,22-triene (7.0%), 2,6-dimethyl-2-octene (6.99%), estra-1,3,5(10)-trien-17-β-ol (6.3%), ergosta-4,7,22-trien-3-β-ol (5.19 %), 1-methylcholest-1,3,5(10)-trien-3-ol (5.06%)[20].

As expected the compositional patterns of the two studied essential oils differ from one another due to factors such as the nature of the plant among others. The compositions of these essential oils were compared with data available for some *Syzygium* essential oils previously reported from Vietnam. The abundance of β-caryophyllene has been reported in the majority of *Syzygium* essential oils analysed from Vietnam. This compound occurred in higher quantity in the leaf (25.6%) and stem (29.3%) of *S. grande*[5], the leaves of *S. caryophyllatum* (64.53%)[6], *S. hancei* (9.11%)[6], *S. lineatum* (64.53%)[6], *S. nervosum* (12.1%)[7], *S. bullockii* (49.65%)[16], and *S. tsoongii* (23.40%)[16]. This compound was identified in a much lower amount in *S. syzygioides* The amount of β-caryphyllene (9.3%) was similar to the quantity identified in *S. hancei* (9.11%)[6]. In addition, caryophyllene oxide (14.8%), the main constituents of *S. syzygioides* in this study was also identified as one of the main compounds in the essential oil of the wax of *S. samarangense* (15.02%)[11]. Moreover, some compounds present in higher quantities in previously analysed essential oils of *Syzygium* plants were either present in much lower amount or conspicuously absent in the present investigated oil samples. These includes sabinene in the leaf and stem of *S. grande*[5], (*E*)-β-ocimene in *S. caryophyllatum*[6], (*Z*)-β-ocimene present in *S. nervosum*[7] and *S. tsoongii*[16], eugenol present in *S. aromaticum*[9], bicyclogermacrene identified in *S. tsoongii*[16], as well as α-cubebene, epizonarene, β-gurjunene and α-selinene from the wax of *S. samarangense*[11].

Interestingly, however, γ-guaiene (24.1%) the

Table 1. Constituents of essential oils from the leaves of *S. petelotti* and *S. syzygioides*

Sr. No.	RT (min.)	Compounds[a]	RI[b]	RI[c]	*S. petelotti* [d]	*S. syzygioides* [d]
1	9.76	α-thujene	930	926	-	0.7 ± 0.00
2	10.02	α-pinene	939	932	7.8 ± 0.00	10.6 ±0.11
3	10.50	Camphene	955	946	0.2 ± 0.00	0.3 ± 0.00
4	11.37	β-pinene	984	982	6.1 ± 0.01	6.6 ± 0.00
5	11.60	Myrcene	991	988	0.2 ± 0.00	1.2 ± 0.00
6	12.82	*o*-cymene	1030	1020	0.1 ± 0.00	4.7 ± 0.01
7	12.98	Limonene	1034	1028	1.2 ± 0.00	2.5 ± 0.00
8	13.12	1,8-cineole	1037	1036	-	1.2 ± 0.01
9	13.49	(*E*)-β-ocimene	1048	1044	0.4 ± 0.00	0.2 ± 0.00
10	15.03	Terpinolene	1093	1090	0.2 ± 0.00	-
11	15.19	Rosefuran	1098	1098	-	0.3 ± 0.00
12	15.29	Linalool	1101	1100	-	0.6 ± 0.00
13	15.38	Perillene	1104	1106	-	0.2 ± 0.00
14	15.51	*o*-guaicol	1107	1109	-	0.4 ± 0.00
15	16.38	α-campholenal	1132	1132	-	0.1± 0.00
16	16.92	*cis*-sabinol	1148	1146	-	0.6 ± 0.00
17	17.06	*trans*-sabinol	1152	1152	-	0.4 ± 0.00
18	18.21	Terpinen-4-ol	1185	1185	-	0.6 ± 0.00
19	18.40	p-cymen-8-ol	1190	1190	-	0.6 ± 0.00
20	18.64	α-terpineol	1197	1198	0.1 ± 0.00	0.6 ± 0.00
21	18.86	Methyl chavicol	1203	1202	-	1.3 ± 0.01
22	18.98	Myrtenal	1206	1208	-	0.2 ± 0.00
23	19.40	Verbenone	1219	1219	-	0.2 ± 0.00
24	19.58	*trans*-carveol	1224	1224	-	0.2 ± 0.00
25	21.83	Linaly oxide actetate (pyranoid)	1290	1291	-	0.8 ± 0.00
26	21.95	Bornyl acetate	1293	1293	-	0.5 ± 0.00
27	24.94	α-ylangene	1384	1382	0.2 ± 0.00	0.4 ± 0.00
28	25.09	α-copaene	1388	1390	2.1 ± 0.01	0.6 ± 0.00
29	25.54	*cis*-β-elemene	1403	1402	0.4 ± 0.00	0.8 ± 0.00
30	25.85	Sesquithujene	1412	1414	0.2 ± 0.00	-
31	26.24	*cis*-α-bergamotene	1424	1424	0.6 ± 0.00	0.3 ± 0.00
32	26.41	α-cedrene	1430	1431	0.3 ± 0.00	-
33	26.54	β-copaene	1434	1435	-	0.5 ± 0.00
34	26.60	β-caryophyllene	1436	1437	9.3 ± 0.01	1.6 ± 0.00
35	26.85	β-gurjunene	1444	1442	-	1.0 ± 0.00
36	26.88	*trans*-α-bergamotene	1445	1445	1.1 ± 0.00	-
37	27.05	α-guaiene	1450	1450	0.7 ± 0.00	-
38	27.20	Aromadendrene	1455	1457	-	1.2 ± 0.00
39	27.35	(*Z*)-β-farnesene	1460	1461	2.3 ± 0.01	-
40	27.43	*cis*-muurola-3,5-diene	1462	1462	3.0 ± 0.00	-
41	27.67	α-humulene	1470	1472	1.4 ± 0.00	2.2 ± 0.00
42	27.91	9-*epi*-(*E*)-caryophyllene	1478	1477	0.8 ± 0.00	0.4 ± 0.00
43	27.95	α-acoradiene	1479	1480	0.2 ± 0.00	-
44	28.22	γ-curcumene	1488	1488	0.2 ± 0.00	-

Table 1 cont.

Sr. No.	RT (min.)	Compounds[a]	RI[b]	RI[c]	*S. petelotti* [d]	*S. syzygioides* [d]
45	28.24	γ-muurolene	1489	1489	-	1.2 ± 0.00
46	28.26	β-chamigrene	1490	1490	1.4 ± 0.00	-
47	28.32	ar-curcumene	1491	1491	3.0 ± 0.01	-
48	28.36	α-amorphene	1492	1493	0.6 ± 0.00	0.6 ± 0.00
49	28.70	α-zingiberene	1503	1501	5.3 ± 0.01	-
50	28.84	γ-amorphene	1508	1510	0.2 ± 0.00	-
51	28.95	α-selinene	1511	1513	3.0 ± 0.00	0.6 ± 0.00
52	28.96	α-muurolene	1512	1514	-	1.1 ± 0.01
53	29.12	β-bisabolene	1517	1520	4.0 ± 0.00	-
54	29.26	γ-guaiene	1522	1523	24.1 ± 0.01	-
55	29.46	γ-cadinene	1529	1531	0.3 ± 0.00	0.7 ± 0.00
56	29.60	β-sesquiphellandrene	1530	1532	2.3 ± 0.00	-
57	29.67	δ-cadinene	1536	1537	1.4 ± 0.00	-
58	29.70	*cis*-calamenene	1537	1538	-	1.4 ± 0.00
59	29.76	Aromadendra-4,9-diene	1552	1555	2.9± 0.01	-
60	30.34	α-calacorene	1558	1560	-	0.4 ± 0.00
61	30.64	(*E*)-nerolidol	1568	1571	0.3 ± 0.00	0.3 ± 0.00
62	30.81	β-calacorene	1574	1573	0.3 ± 0.00	-
63	31.20	Palustrol	1587	1587	-	0.5 ± 0.00
64	31.45	Spathulenol	1595	1597	-	6.4 ± 0.01
65	31.68	Caryophyllene oxide	1603	1601	-	14.8 ± 0.01
66	31.70	Guaiol	1604	1602	1.7 ± 0.00	-
67	31.91	Cubeban-11-ol	1611	1613	-	1.4 ± 0.00
68	32.10	Humulene epoxide I	1618	1620	-	0.7 ± 0.00
69	32.14	Rosifoliol	1620	1622	-	0.3 ± 0.00
70	32.42	Humulene epoxide II	1629	1629	-	4.6 ± 0.01
71	32.99	1-*epi*-cubenol	1649	1648	0.2 ± 0.00	2.7 ± 0.00
72	33.02	Humulene epoxide III	1651	1653	-	0.6 ± 0.00
73	33.16	*epi*-α-cadinol	1656	1660	0.2 ± 0.00	1.0 ± 0.00
74	33.21	*epi*-α-muurolol	1657	1658	-	0.4 ± 0.00
75	33.31	α-muurolol (δ-cadinol)	1661	1663	-	0.5 ± 0.00
76	33.58	α-cadinol	1671	1671	-	1.0 ± 0.00
77	33.68	*neo*-intermedeol	1674	1677	0.2 ± 0.00	0.5 ± 0.00
78	33.97	*trans*-calamenen-10-ol	1684	1682	0.2 ± 0.00	2.3 ± 0.00
79	34.05	14-hydroxyl-9-epi-(*E*)-caryophyllene	1687	1689	-	0.7 ± 0.00
80	34.17	Cadalene	1691	1693	-	1.9 ± 0.00
Total					**90.7**	**91.2**
Monoterpene hydrocarbons (Sr. No. 1-7, 9, 10, 13)					**16.2**	**28.5**
Oxygenated monoterpenes (Sr. No. 8, 11, 14, 26)					**0.1**	**7.1**
Sesquiterpene hydrocarbons (Sr. No. 27-60, 62, 80)					**71.6**	**15.0**
Oxygenated sesquiterpenes (Sr. No. 61-79)					**2.8**	**40.6**

[a] Elution order on HP-5MS column; [b] Experimental retention indices; [c] Literature retention indices on HP-5MS column as seen in NIST[18]; [d] means of three replicate values; Sr. No, serial number; RT, retention times on HP-5MS column

most abundant constituent of *S. petelotii* was reported previously as the main constituent of *S. hancei* (11.07%)[6]. This indicates similarity in the main constituent of essential oils of *S. petelotii* and *S. hancei* from Vietnam. Although caryophyllene oxide (14.8%) was the main compound in the essential oil of *S. syzygioides,* its occurrence in high quantity in the leaf essential oils of *S. nervosum* (12.1%)[7], and wax of *S. samarangense* caryophyllene oxide (15.02%)[11] confers some similarity and may be useful in the delineation of the chemotypes of *Syzygium* essential oils.

In addition, the compositional patterns of main compounds of essential oils of *Syzygium* plants from other parts of the world were found to be different from the data reported for *Syzygium* from Vietnam. Several compounds that were characteristics of *Syzygium* plants from other parts of the world were not identified in the Vietnamese grown samples. These include hexaydrofarnesyl acetate and 2,3-butanediol diacetate of *S. cordatum* from India[17], aristolochene, γ-himachalene, myltayl-4(12)-ene, viridiflorol and (2*E*,6*E*)-farnesyl acetate from *S. malaccense* grown in Brazil[19], (*E*,*E*)-α-farnesene found in *S. jambos* from Brazil[19], as well as sitosteryl acetate, stigmastan-3,5,22-triene, 2,6-dimethyl-2-octene, estra-1,3,5(10)-trien-17-β-ol, ergosta-4,7,22-trien-3-β-ol and 1-methylcholest-1,3,5(10)-trien-3-ol *S. benthamianum* grown in India[20]. These variations in the compositional patterns of *Syzygium* essential oils may also be explained by the nature of the plant parts, differences in the ecological and climatic conditions at the points of collection among others.

Antimicrobial activity of the essential oils

The essential oil of *S. petelotii* showed pronounced antimicrobial activity against *P. aeruginosa* and *E. faecalis*, with minimum inhibitory concentration (MIC) values of 16.0 µg/mL and 64.0 µg/mL, respectively. The essential oil of *S. syzygioides* only displayed activity towards *P. aeruginosa* with MIC value of 32.0 µg/mL. Both essential oils exhibited antimicrobial activity towards *B. cereus* (MIC value, 128.0 µg/mL), and anti-candidal action against *C. albicans* with MIC value of 64.0 µg/mL. The essential oils of *S. petelotii* and *S. syzygioides* did not exhibited antimicrobial activity towards the Gram-negative bacteria, *E. coli*, and *S. enterica*.

The antimicrobial susceptibility of any essential oil was attributed to the chemical substances and compounds produced by each plant. The mechanism of the antimicrobial action of essential oils depends solely on the interaction of the hydrophobic components of the compounds of essential oils with the lipids present in the cell membrane of the microorganisms. This will result in the reduction of cell membrane viability, thereby disrupting the functioning of the electron transport chain. In addition, a change in the absorption of nutrients, reduced protein and nucleic acid synthesis, changes in the coagulation of cellular content and the inhibition of enzymes essential for energy metabolism also occur, resulting in cell death[21].

The studies on the mechanisms of the antimicrobial mode of action of essential oil and their constituents have focused on bacteria, with lesser-known reports about their action on yeast and molds. It is well known that Gram-negative bacteria are generally less susceptible than Gram-positive bacteria[22]. This is due to the fact that the outer membrane of Gram-negative bacteria contains hydrophilic lipopolysaccharides (LPS), whereby barriers are created toward macromolecules and hydrophobic compounds. In effect, the Gram-negative bacteria enjoys higher tolerance toward hydrophobic antimicrobial compounds commonly found in essential oils. Because of the nature of their structural patterns, a host of essential oil constituents have several targets of activities. This makes it difficult to predict the susceptibility pattern of a microorganism towards a particular compound and why the susceptibility varies from strain to strain. To predict the mode of action of an essential oil in the antimicrobial testing therefore requires absolute study of the target site of the constituents, mechanism of action and their interactions with the surrounding environment[23,24].

The essential oils of the studied *Syzygium* plants showed antimicrobial activity towards

both Gram-positive (*B. cereus* and *E. faecalis*) and Gram-negative bacteria (*P. aeruginosa*) as seen in Table 2. The antimicrobial activity of essential oils of *S. petelotii* and *S. syzygioides* were being reported for the first time. The observed antimicrobial activity of the studied essential oils reinforces the fact that *Syzygium* essential oils selectively inhibited the growth of different microorganisms. The observed data are comparable to the previously reported antimicrobial activity of other *Syzygium* essential oils. For example, *S. aromaticum* essential oil from Vietnam was found to inhibit five bacteria strains including *B. cereus*, *S. aureus*, *E. coli*, *S. typhimurium* and *P. aeruginosa*[9]. The essential oil of *S. nervosum* only showed antibacterial activity to *E. faecalis* with (MIC value 32 µg/mL) and anti-candidal property towards *C. albicans* (MIC 128 µg/mL)[7], while *S. samarangense* only showed pronounced activity towards *S. aureus*[11]. Undoubtedly, essential oils from *Syzygium* plants can be exploited for their antimicrobial activity.

Pseudomonas aeruginosa, an opportunistic pathogen, is a Gram-negative microorganisms commonly habiting soil, water, and animals. The effect of *P. aeruginosa* in human beings could be seen in the infections of respiratory tract, urinary tract, wounds, and burns etc. It is known to evade treatments by forming biofilms which prevent antibiotics from reaching it. *Staphylococcus aureus* is a Gram-positive pathogen. *S. aureus* is known to cause diseases such as skin infections and systemic failures. *S. aureus* is now known to possess antibiotic resistance[23].

Bacillus cereus is commonly found in food products and dairy products. *B. cereus* is s spore-forming food-borne pathogen and accounted for a proportion of a number of food infection in the world. The damage of the cell was the main factor for potential effect of essential oils in inhibiting the growth of *B. cereus*. The damaged cell leads to reduced cell viability, protein changes, decreased intracellular ATP concentration and other cellular morphological changes[25-27]. *Enterococci* species are Gram-positive anaerobic cocci. They are very resilient by nature and can survive wide variety of hostile environmental over a long period of time. The two common species which are *Enterococcus faecalis* and *Enterococcus faecium* are known as opportunistic pathogens causing nosocomial infections[28]. They are believed to cause a number of ailments such as urinary tract infections, bacteremia, meningitis, wound infections and neonatal complications[29]. Essential oils are reported to have effectively and totally inhibited the biofilm formation of *E. faecalis* by affecting cell adherence and synthesis exopolysaccharide (EPS)[8,29].

It is well known that the constituents present in substances have direct correlation with the observed biological activity of the extracts and essential oils from the plants. Thus the antimicrobial activities of the essential oils of *S. petelotti* and *S. syzygioides* can be correlated with the main compounds or synergy among the

Table 2. Antimicrobial activity of the essential oils from the leaves of *S. petelotti* and *S. syzygioides*

Microorganisms	MIC µg/mL[a]		IC_{50} µg/mL[a]	
	S. petelotti	***S. syzygioides***	***S. petelotti***	***S. syzygioides***
Enterococcus faecalis ATCC29212	64.0±0.00	128.0±0.150	33.23±0.00	65.33±0.10
Staphylococcus aureus ATCC25923	256.0±0.10	128.0±0.10	78.99±0.50	55.45±0.00
Bacillus cereus ATCC14579	128.0±0.05	128.0±0.02	99.67 ±0.10	78.99±0.10
Pseudomonas aeruginosa ATCC27853	16.0±0.00	32.0 ±0.00	3.23±0.00	12.44±0.00
Candida albicans ATCC10231	64.0±0.00	64.0±0.01	33.56±0.01	33.23±0.00
Escherichia coli ATCC25922	-	-	-	-
Salmonella enterica ATCC13076	-	-	-	-

- No activity; [a] Mean value of three replicate assays

other constituents present in the essential oils. The antimicrobial potential of some of these compounds has already been reported. α-Pinene and β-pinene are known for their antibacterial activity[30], while the sesquiterpene, β-caryophyllene showed antibacterial activity against *S. aureus*[31] and antifungal effect[32]. β-Ocimene was described as the component responsible for the inhibitory activity of an essential oil against *S. aureus*[33]. Likewise, caryophyllene oxide and spathulenol have been reported as antimicrobial agent[34]. The antimicrobial potential of γ-guaiene has been documented[35].

Conclusion

The present study revealed that γ-guaiene (24.1%), β-caryphyllene (9.3%), α-pinene (7.8%), and β-pinene (6.1%) were the major compounds of *S. petelotii*, while the main constituents of *S. syzygioides* were caryophyllene oxide (14.8%), α-pinene (10.6%), β-pinene (6.6%) and spathulenol (6.4%). The essential oil of *S. petelotii* showed pronounced antimicrobial activity against *P. aeruginosa* and *E. faecalis*, with MIC values of 16.0 µg/mL and 64.0 µg/mL, respectively. The essential oil of *S. syzygioides* only displayed activity towards *P. aeruginosa* with MIC value of 32.0 µg/mL. Both essential oils exhibited antimicrobial activity towards *B. cereus* with MIC value of 128.0 µg/mL, and anti-candidal action against *C. albicans* with MIC value of 64.0 µg/mL. The observed antimicrobial effects of the essential oils confirm the ethnomedical information of both *S. petelotti* and *S. syzygioides* in the treatment of microbial infections.

Competing interests

The authors declare that no competing interest exists.

Acknowledgements

The authors acknowledged financial support from the Ministry of Education and Training, Vietnam, with grant number B2022-TDV-07.

References

1. **Dao, K.M. (2003).** List of plants in Vietnam, Myrtaceae. Agricultural Publishin House, pg 891-910.
2. **Huong, L.T., Chau, D.T.M., An, N.T.H., Dai, D.N. and Ogunwande, I.A. (2022).** Essential oils of Lauraceae: Antimicrobial activity and constituents of *Phoebe macrocarpa* C. Y. Wu leaf essential oil from Vietnam. J. Essent. Oil Bearing Plants. 25(2): 297-304.
3. **Shono, K., Davies, S.J. and Chua, Y.K. (2007).** Performance of 45 native tree species on degraded lands in Singapore. J. Trop. For. Sci. 19: 25-34.
4. **Soh, W.-K. and Parnell, J. (2015).** A revision of *Syzygium* Gaertn. (Myrtaceae) in Indochina (Cambodia, Laos and Vietnam**).** Adansonia ser. 3: 179-275.
5. **Huong, L.T., Hung, N.V., Chac, L.D., Dai, D.N. and Ogunwande, I.A. (2017).** Essential oils from *Syzygium grande* (Wight) Walp. and *Syzygium sterrophyllum* Merr. et Perry. J. Essent. Oil Bearing Plants. 20(6): 1620-1626.
6. **Khanh, T.H. and Ban, P.H. (2020).** Analysis of essential oils from leaf of *Syzygium hancei* Merr. & Perry, *Syzygium caryophyllatum* (L.) Alston and *Syzygium lineatum* (DC.) Merr. & Perry from Vietnam. J. Essent. Oil Bearing Plants. 23(3): 545-558.
7. **An, N.T.G., Huong, L.T., Satyal, P., T.A. Tai, 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: 1-21.
8. **Teles, A.M., Silva-Silva, J.V., Fernandes, J.M.P., Abreu-Silva, A.L., da Silva, K.C., Filho, N.E.M., Mouchrek, A.N. and Almeida-Souza, F. (2021).** GC-MS characterization of antibacterial, antioxidant, and antitrypanosomal activity of *Syzygium aromaticum* essential oil and eugenol. Evid-Based Complement. Altern. Med. 2021: 1-14 Article ID 6663255.
9. **Toan, T.Q., Inh, C.T., Bich, H.T., Long, P.Q., Ngan, T.T.K. and Ha, P.T.H. (2020).** Chemical composition analysis and evaluation of antibacterial activities

of essential oil from clove (*Syzygium aromaticum*) buds. Asian J. Chem. 32: 1649-1652.

10. **Lawal, O.A., Ogunwande, I.A., Bullem, C.A., Taiwo, O.T. and Opoku, A.R. (2014).** Essential oil composition and *in vitro* biological activities of three *Syzygium* species from Nigeria. In: New Developments in Terpene Research (Jinnan, H. ed.), Nova Science Publisher Inc, New York, pp. 93-112.
11. **Van, H.T., Tran, Q.T., Tran, T.T.H., Tran, N.B., Huynh, N.T., Nguyen, K.B., Nguyen, B.T. and Phan, U.T.X. (2020).** Chemical Constituents and bacterial activity of essential oils of five wax apples (*Syzygium samarangense*) from Dong Thap Province, Vietnam. Agric. Conspec. Sci. 85: 145-152.
12. **Hoang, S. V. (2008).** Uses and conservation of plant species in a National Park-A case study of Ben En, Vietnam. Econ Bot. 62: 574-593.
13. **National Institute of Science and Technology**. **(2018).** NIST Chemistry Webbook. Data from NIST Standard.
14. **Huong, L.T., Huong, T.T., Dai, D.N., and Ogunwande, I.A. (2022).** Chemical compositions, mosquito larvicidal and antimicrobial activities of essential oils of *Hedychium stenopetalum* Lodd. and *Hedychium villosum* Wall. of Zingiberaceae family from Vietnam. J. Essent. Oil Bearing Plants. 25(4): 924-938.
15. **Huong, L.T., Huong, T.T., Bich, N.T., Viet, N.T. and Ogunwande, I.A. (2022).** Chemical compositions, larvicidal and antimicrobial activities of *Zingiber castaneum* (Škorničk. & Q.B. Nguyễn) and *Zingiber nitens* (M.F. Newman) essential oils. Braz. J. Pharm. Sci. 58: 1-17.
16. **Khanh, T.H., Ban, P.H. and Hoi, T.M. (2021).** Chemical composition of essential oils from the leaves of *Syzygium bullockii* and *Syzygium tsoongii* in Ke Go Nature Reserve, Ha Tinh Province. VNU J. Sci. 37: 201-203.
17. **Chalannavar, R., Baijnath, H. and Odhav, B. (2011).** Chemical constituents of the essential oil from *Syzygium cordatum* (Myrtaceae). Afr. J. Biotechnol. 10: 2741-2745.
18. **Sarma, N., Begum, T., Pandey, S. K., Gogoi, R., Munda, S., and Lal, M. (2020).** Chemical composition of *Syzygium cumini* (L.) Skeels leaf essential oil with respect to its uses from North East region of India. J. Essent. Oil Bearing Plants. 23(3): 601-607.
19. **Reis, A.S., de Sousa Silva, L., Martins, C.F. and de Paula, J.R. (2021).** Analysis of the volatile oils of three species of the gender *Syzygium*. Res. Soc. Dev. 10: 21-29.
20. **Deepika, N., Eganathan, P., Sujanapal, P. and Parida, A. (2013).** Chemical composition of *Syzygium benthamianum* (Wt. ex Duthie) Gamble essential oil - an endemic and vulnerable tree species. J. Essent. Oil Bearing Plants. 16: 289-293.
21. **da Silva, D., Bernardes, P.C., Pinheiro, P.F., Fantuzzi, E. and Roberto, C.D. (2021).** Chemical composition, extraction sources and action mechanisms of essential oils: Natural preservative and limitations of use in meat products. Meat Science. 176: 23-47.
22. **Hyldgaard, M., Mygind, T. and Meyer, R. L. (2012).** Essential oils in food preservation: mode of action, synergies, and interactions with food matrix components. Front. Microbiol. 3: 10-27.
23. **Kavanaugh, L.N. and Ribbeck. K. (2012).** Selected antimicrobial essential oils eradicate *Pseudomonas* spp. and *Staphylococcus aureus* biofilms. Appl. Environ. Microbiol. 78: 4057-4061.
24. **Anupama, G., Das, K.K. and Avinash, M. (2019).** Essential oils: A novel source for food preservation. J. Pharmacog. Phytochem. 8: 2098-2101.
25. **Ultee, A., Kets, E.P.W. and Smid, E.J. (2009).** Mechanisms of action of carvacrol on the food-borne pathogen *Bacillus cereus*. Appl. Environ. Microbiol. 65: 4606-4610.
26. **Man, A., Santacroce, L., Iacob, R., Mare, A. and Man, L. (2019).** Antimicrobial activity of six essential oils against a group of human pathogens: A comparative study.

Pathogens. 8: 15-22.
27. **Kang, J., Liu, L., Wu, X., Sun, Y. and Liu, Z. (2018).** Effect of thyme essential oil against Bacillus cereus planktonic growth and biofilm formation. Appl. Microbiol. Biotechnol. 102: 10209-10218.
28. **Anderson, A.C., Jonas, D., Huber, I., Karygianni, l., Woelber, J., Hellwing, E., Arweller, N., Vach, K., Wittmer, A. and Al-Ahmad, A (2016).** *Enterococcus faecalis* from food, clinical specimens, and oral sites: prevalence of virulence factors in association with biofilm formation. Front. Microbiol. 6: 1534-1544.
29. **Adrian, M., Santacroce, L., Romeo, I., Mare, A. and Man, L. (2019).** Antimicrobial activity of six essential oils against a group of human pathogens: A comparative study. Pathogens. 8: 15-32.
30. **Asakawa, Y. (2021).** Dietary Monoterpenoids. In. Handbook of Dietray Phytochemicals. (Xiao, J.; Sarker, S. D.; Asakawa, Y. eds.). Springer, Singapore, vol. 2: 607-622.
31. **Dahham, S.S., Tabana, Y.M., Iqbal, M.A., Ahamed, M.B., Ezzat, M.O., Majid, A.S. and Majid, A.M. (2015).** The anticancer, antioxidant and antimicrobial properties of the sesquiterpene β-caryophyllene from the essential oil of *Aquilaria crassna*. Molecules. 20: 11808-11829.
32. **Sobrinho, A.C.N., de Morais, S.M., de Souza, E.B., Albuquerque, M.R.S., dos Santos, H.S., de Paula Cavalcante, C.S., de Sousa, H.A. and dos Santos Fontenelle, R.O. (2020).** Antifungal and antioxidant activities of *Vernonia chalybaea* Mart. ex. DC. essential oil and their major constituents β-caryophyllene. Braz. Arch. Biol. Technol. 63: 1-17.
33. **Chen, Y.Y., Liu, L., Wang, H., Ma, J.H., Peng, W.X., Li, X.M., Lai, Y., Zhang, B. and Zhangad, D. (2021).** Environmentally friendly plant essential oil: Liquid gold for human health: Adv. Agron. 170: 289-337.
34. **Şener, N., Özkinali, S., Mahmut, G., Kerim, G., Özkan, O.E. and Moustafa, M.K. (2017).** Determination of antimicrobial activity and chemical composition of pimento & ginger essential oil. Ind. J. Pharm. Edu. Res. 51: S230-S232.
35. **Chávez-González, M.L., Rodríguez-Herrera, R. and Aguilar, C.N. (2016).** Essential Oils: A Natural Alternative to Combat Antibiotics Resistance. Chapter 11- In: Antibiotic Resistance, Mechanisms and New Antimicrobial Approaches. Science Publisher, UK. pp. 227-237.