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Essential oil of *Syzygium boisianum* (Gagnep.) Merr. & L.M.Perry: Chemical compositions, antimicrobial activity, and molecular docking

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Abstract

Essential oil obtained from hydrodistillation of the Vietnamese *Syzygium boisanum* leaves was analyzed by the GC/FID-MS (gas chromatography/flame ionization detection-mass spectrometry). Forty-five compounds (90.08%) were namely identified, in which sesquiterpene hydrocarbons (73.07%) were the major chemical class. Among identified compounds, *β*-caryophyllene (18.21%) and bicyclogermacrene (25.47%) were the principal compounds. This essential oil was comparable with the positive control streptomycin in an antimicrobial assay against three Gram-positive bacteria *Enterococcus faecalis* ATCC299212, *Staphylococcus aureus* ATCC25923, and *Bacillus cereus* ATCC14579. Docking study of two major sesquiterpene hydrocarbons *β*-caryophyllene and bicyclogermacrene against bacterial DNA gyrase B showed that these two compounds mainly interacted with hydrophobic residues at the rim of the pocket, thereby they are possible inhibitors.

Keywords. *Syzygium boisanum*, essential oil, sesquiterpene hydrocarbon, antimicrobial activity, molecular docking.

1. INTRODUCTION

Syzygium is a large genus in the family Myrtaceae, containing about 1200 evergreen trees and shrubs. Its native range stretches from Southern Asia to the Pacific to Africa and Madagascar.[1] The fruits of a few species can be consumed fresh or used to make jams and jellies, and many are kept as decorative plants for their lovely glossy foliage.[2] The clove *S. aromaticum*, whose unopened flower buds constitute a significant spice, is the most essential species to the economy.[3] *Syzygium* species that are edible are grown as ornamentals all over the tropics, as well as several of them have spread invasively into some island environments.[1]

A vast number of phytochemical investigations focused on the chemical identification of *Syzygium* essential oils utilizing GC-MS analysis, especially in terms of Vietnamese *Syzygium* plants. For instance, *β*-caryophyllene (> 40.0%) can be seen as the principal compound in the leaf oils of *S. hancei* and *S. lineatum*, collected from Hatinh.[4] This compound (25.6-29.3%) was also characteristic of the essential oils of *S. sterrophyllum* leaf and stem, gathered from Nghe An.[5]

Syzygium boisianum (Gagnep.) Merr. & L.M.Perry, locally named Tram bois, is widely distributed in primary or secondary forests of Vietnam, Thailand, and China.[6] For the first time, the current report provides information on chemical compositions in essential oil of *S. boisianum* leaves, collected from the northern part of central Vietnam. The obtained oil was also used to assess its antibacterial effects. The biological potential is highlighted by the molecular docking study.

2. MATERIALS AND METHODS

2.1. Materials

S. boisanum fresh leaves were collected from Vu Quang Nature park, Ha Tinh, Viet Nam in July 2021. The plant material was identified by our co-author Assoc. Prof. Dr. Le Thi Huong, Vinh university. The herbal specimen SB-2021 has been deposited at Vinh university.

2.2. Hydro-distilled procedure

The fresh leaves (2.0 kg) were chopped into pieces, and then hydro-distilled using a Clevenger-type for 3.5 h, to yield 0.25% of a yellowish oil (w/w). This oil was then dried over anhydrous Na_2SO_4 before analysis.

2.3. GC/FID-MS analysis

The GC analysis of *S. boisanum* leaf oil was carried out using an Agilent Technologies GC HP7890A system [HP-5MS column (5% phenyl, 95% dimethyl polysiloxane; 30 m × 0.25 mm, film thickness 0.25 m)], which was aided by the FID (flame ionization detector).[7] The oven temperature was performed from 50 to 260ºC at a rate of 4 ºC/min, then kept isothermal at 260ºC for 12 min. Temperatures for the detector and injector were held at 280 and 270 ºC, respectively. Carrier gas He was used at a flow rate of 1.0 mL/min, with a split of 1:10.

Similar to the GC-FID procedure, the GC-MS analysis was performed using an Agilent Technologies 7890A GC coupled with the MSD (mass spectrum detector) and the HP-5MS column. Temperature of the detector interface was set at 280 ºC, and the ionization voltage of 70 eV. Acquisition mass was ranged from 50 to 450 amu.

Peak areas obtained through the FID response factor correction were used to determine the relative amounts of each component. By employing the logarithmic equation and the homologous series of *n*-alkanes (C-7 to C-30, Niles, USA) as standards, the retention indices (RIs) were computed from gas chromatograms. The name identification of each compound relied on matching with mass spectra and RIs in the NIST Webbook, and Adams book.[8,9]

2.4. Antimicrobial activity

Antimicrobial effect of *S. boisanum* leaf oil was performed using the broth dilution method.[10] Seven pathogenic bacterial strains were used, including three Gram-positive bacterial strains *Enterococcus faecalis* ATCC299212, *Staphylococcus aureus* ATCC25923, and *Bacillus cereus* ATCC14579, three strains of Gram-negative bacterial strains *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC27853, and *Salmonella enterica* ATCC13076, and one yeast strain *Candida albicans* ATCC 10231.

The selection of investigated concentrations was based on our previous publication,[10] 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 16.384 to 2 µg/mL (2^{14}, 2^{13}, 2^{12}, 2^{11}, 2^{10}, 2^9, 2^7, 2^5, 2^3 and 2^1 µg/mL) in by 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).

2.5. Molecular docking

The docking study was used for predicting the binding affinity of major compounds in essential oil of *S. boisianum* leaves, *viz.* *β*-caryophyllene and bicyclogermacrene, against bacterial DNA gyrase B (PDB ID: 4GEE).[11] Streptomycin was used as a reference drug. The selection and preparation of the protein were carried out following the same procedure previously reported.[7] 3D structures of all the compounds were downloaded from Pubchem database. The docking study was performed using Autodock vina 1.2,[12] setting the grid box center on the binding site with a dimension of 40×40×40 Å. Conformers with the lowest energy value (dG, kcal/mol) were selected for further analysis.

Table 1: Chemical compositions in essential oil of *S. boisianum* leaves

[a]Rt	[b]RI_E	[c]RI_L	Constituents	Classification	Leaves (%)
18.19	1168	1163	Benzenpropanal	Non terpenic compound	0.52
24.35	1348	1335	*δ*-Elemene	Sesquiterpene hydrocarbon	0.78

[a]Rt	[b]RI_E	[c]RI_L	Constituents	Classification	Leaves (%)
24.75	1360	1345	*α*-Cubebene	Sesquiterpene hydrocarbon	0.46
25.61	1387	1374	Isoledene	Sesquiterpene hydrocarbon	0.16
25.71	1390	1374	*α*-Copaene	Sesquiterpene hydrocarbon	0.57
26.12	1402	1387	*β*-Cubebene	Sesquiterpene hydrocarbon	0.61
26.16	1404	1389	*Cis*-*β*-elemene	Sesquiterpene hydrocarbon	0.61
26.87	1420	1409	*α*-Gurjunene	Sesquiterpene hydrocarbon	1.19
27.25	1428	1417	*β*-Caryophyllene	Sesquiterpene hydrocarbon	18.21
27.74	1454	1440	*α*-Maalinene	Sesquiterpene hydrocarbon	0.21
27.85	1457	1443	Aromadendrene	Sesquiterpene hydrocarbon	6.37
27.95	1461	1454	Selina-5,11-diene	Sesquiterpene hydrocarbon	0.33
28.23	1469	1457	*Allo*-aromadendra-4(15),10(14)-diene	Sesquiterpene hydrocarbon	1.68
28.31	1472	1460	*α*-Humulene	Sesquiterpene hydrocarbon	3.16
28.56	1480	1464	9-*Epi*-(*E*)-Caryophyllene	Sesquiterpene hydrocarbon	6.09
28.82	1488	1475	*Trans*-cadina-1(6),4-diene	Sesquiterpene hydrocarbon	0.18
28.90	1491	1477	Eudesma-1,4(15),11-triene	Sesquiterpene hydrocarbon	0.80
29.13	1498	1484	Germacrene D	Sesquiterpene hydrocarbon	0.59
29.34	1505	1489	*β*-Selinene	Sesquiterpene hydrocarbon	0.56
29.41	1507	1490	*Allo*-aromadendr-9-ene	Sesquiterpene hydrocarbon	1.23
29.66	1516	1500	Bicyclogermacrene	Sesquiterpene hydrocarbon	25.47
29.82	1521	1507	Aromadendra-1(10),4(15)-diene	Sesquiterpene hydrocarbon	0.75
29.93	1524	1505	7-*Epi*-eremophila-1(10),8,11-triene	Sesquiterpene hydrocarbon	0.27
30.08	1530	1513	*γ*-Cadinene	Sesquiterpene hydrocarbon	0.31
30.29	1537	1521	*δ*-Cadinene	Sesquiterpene hydrocarbon	1.53
30.34	1538	1522	*Trans*-Calamenene	Sesquiterpene hydrocarbon	0.20
30.42	1541	1528	Zonarene	Sesquiterpene hydrocarbon	0.23
30.62	1548	1533	*Trans*-cadina-1,4-diene	Sesquiterpene hydrocarbon	0.13
30.98	1560	1557	*Trans*-cadinene ether	Sesquiterpene hydrocarbon	0.29
31.06	1563	1564	*β*-Calacorene	Sesquiterpene hydrocarbon	0.10
31.86	1586	1567	Palustrol	Oxygenated sesquiterpene	0.57
32.09	1592	1577	Spathulenol	Oxygenated sesquiterpene	2.33
32.18	1600	1578	Guaiol	Oxygenated sesquiterpene	1.74
32.30	1605	1582	Caryophyllene oxide	Oxygenated sesquiterpene	4.67
32.57	1614	1595	Cubeban-11-ol	Oxygenated sesquiterpene	1.08
32.70	1619	1612	*β*-Biotol	Oxygenated sesquiterpene	0.22
32.89	1626	1616	Ledol	Oxygenated sesquiterpene	1.04
33.05	1631	1619	Humulene epoxide II	Oxygenated sesquiterpene	0.18
33.49	1637	1627	1-*Epi*-cubenol	Oxygenated sesquiterpene	0.31
33.81	1658	1640	*Epi*-*α*-cadinol	Oxygenated sesquiterpene	1.02
33.84	1659	1644	*Epi*-*α*-muurolol	Oxygenated sesquiterpene	0.39
33.95	1663	1668	*α*-Muurolol	Oxygenated sesquiterpene	1.12
34.22	1673	1678	*α*-Cadinol	Oxygenated sesquiterpene	0.65
34.67	1688	1690	(Z)-*α*-*Trans*-bergamotol	Oxygenated sesquiterpene	0.54
45.38	2116	2108	Phytol	Oxygenated diterpene	0.63
			Total		90.08
			Non terpenic compound		0.52
			Sesquiterpene hydrocarbons		73.07
			Oxygenated sesquiterpenes		15.86
			Oxygenated diterpene		0.63

[a]Retention time, [b]Retention indices relative to *n*-alkanes (C_7-C_{30}) on HP-5 MS column, [c]Retention indices from Adam book and the NIST Webbook.

3. RESULTS AND DISCUSSION

The GC/FID-MS analysis of the leaf oil of *S. boisianum* has resulted in the identification of 45 compounds, which accounted for 90.08% (table 1). Sesquiterpene hydrocarbons (29 compounds,

73.07%) were the main chemical class, followed by oxygenated sesquiterpenes (14 compounds, 15.86%). Two remaining compounds, including oxygenated diterpene phytol (0.63%) and non-terpenic compound benzenpropanal (0.53%), were obtained as minor compounds.

Also in table 1, two sesquiterpene hydrocarbons bicyclogermacrene, and *β*-caryophyllene were the principal compounds, which represented 25.47 and 18.21%, respectively. The leaf oil *S. boisianum* was found to be characterized by the presence of several compounds with an amount of greater than 1.00%, consisting of aromadendrene (6.37%), 9-*epi*-(*E*)-caryophyllene (6.09%), caryophyllene oxide (4.67%), *α*-humulene (3.16%), spathulenol (2.33%), *allo*-aromadendra-4(15),10(14)-diene (1.68%), *δ*-cadinene (1.53%), *allo*-aromadendr-9-ene (1.23%), *α*-gurjunene (1.19%), *α*-muurolol (1.12%), cubeban-11-ol (1.08%), ledol (1.04%), and *epi*-*α*-cadinol (1.02%).

As mentioned above, Vietnamese *S. hancei, S. lineatum*, and *S. sterrophyllum* were dominated by *β*-caryophyllene.[4,5] Meanwhile, bicyclogermacrene (21.23%), and *β*-caryophyllene (23.40%) were also characteristics of another Vietnamese *Syzygium*, *S. tsoongii*.[13] The current result indicated a close relationship among these plants, as well as the role of geographic factor.

Table 2: Antimicrobial activity of *S. boisianum*

Microbial strains		Minimum Inhibitory concentration (MIC: µg/mL)		
		Leaf oil	Streptomycin	Nystatin
Gram (+)	*E. faecalis*	256	256	
	S. aureus	128	128	
	B. cereus	128	128	
Gram (-)	*E. coli*	-	256	
	P. aeruginosa	-	256	
	S. enterica	-	256	
Yeasts	*C. albicans*	-		8.0

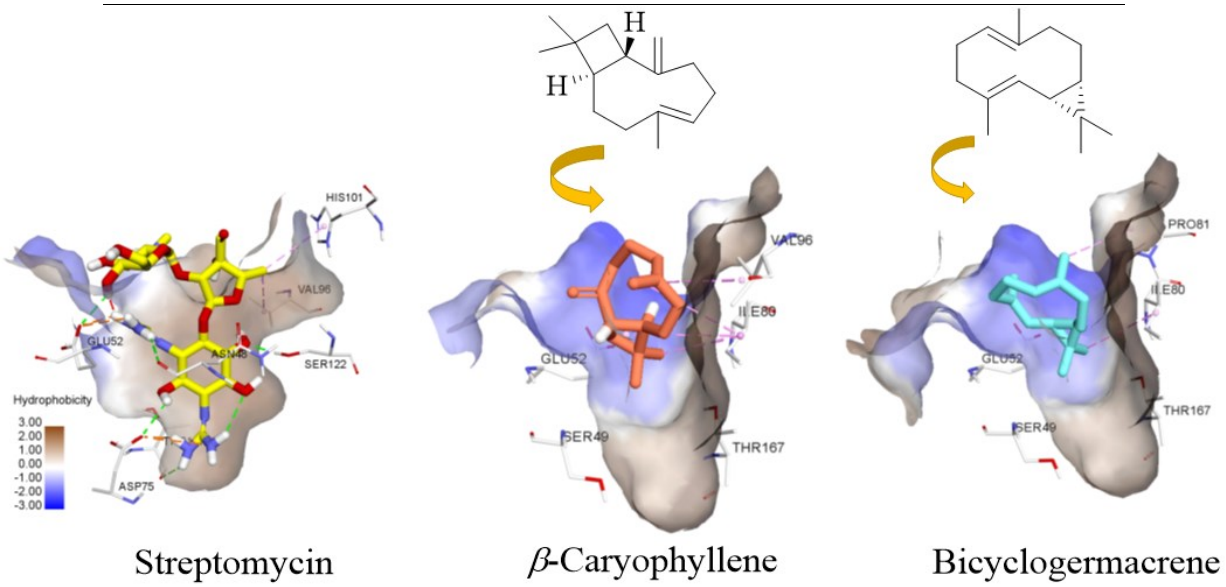

Figure 1: 3D interactions of *β*-caryophyllene and bicyclogermacrene in comparison with streptomycin

This oil was further subjected to antimicrobial assay. Significantly, the essential oil of Vietnamese *S. boisianum* leaf successfully controlled the growth of three Gram-positive bacteria *E. faecalis*, *S. aureus*, and *B. cereus* with the MIC values of 256, 128, and 128 µg/mL, respectively. Obviously, these values were equivalent to those of the standard streptomycin (table 2).

Nazzaro et al suggested that antimicrobial activity of *S. aromaticum* essential oil was due to the action of its major compound eugenol.[14] The current study also highlights antimicrobial activity of Vietnamese *S. boisianum* leaf by molecular docking approach, mostly based on the behavior of its two major sesquiterpene hydrocarbons *β*-caryophyllene and bicyclogermacrene.

We continued examining the inhibitory activity of these two compounds against an important target of Gram-positive bacteria, DNA gyrase B (4GEE). To do so, in the first step, we developed and validated a docking protocol by redocking the co-crystal ligand of 4GEE. As the result, the re-docked and co-crystal ligands were highly overlapped (r.m.s.d of 0.651Å) with dG of -8.2 kcal/mol, suggesting the validity of the docking method developed. The next steps were to dock two compounds *β*-caryophyllene and bicyclogermacrene, and streptomycin into the binding site of DNA gyrase B. As can be seen in figure 1, streptomycin easily penetrated deep inside into the binding pocket of DNA gyrase with a complex interaction network with residues of the target. There are 5 H-bonds formed streptomycin towards Asn48, Glu52, Asp75, Ser122, and Thr167. The binding energy was -7.6 kcal/mol. In contrast, *β*-Caryophyllene and bicyclogermacrene were more difficult to bind with DNA gyrase B compared to streptomycin. These compounds, being more lipophilic than streptomycin, could not interact with the residues at the bottom of the pocket. The only interaction encountered was pi-alkyl stacking interaction with hydrophobic residues such as Ile80, Pro61, and Val96 which are located at the rim of the pocket (figure 1). In addition, *β*-caryophyllene showed binding energy with dG of -6.3 kcal/mol, slightly better than bicyclogermacrene (dG of -5.5 kcal/mol). Overall, these two compounds could be considered as DNA gyrase inhibitors with a lower binding affinity.

4. CONCLUSION

For the first time, chemical compositions of the leaf oil of the Vietnamese *S. boisianum* were identified by the GC/FID-MS analysis. Forty-five compounds were identified, representing 90.08%. This leaf oil was dominated by two sesquiterpene hydrocarbons *β*-caryophyllene (18.21%) and bicyclogermacrene (25.47%). With the MIC values of 128-256 µg/mL, this essential oil was comparable with the positive control streptomycin against three Gram-positive bacteria *E. faecalis*, *S. aureus*, and *B. cereus*. Docking study was performed for two major sesquiterpenes *β*-caryophyllene and bicyclogermacrene against bacterial DNA gyrase B. As the results, these compounds showed appropriate interactions with the residues in the binding site of DNA gyrase.

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