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Research Article

The leaf essential oils of *Meiogyne virgata* (Blume) Miq., *M. vietnamica* Jaikhamseub, T.A.Le & Chaowasku, and *Orophea polycarpa* A.DC.: Chemical composition, antimicrobial activity, molecular docking, and toxicity profiling

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Abstract
The present study aims to describe the chemical composition of essential oils from three Vietnamese Annonaceae species, *Meiogyne virgata*, *M. vietnamica*, and *Orophea polycarpa*, and their antimicrobial activity. Essential oils were extracted by hydro-distillation. From the GC-FID/MS (gas chromatography-flame ionization detection and mass spectrometry) analysis, the leaf essential oil of *M. virgata* was reported to contain the major compound germacrene D (42.48%). Spathulenol (24.51%), bicyclogermacrene (18.58%), β-selinene (11.14%), and germacrene D (8.20%) can be seen as the main compound in *M. vietnamica* leaf essential oil. α-Phellandrene (39.35%), bicyclogermacrene (13.07%), β-phellandrene (10.87%), and limonene (8.27%) represented *O. polycarpa* leaf essential oil. Essential oil of *M. virgata* leaves exhibited strong antimicrobial activity against the Gram (+) bacterium *Bacillus subtilis* ATCC 5230, the Gram (-) bacterium *Escherichia coli* ATCC 8739, and the fungus *Aspergillus niger* ATCC 9587 with the same MIC value of 16 µg/mL. The docking method showed that germacrene D has the $\Delta G_{binding}$ (binding affinity) values of –6.68, –5.265, and –5.602 kcal/mol with the proteins *E. coli* DNA gyrase, *B. subtilis* TasA, and *A. niger* PhyA, respectively. Alkyl and pi-alkyl interactions are the main contributors to the binding affinity between the studied proteins and ligands. Furthermore, germacrene D is predicted to have low toxicity and is not active against any of the considered organ targets.

Keywords
Meiogyne virgata, *Meiogyne vietnamica*, *Orophea polycarpa*, Essential oil, Antimicrobial.

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INTRODUCTION

The family *Annonaceae*, also known as the custard apple family or soursop family, contains about 108 accepted genera and 2400 flowering plants[1]. *Annonaceae* species are widely distributed in the tropics, and few species are found in temperate areas[2]. Almost all of the plants in this family are odoriferous, due to the appearance of essential oils, which of several species have been used in the cosmetics and perfumery industries, and for medicinal purposes[3]. It should be noted that the primary chemical compounds in the fruits and seeds of *Annonaceae* plants are monoterpene hydrocarbons while sesquiterpene hydrocarbons are predominant in the leaves, and oxygenated sesquiterpenes are highly concentrated on the barks and roots[4].

The genus *Meiogyne* contains about 33 species, and its distribution ranges from India and Indochina to Australia[5]. Studies on the

chemical compositions of *Meiognye* essential oils were only emphasized on Vietnamese species, to date. For instance, the essential oils of *M. monogyna* leaves and stems were characterized by bicyclogermacrene (38.1-49.3%), β-caryophyllene (15.0-16.8%), and linalool (6.6-8.0%)[6]. δ-Cadinene (36.6-49.3%) acted as the major compound in the leaf and stem essential oils of *M. hainanensis*[6]. The genus *Orophea* is native to Southeast Asia, consisting of about 60 species[7]. Phytochemical investigations on this genus were recorded on the isolations of secondary metabolites, but the results in essential oil compositions have not yet been carried out. The current research focuses on describing the chemical identification and antimicrobial effects of essential oils from the leaves of three Vietnamese Annonaceae species, *M. virgata*, *M. vietnamica*, and *O. polycarpa*. The obtained experiments have been further aided by *in silico* approach.

Materials and Methods

Plant material

The fresh leaves of studied plants (*M. virgata* at 20°31'17"N and 105°4'58"E, *M. vietnamica* at 19°26'45"N and 104°54'30"E, and *O. polycarpa* at 19°23'41"N and 104°57'4"E) were collected from Pu Luong Nature Reserve, Thanh Hoa, Vietnam in December 2023. The voucher specimens DN-01, DN-02, and DN-03 were assigned to *M. virgata*, *M. vietnamica*, and *O. polycarpa*, respectively, and were deposited at Nghe An University of Economics.

Hydro-distillation procedure

The fresh leaves of each plant (2.0 kg) were cut into small pieces and hydrodistilled using a Clevenger-type apparatus for 2.5 h. Before being utilized for analysis and the bioactive tests, the obtained essential oils were dried over Na_2SO_4 and kept in a sealed glass vial at 5°C. The mean yield was calculated following the dried weight material, including DN-01, 0.23% v/w (dry weight); DN-02, 0.45% v/w; and DN-03, 0.36% v/w.

GC-FID and GC-MS analyses

The GC-FID analysis was carried out using a Shimadzu GC2010 with the FID detector[8-10]. The HP5-MS column (30 x 0.25 mm, 0.25 m film thickness) was used. Operating conditions included: column temperature rising from 50 to 250°C at 4°C/min, and then held at 250°C for 4 min; the helium (99.999%) was used as a carrier gas with a 1.0 mL/min flow rate; injection volume, 0.1 µL (split ratio of 1:20); and the injector and detector temperatures = 250 and 270°C, respectively. The relative percentage of each component in essential oil was obtained by the normalized peak area (%), and displayed as the mean of three replicates.

The GC-MS analysis was carried out by a Shimadzu GC2010. The column was an HP5-MS fused silica capillary one (30 m x 0.25 mm i.d. x 0.25 µm film thickness). The EI (electron ionization) mode happened at 70 eV. Helium was employed as a carrier gas at a flow rate of 1.0 mL/min. The injection volume was 0.1 µL (split ratio of 1:20). Injector and ion-source temperatures were established at 250 and 270°C, respectively. The oven temperature program was the same as the one used for the GC. Mass spectra were taken at a scan interval of 0.5 s, in a mass range from 50 to 550 Da. Identification of constituents in essential oils was based on their RI (retention indices) on an HP-5MS capillary column, under the same operating conditions as those used in the GC-FID analysis, involving a homologous series of *n*-alkanes (C7-C30). Chemical structures were matched with the W09N08 library, Adams book[11], and NIST Chemistry WebBook[12].

Antimicrobial assay

The pathogenic ATCC (American Type Culture Collection) strains, consisting of three Gram (+) bacteria *Bacillus subtilis* ATCC 5230, *Staphylococcus aureus* ATCC 33591 and *Clostridium sporogenes* ATCC 7955, two Gram (–) bacteria *Escherichia coli* ATCC 8739 and *Pseudomonas aeruginosa* ATCC 27853, three fungi *Aspergillus brasiliensis* ATCC 9642, *A. niger* ATCC 9587 and *Fusarium oxysporum* ATCC 11739, and two yeasts *Candida albicans* ATCC 12354 and *Saccharomyces cerevisiae* ATCC 4078, have been used in this study. All pathogenic strains were cultured on Muller

Hilton Agar (MHA, Merck) plates for 24 h at 37°C. The protocol was identical to our previous publication (Supplementary data)[13].

Molecular docking and toxicity prediction

In this study, the selected target proteins represent bacteria *E. coli*, *B. subtilis*, and the fungus *A. niger*. These protein structures were obtained from the RCSB PDB database with PDB ID codes 6F86, 5OF2, and 3K4Q, respectively[14-17]. They were then processed by removing water molecules, co-crystallized molecules, adding hydrogen, estimating Kollman charges, and converting the structure files to *pdbqt format using AutoDockTools software. For ligand preparation, germacrene D was downloaded from the PubChem database in *sdf format (3D Standard Data Format). Avogadro2 software was used to optimize the energy using the MMFF94s force field and to convert the *sdf format to *pdb format[18]. The structure of this compound was then imported into AutoDockTools v1.5.6 to prepare the ligand by adding Gasteiger charges. To perform docking, the grid box parameters were set based on the active site of the specific proteins under study: 6F86 (X = 62.4, Y = 28.2, Z = 64.7, and X x Y x Z = 24 x 24 x 24), 5O2F (X = –11.0, Y = –1.9, Z = –23.2, and X x Y x Z = 30 x 30 x 30), 3K4Q (X = 27.8, Y = -30.2, Z = 6.8, and X x Y x Z = 24 x 24 x 24), and the exhaustiveness parameter was set to 400. AutoDock Vina v1.2.3 was used for all molecular docking simulations, and Discovery Studio Visualizer software was used to represent the protein-ligand interactions[19,20].

Results and Discussion

Chemical analysis

Hydro-distillation of the fresh leaves of *M. virgata* resulted in a yellow oil with a yield of 0.23% v/w. By the GC-FID/MS analysis, a total of 41 compounds were identified, which accounted for 90.66% (Table 1 and Fig. S1). The obtained essential oil was dominated by sesquiterpene hydrocarbons (74.72%), followed by their oxygenated derivatives (11.9%) and non-terpenic compounds (4.04%). Germacrene D was the major compound with 42.48%. Several analogous compounds have possessed significant percentages, such as bicyclogermacrene (6.61%), spathulenol (6.03%), α-copaene (4.19%), β-cubebene (4.18%), δ-cadinene (2.40%), and γ-muurolene (2.02%). The major compound in this study, germacrene D, was absent in *M. virgata* leaf essential oil, collected from Bach Ma National Park, Thua Thien Hue, Vietnam[21]. It reflected a great role of geographic factors, time collection, and extraction methods.

M. vietnamica was recently described as a new species, which can be found in central Vietnam[5]. The current study provides first information regarding the chemical analysis of its essential oil. Hydro-distilled extraction of its fresh leaves gave a yellow essential oil (0.45% v/w yield). This sample was found to contain 21 identified compounds, which represented 87.82% (Table 1 and Fig. S2). The obtained essential oil was associated with the presence of two main chemical classes, sesquiterpene hydrocarbons (59.87%) and their oxygenated derivatives (27.95%). Spathulenol (24.51%), bicyclogermacrene (18.58%), β-selinene (11.14%), and germacrene D (8.20%) were the principal agents. Some other compounds also possessed remarkable percentages, such as (*E*)-caryophyllene (5.08%), *cis*-β-elemene (4.05%), and aromadendrene (1.92%). Various compounds have been only found in *M. virgata*, but absent in *M. vietnamica*, and *versus* (Table 1). The percentage of germacrene D drastically decreased from *M. virgata* to *M. vietnamica*, but that of spathulenol and bicyclogermacrene was in contrast. β-Selinene was absent in *M. virgata*. Considering *O. polycarpa*, its leaf essential oil was recorded to consist of 31 identified compounds, which accounted for 98.16% (Table 2 and Fig. S3). The main chemical classes in this essential oil have included monoterpene hydrocarbons (70.66%), and sesquiterpene hydrocarbons (24.04%). In the meantime, their oxygenated derivatives and non-terpenic compounds have occurred occasionally with less than 3.00%. α-Phellandrene (39.35%), bicyclogermacrene (13.07%), β-phellandrene (10.87%), and limonene (8.27%) were the primary compounds. The percentage of some

Table 1. Essential oil compositions (%) in the leaves of *M. virgata* and *M. vietnamica*

No	Constituents	[a]Rt	[b]RI_E	[c]RI_L	*M. virgata*	*M. vietnamica*	Identification
1	2-Butylfuran	8.29	885	890	0.35	-	RI and MS
2	Safrole	21.93	1298	1285	1.95	-	RI and MS
3	δ-Elemene	23.58	1347	1335	0.12	0.28	RI and MS
4	α-Cubebene	23.99	1360	1345	1.25	0.60	RI and MS
5	Cyclosativene	24.69	1381	1363	1.96	-	RI and MS
6	α-Copaene	24.95	1389	1374	4.19	1.79	RI and MS
7	β-Bourbonene	25.29	1399	1387	1.22	1.42	RI and MS
8	β-Cubebene	25.37	1402	1391	4.18	1.42	RI and MS
9	*cis*-β-Elemene	25.41	1403	1395	-	4.05	RI and MS
10	Ylanga-2,4(15)-diene	25.93	1419	1405	0.15	-	RI and MS
11	α-Gurjunene	26.09	1424	1408	-	0.75	RI and MS
12	β-Ylangene	26.40	1434	1416	0.47	-	RI and MS
13	(*E*)-Caryophyllene	26.45	1436	1417	1.59	5.08	RI and MS
14	β-Gurjunene	26.71	1444	1431	0.99	1.37	RI and MS
15	Aromadendrene	27.07	1456	1439	0.27	1.92	RI and MS
16	*cis*-Muurola-3,5-diene	27.38	1465	1448	0.15	-	RI and MS
17	α-Humulene	27.53	1470	1452	0.52	0.79	RI and MS
18	9-epi-(*E*)-Caryophyllene	27.77	1478	1464	-	0.30	RI and MS
19	*cis*-Muurola-4(14),5-diene	27.80	1479	1465	0.24	-	RI and MS
20	*trans*-Cadina-1(6),4-diene	28.08	1488	1475	0.27	-	RI and MS
21	γ-Muurolene	28.18	1491	1478	2.02	1.57	RI and MS
22	Germacrene D	28.47	1500	1484	42.48	8.20	RI and MS
23	β-Selinene	26.61	1505	1489	-	11.14	RI and MS
24	Asaricin	28.68	1507	1495	1.74	-	RI and MS
25	*trans*-Muurola-4(14),5-diene	28.78	1510	1497	1.33	-	RI and MS
26	Bicyclogermacrene	28.88	1514	1500	6.61	18.58	RI and MS
27	δ-Amorphene	29.09	1521	1511	0.18	-	RI and MS
28	γ-Cadinene	29.35	1529	1513	0.83	0.23	RI and MS
29	δ-Cadinene	29.56	1536	1522	2.40	0.38	RI and MS
30	Zonarene	29.69	1541	1528	0.44	-	RI and MS
31	*trans*-Cadina-1,4-diene	29.88	1547	1533	0.19	-	RI and MS
32	α-Cadinene	30.03	1552	1537	0.21	-	RI and MS
33	α-Calacorene	30.24	1559	1544	0.24	-	RI and MS
34	Salviadienol	30.55	1570	1549	0.35	-	RI and MS
35	Germacrene B	30.75	1576	1559	0.22	-	RI and MS
37	Mintoxide	31.05	1586	1570	0.34	1.56	RI and MS
38	Spathulenol	31.38	1597	1577	6.03	24.51	RI and MS
39	Caryophyllene oxide	31.57	1604	1589	1.35	1.88	RI and MS

Table 1 ***cont***.

No	Constituents	[a]Rt	[b]RI_E	[c]RI_L	*M. virgata*	*M. vietnamica*	Identification
40	Salvial-4(14)-en-1-one	31.85	1614	1599	0.84	-	RI and MS
41	1-*epi*-Cubenol	32.77	1646	1638	0.69	-	RI and MS
42	*epi*-α-Cadinol	33.11	1658	1645	0.87	-	RI and MS
43	*epi*-α-Muurolol	33.16	1660	1650	0.73	-	RI and MS
44	α-Muurolol	33.25	1663	1651	0.34	-	RI and MS
45	α-Cadinol	33.53	1673	1652	0.36	-	RI and MS
Total					90.66	87.82	
Sesquiterpene hydrocarbons					74.72	59.87	
Oxygenated sesquiterpenes					11.9	27.95	
Non-terpenic compounds					4.04	-	

The MS spectrum of each compound was based on the EI-MS mode

Table 2. Essential oil compositions (%) in the leaves of *O. polycarpa*

No	Constituents	[a]Rt	[b]RI_E	[c]RI_L	*O. polycarpa*	Identification
1	α-Thujene	9.53	930	924	0.24	RI and MS
2	α-Pinene	9.79	939	932	2.64	RI and MS
3	Sabinene	10.98	978	969	0.85	RI and MS
4	β-Pinene	11.15	984	974	0.31	RI and MS
5	6-Methylhept-5-en-2-one	11.25	988	987	0.50	RI and MS
6	Myrcene	11.40	992	988	2.28	RI and MS
7	α-Phellandrene	12.02	1012	1002	39.35	RI and MS
8	3-δ-Carene	12.19	1016	1008	0.15	RI and MS
9	o-Cymene	12.65	1030	1022	4.57	RI and MS
10	Limonene	12.81	1035	1024	8.27	RI and MS
11	β-Phellandrene	12.87	1036	1025	10.87	RI and MS
12	(*E*)-β-Ocimene	13.31	1049	1044	1.13	RI and MS
13	Perillene	15.68	1118	1110	0.14	RI and MS
14	δ-Elemene	23.58	1347	1335	0.44	RI and MS
15	α-Copaene	24.93	1388	1374	0.24	RI and MS
16	Bourbon-7-ene	25.11	1394	1384	0.19	RI and MS
17	*cis*-β-Elemene	25.40	1403	1389	0.71	RI and MS
18	(*E*)-Caryophyllene	26.45	1436	1417	3.77	RI and MS
19	Guaia-6,9-diene	27.08	1456	1447	0.60	RI and MS
20	α-Humulene	27.54	1471	1469	2.49	RI and MS
21	Germacrene D	28.37	1497	1487	1.24	RI and MS
22	β-Selinene	28.56	1503	1491	0.33	RI and MS
23	Bicyclogermacrene	28.89	1514	1503	13.07	RI and MS
24	β-Bisabolene	28.98	1517	1508	0.82	RI and MS
25	δ-Cadinene	29.55	1536	1530	0.14	RI and MS
26	(*E*)-Nerolidol	30.56	1570	1561	0.14	RI and MS
27	Dendrolasin	30.95	1583	1574	0.28	RI and MS

Table 2 ***cont***.

No	Constituents	[a]Rt	[b]RI_E	[c]RI_L	*O. polycarpa*	Identification
28	Spathulenol	31.36	1597	1579	1.68	RI and MS
29	Cubeban-11-ol	31.82	1613	1599	0.18	RI and MS
30	*epi*-*α*-Cadinol	33.09	1658	1648	0.32	RI and MS
31	*α*-Muurolol	33.24	1663	1659	0.22	RI and MS
Total					98.16	
Monoterpene hydrocarbons (Sr. no. 1-7, 9, 10)					70.66	
Oxygenated monoterpenes (Sr. no. 8, 11-22)					0.14	
Sesquiterpene hydrocarbons (Sr. no. 23-26)					24.04	
Oxygenated sesquiterpenes					2.82	
Non-terpenic compounds					0.50	

The MS spectrum of each compound was based on the EI-MS mode

other compounds exceeds 1.00%, consisting of *o*-cymene (4.57%), (*E*)-caryophyllene (3.77%), α-pinene (2.64%), α-humulene (2.49%), myrcene (2.28%), spathulenol (1.68%), germacrene D (1.24%), and (*E*)-β-ocimene (1.13%). To date, this is the first time that essential oils of the genus *Orophea* have been studied.

Antimicrobial activity

Three studied essential oils have been subjected to antimicrobial assay, and the results are outlined in Table 3. *M. virgata* leaf essential oil strongly inhibited the Gram (+) bacterium *B. subtilis* with the MIC value of 16 µg/mL, as compared with that of *M. vietnamica* (MIC 32 µg/mL), and the standard streptomycin (MIC 4 µg/mL). Both two *Meiognye* essential oils showed the same MIC values of 128 and 256 µg/mL against the Gram (+) bacteria *C. sporogenes* and *S. aureus*, respectively. The leaf essential oil of *M. virgata* with the MIC values of 16-32 µg/mL was better than the leaf essential oil of *M. vietnamica* (MIC 64-128 µg/mL) in antimicrobial assay against the Gram (–) bacteria *E. coli* and *P. aeruginosa*.

In the case of fungi, again, *M. virgata* leaf essential oil was strongly active against the fungus *A. niger* with a MIC value of 16 µg/mL, in comparison with that of *M. vietnamica* (MIC 32 µg/mL), and the standard nystatin (MIC 8 µg/mL). Both two *Meiognye* samples showed the same MIC values of 128 and 256 µg/mL against the fungus *A. brasiliensis* and *C. albicans*, respectively, but they failed to control two analogous strains *F. oxysporum* and *S. cerevisiae* (MIC > 512 µg/mL). *O. polycarpa* leaf essential oil exhibited antibacterial activity against the Gram (+) bacterium *B. subtilis* with a MIC value of 256 µg/mL, but did not show inhibitory activity against the remaining pathogenic strains (Table 2).

Docking results and toxicity profiles

It is assumed that the antimicrobial activity of the essential oil of *M. virgata* leaves is due to the great role of its major compound, germacrene D. Hence, in this section, the evaluations of the affinities and interaction modes between germacrene D and the essential proteins DNA gyrase, TasA, and PhyA of *E. coli*, *B. subtilis*, and *A. niger*, respectively, were performed. The proteins used as molecular targets are essential for the metabolism of these microorganisms and were used as targets for natural and synthetic products against these pathogens[14-17]. Before the docking process, a successful redocking process was conducted to verify the docking protocol by calculating the RMSD value between the native ligand and the re-docked ligand (Fig. S4). As a result, the RMSD value of the co-crystallized ligand in the representative protein 6F86 was 1.61771 Å < 2 Å, indicating the high reliability of the protocol[22]. The binding affinity ($\Delta G_{binding}$)

Table 3. Antimicrobial activity of the studied essential oils

Microbial strains		Minimum inhibitory concentration (MIC: mg/mL)					
		M. virgata	***M. vietnamica***	***O. polycarpa***	**Streptomycin**	**Tetracycline**	**Nystatin**
Gram (+)	*B. subtilis*	16	32	256	4		
	C. sporogenes	128	256	>512	8		
	S. aureus	128	256	>512	8		
Gram (–)	*E. coli*	16	128	>512		4	
	P. aeruginosa	32	64	>512		4	
Fungi	*A. niger*	16	32	>512			8
	A. brasiliensis	128	128	>512			8
	F. oxysporum	>512	>512	>512			8
Yeasts	*C. albicans*	256	256	>512			4
	S. cerevisiae	>512	>512	>512			8

and amino acid interactions of the compound germacrene D and reference inhibitors with the selected bacterial and fungal target proteins are presented in Table 4. The $\Delta G_{binding}$ of germacrene D with the proteins DNA gyrase, TasA, and PhyA were –6.68, –5.265, and –5.602 kcal/mol, respectively. In comparison, the control compound streptomycin exhibited binding affinities with DNA gyrase and TasA of –6.871 and –5.733 kcal/mol, respectively, and cycloheximide had a $\Delta G_{binding}$ value of –6.277 kcal/mol with PhyA (Fig. S5). The docked structure was analyzed for the interactions of the germacrene D compound with the amino acid residues of the studied proteins as observed in Fig. 1-3. Specifically, germacrene D formed alkyl interactions with the residues Ile94, Ile78, Val167, and Ala47 of the DNA gyrase protein. Notably, Ile94 and Ile78 are considered important amino acids for this protein. For the TasA protein, germacrene D established alkyl and pi-alkyl interactions with Phe70, Ala53, Val55, Leu66, and Lys68 residues. Additionally, these interactions were formed with the residues Lys278, Lys277, His282, Ala201, and Phe195 of the PhyA protein. Furthermore, a toxicological feature of germacrene D was predicted using the ProTox 3.0 web server (Table 5). The result showed that the LD_{50} value of this compound was 5300 mg/kg, which was higher than that of streptomycin (LD_{50} = 500 mg/kg) and cycloheximide (2 mg/kg). This is classified as toxicity class 5 according to the Globally Harmonized System classification[23]. It showed that germacrene D has low oral toxicity. In addition, the toxicity of this compound to the organ was considered. Interestingly, germacrene D was inactive against all considered targets, including hepatotoxicity, neurotoxicity, nephro-toxicity, respiratory toxicity, and cardiotoxicity, with calculated probability (p) values of 0.8, 0.51, 0.89, 0.71, and 0.71, respectively. This indicates that germacrene D is estimated to have no toxic effects on the targets, with notable predicted p-values greater than 0.5. Therefore, this potential compound needs to be verified and further tested in the future.

Table 4. The obtained docking results of germacrene D with the studied proteins

Compounds	Target Proteins	Binding affinity (ΔG, kcal/mol)	Alkyl and pi-alkyl interactions
Germacrene D	DNA gyrase (PDB ID: 6F86)	–6.68	Ile94, Ile78, Val167, Ala47
	TasA (PDB ID: 5OF2)	–5.265	Phe70, Ala53, Val55, Leu66, Lys68
	Phytase (PDB ID: 3K4Q)	–5.602	Lys278, Lys277, His282, Ala201, and Phe195
Cycloheximide	Phytase (PDB ID: 3K4Q)	–6.277	-
Streptomycin	DNA gyrase (PDB ID: 6F86)	–6.871	-
	TasA (PDB ID: 5OF2)	–5.733	Lys201

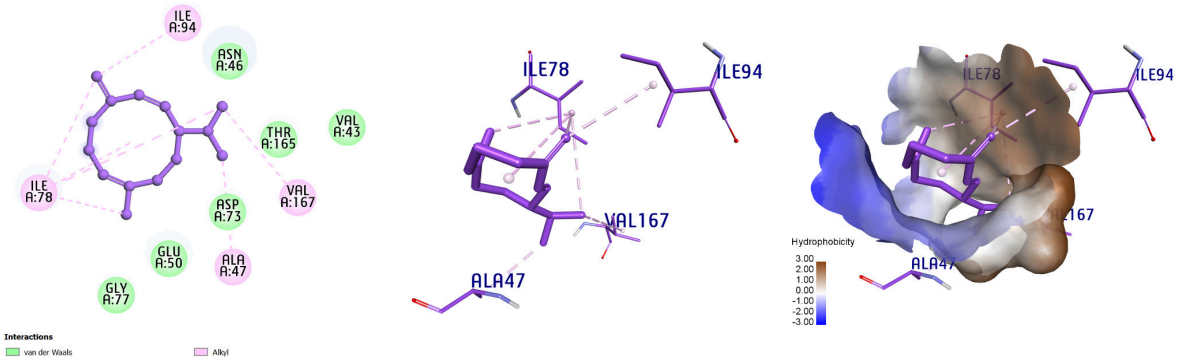

Figure 1. 2D and 3D interactions of germacrene D with the amino acid residues of the *E. coli* DNA gyrase protein (PDB ID: 6F86)

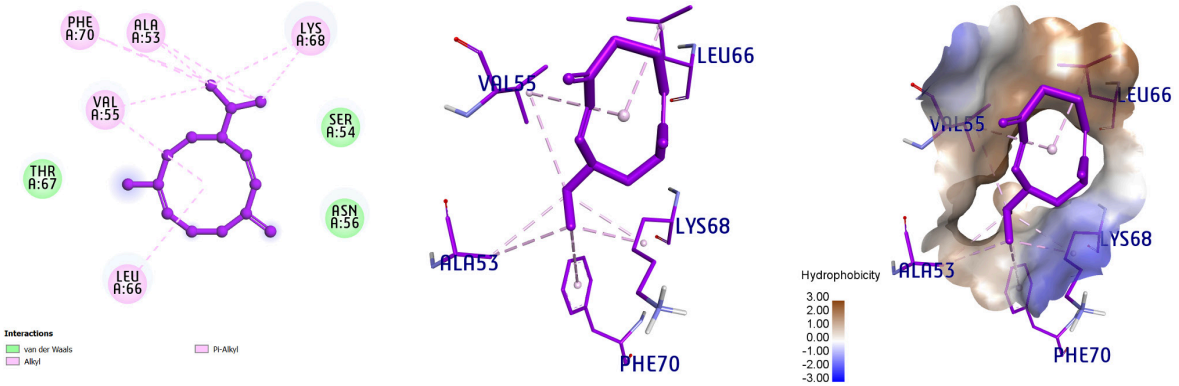

Figure 2. 2D and 3D interactions of germacrene D with the amino acid residues of the *B. subtilis* TasA protein (PDB ID: 5OF2)

Conclusion

The current research provides a chemical analysis of three Annonaceae essential oils, collected from Vietnam. Generally, monoterpene hydrocarbons, sesquiterpene hydrocarbons, and their oxygenated derivatives are the main chemical classes. *M. virgata* leaf essential oil was rich in germacrene D, whereas *M. vietnamica* leaf essential oil was dominated by spathulenol, bicyclogermacrene, β-selinene, and germacrene D. Meanwhile, the leaf essential oil of *O. polycarpa* was characterized by α-phellandrene, bicyclogermacrene, β-phellandrene, and limonene. *Meiognye* essential oil showed good

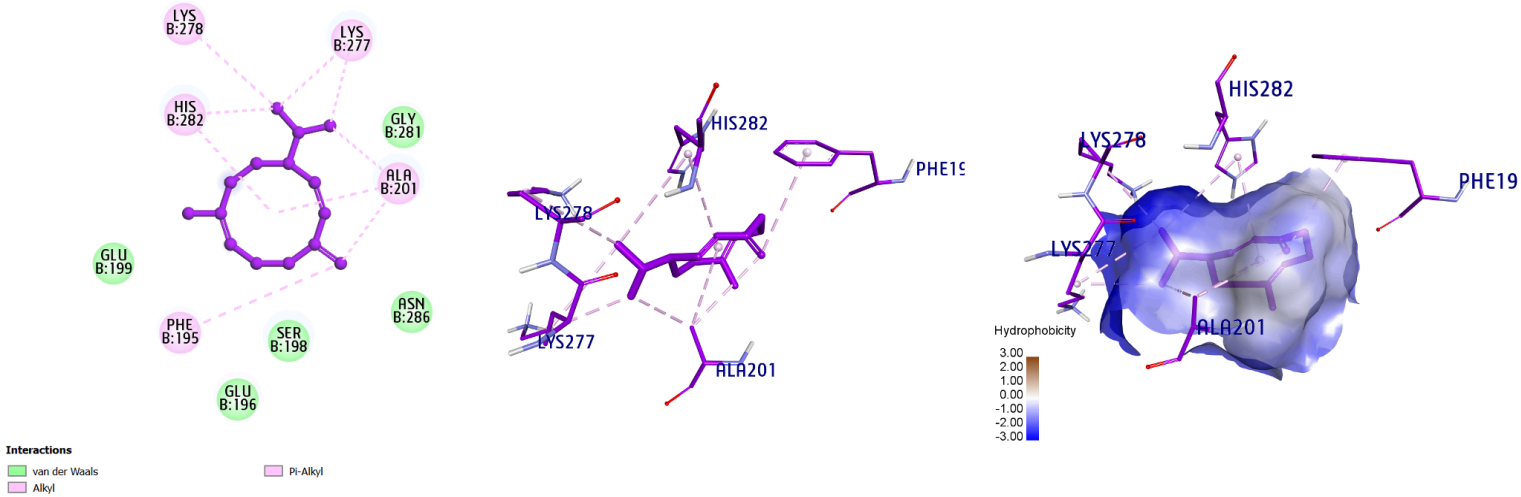

Figure 3. 2D and 3D interactions of germacrene D with the amino acid residues of the *A. niger* PhyA protein (PDB ID: 3K4Q)

Table 5. The oral toxicity of germacrene D and the positive controls

Compounds	Predicted LD_{50} (mg/kg)	Predicted Toxicity Class	Prediction accuracy	Average similarity	Organ toxicity				
					Hepato-toxicity	Neuro-toxicity	Nephro-toxicity	Respiratory toxicity	Cardio-toxicity
Germacrene D	5300	5	70.97%	80.77%	Inactive (p = 0.80)	Inactive (p = 0.51)	Inactive (p = 0.89)	Inactive (p = 0.71)	Inactive (p = 0.71)
Streptomycin	500	3	70.54%	69.26%	Inactive (p = 0.95)	Active (p = 0.79)	Active (p = 0.72)	Active (p = 0.68)	Inactive (p = 0.80)
Cyclohexamide	2	1	100%	100%	Inactive (p = 0.79)	Inactive (p = 0.52)	Active (p = 0.55)	Active (p = 0.62)	Inactive (p = 0.61)

antimicrobial activity, especially *M. virgata* leaf essential oil, which strongly controlled the growth of *B. subtilis, E. coli,* and *A. niger* with the same MIC value of 16 µg/mL. By the molecular docking approach, germacrene D showed effective binding capabilities compared to two positive controls, and did not show toxicity towards the considered organ targets.

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Competing interests
No potential conflict of interest was reported by the authors.

Data availability statement
Data available on request from the corresponding author.

Supplementary data
Figures S1 to S5 are given as supplementary data.

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