Natural Product Communications
Volume 18(7): 1–7

DOI: 10.1177/1934578X231190689
journals.sagepub.com/home/npx

Essential Oils of Two Vietnamese Plants *Piper betle* f. *densum* (Piperaceae) and *Disepalum plagioneurum* (Annonaceae): Chemical Composition, Antimicrobial, and Cytotoxic Activities

Do Ngoc Dai[1], Ty Viet Pham[2], Nguyen Dinh Luyen[3], Vo Thi Dung[1], Le Thi Huong[4] and Ninh The Son[5]

Abstract

The chemical compositions were determined by the leaf essential oils of 2 Vietnamese plants, *Piper betle* f. *densum* and *Disepalum plagioneurum*. The main chemical classes in the *P densum* leaf essential oil were sesquiterpene hydrocarbons (30.1%) and oxygenated sesquiterpenes (60.1%), with γ-elemene (12.7%), valeranone (9.3%), and ishwarone (6.0%) being the principal compounds. *Disepalum plagioneurum* leaf essential oil was dominated by monoterpene hydrocarbons (23.9%) and sesquiterpene hydrocarbons (59.8%). Bicyclogermacrene (26.8%), (*E*)-caryophyllene (12.7%), (*E*)-*β*-ocimene (8.4%), and (*Z*)-*β*-ocimene (6.0%) were the major compounds of this essential oil. With the same MIC of 64 μg/mL, the leaf essential oils of *P betle* f. *densum* and *D plagioneurum* strongly controlled the growth of the Gram (+) bacterium *Clostridium sporogenes* NCTC 12935 and the fungus *Aspergillus niger* ATCC 1015, respectively. It was also found that *D plagioneurum* leaf essential oil was cytotoxic to cancer cell lines MCF7 and HeLa with IC_{50} values of 63.2 and 41.4 μg/mL, respectively.

Keywords

piperaceae, annonaceae, *Piper betle* f. *densum*, *Disepalum plagioneurum*, essential oil, antimicrobial activity, cytotoxicity

Received: June 3rd, 2023; Accepted: July 11th, 2023.

Introduction

Piperaceae (or pepper) is a large family of small trees, shrubs, and herbs. This family is divided into 5 genera, namely *Macropiper*, *Zippelia*, *Piper*, *Peperomia*, and *Manekia*, with about 3600 accepted species.[1] These are now widely distributed in both pantropical and neotropical zones.[1] Piperaceae species, especially *Piper* sp., are aromatic and can produce essential oils.[1] Essential oils extracted from different organs of many *Piper* species have generally been constituted of terpenoids, phenylpropanoids, and alkaloids.[1] Of the *Piper* species, *P betle* is among the most attractive for essential oil studies. Phytol, carvacrol, chavicol, chavibetol, and especially eugenol, are the major compounds in *P betle* leaf oils.[2] For instance, eugenol constituted 14% to 64% of the leaf oil from India.[3,4] Eugenol (22.7%) was also one of the major compounds in Vietnamese *P betle* leaf essential oil.[5]

The Annonaceae is the largest family in the Magnoliales order.[2] About 112 genera and 2440 species have been recorded, which are concentrated in the tropics, with few species found in temperate areas.[6,7] Numerous Annonaceae species are of economic value since their extracts and metabolites, especially essential oils, are used as raw materials in the cosmetic and perfumery industries, and for medicinal use.[8] For instance, Ylang-ylang (*Cananga odorata*) essential oil is one of the most extensively used natural ingredients in the perfume industry, earning the name "Queen of Perfumes."[9]

[1]Faculty of Agriculture, Forestry and Fishery, Nghe An University of Economics, Vinh City, Vietnam
[2]Faculty of Chemistry, University of Education, Hue University, Hue City, Vietnam
[3]Institute of Natural Products Chemistry, Vietnam Academy of Science and Technology, Hanoi, Vietnam
[4]Faculty of Biology, College of Education, Vinh University, Vinh City, Vietnam
[5]Institute of Chemistry, Vietnam Academy of Science and Technology, Hanoi, Vietnam

Corresponding Author:
Ninh The Son, Institute of Chemistry, Vietnam Academy of Science and Technology, 18 Hoang Quoc Viet, Caugiay, Hanoi, Vietnam.
Email: ntson@ich.vast.vn

As can be seen, the plants of these 2 families are thought to be rich resources of essential oils. In Vietnam, there have been plenty of phytochemical investigations using GC-MS (gas chromatography-mass spectrometry) analyses of Piperaceae and Annonaceae essential oils. *Piper nigrum* (black pepper) seed essential oil, collected from Gialai was dominated by 3-carene (29.2%), δ-limonene (20.9%), caryophyllene (15.0%), and β-pinene (9.8%).[10] The main compounds in *Piper laosanum* fresh leaf essential oil were α-curcumene (12.0%), germacrene D (6.3%), and sabinene (6.1%).[11] *Piper albispicum* leaf oil, gathered from Hatinh, showed the best antimicrobial activity against *Pseudomonas aeruginosa* with a MIC of 5.82 μg/mL due to the role of chavicol acetate (16.3%) and bicyclogermacrene (13.8%).[12] Our previous report indicated that *Polyalthia viridis* leaf and stem essential oils (Annonaceae), collected from Quangtri, have cytotoxic, antimicrobial, and anti-inflammatory effects.[13]

Piper betle f. *densum* (Blume) Fosberg (synonyms: *Piper betle* var. *densum* (Blume) C. DC., or *Piper densum* Blume), locally named Tieu day, is an endemic species found in Kontum and Lamdong provinces of Vietnam.[14] *Disepalum plagioneurum* (Diels) D. M. Johnson (synonym: *Polyalthia pingpienensis*), locally named Nhoc trai khop la thuon, is an Annonaceae species found in China, Laos, and Vietnam.[15] To date, only one phytochemical report has dealt with the isolation of acetogenin derivatives from the leaves of *D plagioneurum*.[15] The current study aimed to describe the chemical composition of the essential oils from these 2 plants. These oils have been further taken into consideration for their antimicrobial and cytotoxic properties.

Results and Discussion

The yellow essential oil from *P betle* f. *densum* fresh leaves was obtained with a yield of 0.15% (v/w). A total of 45 compounds were identified, which represented 92.5% of the composition (Table 1). Sesquiterpene hydrocarbons (30.1%) and their oxygenated derivatives (60.3%) were the primary chemical classes. Other types were present in trace amounts, including monoterpene hydrocarbons (0.7%), oxygenated monoterpenes (0.8%), diterpene hydrocarbons (0.4%), and nonterpenic compounds (0.2%). γ-Elemene (12.7%), valeranone (9.3%), and ishwarone (6.0%) were the principal compounds. *Piper betle* f. *densum* leaf essential oil was further characterized by the appearance of other compounds with an amount greater than 1.0%, consisting of *trans*-cycloisolongifol-5-ol (4.7%), *trans*-calamenene (4.7%), *cis*-sesquisabinene hydrate (4.7%), *epi*-cedrol (4.6%), *cis*-cadinene ether (4.4%), occidentalol (3.5%), *p*-menth-1-ene-7,8-diol (3.3%), germacrene B (3.2%), α-amorphene (2.9%), (*Z*)-α-santalol acetate (2.5%), β-bisabolenal (2.4%), eudesm-7(11)-en-4-ol acetate (1.8%), 14-hydroxy-α-humulene (1.7%), longipinanol (1.6%), cedr-8-en-13-ol (1.5%), β-vetivenene (1.3%), *epi*-cubebol (1.2%), β-(*Z*)-curcumen-12-ol (1.2%), (*E*)-caryophyllene (1.1%), and germacra-4(15),5,10(14)-trien-1-α-ol (1.1%). As mentioned above, the chemical compositions of essential oils of *P betle* f. *densum* and *P betle* are quite different.[2-5]

Powdered *D plagioneurum* leaves were hydrodistilled to give a yellow essential oil with a yield of 0.17% v/w. A total of 42 compounds were identified, which accounted for 99.9% of the composition (Table 2). This essential oil was predominated by sesquiterpene hydrocarbons (59.8%) and monoterpene hydrocarbons (23.9%). Oxygenated monoterpenes, oxygenated sesquiterpenes, and nonterpenic compounds formed 9.6%, 6.3%, and 0.3%, respectively. Bicyclogermacrene (26.8%), (*E*)-caryophyllene (12.7%), (*E*)-β-ocimene (8.4%), and (*Z*)-β-ocimene (6.0%) were the major compounds in this essential oil. Some compounds reached more than 1.0%, including germacrene D (4.3%), 1,8-cineole (3.7%), (*E*)-nerolidol (3.7%), bicycloelemene (2.9%), β-myrcene (3.6%), α-pinene (2.3%), perillene (2.3%), α-copaene (2.3%), α-humulene (2.0%), sabinene (1.8%), α-terpinyl acetate (1.7%), β-elemene (1.4%), β-selinene (1.2%), aromadendrene (1.1%), lianalool acetate (1.0%), δ-cadinene (1.0%), and spathulenol (1.0%). This result matches well with previous reports since bicyclogermacrene can be seen as the major compound of the essential oils of Vietnamese *Polyalthia* species, for example, *P viridis* leaf (17.1%),[13] *P harmandii* leaf (20.9%),[18] *P harmandii* stem (27.9%),[18] and Polyalthia *suberosa* leaf (26.3%).[19]

In the antimicrobial assay (Table 3), *P betle* f. *densum* leaf essential oil showed strong activity against the Gram (+) bacterium *Clostridium sporogenes* with a MIC of 64 μg/mL, whereas *D plagioneurum* leaf oil controlled the growth of *Bacillus subtilis* and *Staphylococcus aureus* with the same MIC of 128 μg/mL. Both oils suppressed the growth of 2 Gram (–) bacteria *Escherichia coli* and *Pseudomonas aeruginosa* with the same MIC of 128 μg/mL. The 2 studied oils were inactive against the fungus *Aspergillus brasiliensis* (MIC > 256 μg/mL) and moderately active against the fungus *Fusarium oxysporum* with the same MIC of 128 μg/mL. In contrast to *P betle* f. *densum* leaf oil (MIC > 256 μg/mL), *D plagioneurum* leaf oil successfully inhibited the growth of the fungus *Aspergillus niger* with a MIC of 64 μg/mL. In the last case, these 2 essential oils failed against 2 yeasts *Candida albicans* and *Saccharomyces cerevisiae* (MIC > 256 μg/mL).

Huong et al reported that *P albispicum* leaf and stem essential oil possessed MIC values of 9.07 to 10.91 μg/mL against *Enterococcus faecalis* and *C albicans*.[12] Piper *pendulispicum* leaf and stem essential oils (MIC 16-32 μg/mL) were equivalent to the positive control cycloheximide (MIC 32 μg/mL) against *C albicans*.[20] Polyalthia *suberosa* twig essential oil successfully controlled *P aeruginosa*, *A niger*, and *C albicans* with the same MIC of 50 μg/mL.[18] Likewise, *P viridis* stem essential oil produced the same MIC of 50 μg/mL against *A niger* and *C albicans*.[13] Collectively, it is expected to use Vietnamese Piperaceae and Annonaceae essential oils in antimicrobial treatments.

Both essential oil samples were submitted to cytotoxic assay against the growth of HepG2, MCF7, and HeLa cells, with ellipticine used as a positive control (HepG2: $IC_{50} = 0.69$ μg/mL, MCF7; $IC_{50} = 0.81$ μg/mL, and HeLa: $IC_{50} = 0.78$ μg/mL). *Disepalum plagioneurum* leaf essential oil showed cytotoxicity

Table 1. Chemical Compounds in *Piper betle* f. *densum* Leaf Oil.[a]

No	Compounds	Rt	RI_E	RI_L	Concentration (%)
1	α-Pinene	6.70	932	932	0.2
2	β-Pinene	8.11	975	974	0.5
3	Geranyl acetate	25.42	1384	1379	0.2
4	β-Elemene	25.77	1393	1389	0.7
5	(*E*)-Caryophyllene	26.92	1420	1417	1.1
6	(*E*)-α-Ionone	27.19	1426	1428	0.6
7	β-Copaene	27.33	1430	1430	0.2
8	γ-Elemene	27.59	1436	1434	**12.7**
9	*cis*-Thujopsene	27.94	1445	1429	0.4
10	α-Humulene	28.33	1454	1452	0.3
11	Macrocarpene	29.05	1472	1470	1.5
12	α-Amorphene	29.55	1484	1483	2.9
13	*p*-Menth-1-ene-7,8-diol	30.10	1497	1502	3.3
14	β-Himachalene	30.24	1501	1500	0.5
15	β-Bisabolene	30.59	1510	1508	0.6
16	*trans*-Cycloisolongifol-5-ol	30.94	1519	1513	4.7
17	*trans*-Calamenene	31.16	1524	1521	4.7
18	(*Z*)-Nerolidol	31.76	1540	1531	0.9
19	*cis*-Sesquisabinene hydrate	31.96	1545	1542	4.7
20	Occidentalol	32.17	1550	1550	3.5
21	*cis*-Cadinene ether	32.30	1553	1552	4.4
22	β-Vetivenene	32.41	1556	1554	1.3
23	Germacrene B	32.51	1559	1559	3.2
24	β-Copaen-4-α-ol	32.97	1565	1565	0.9
25	Longipinanol	32.97	1570	1567	1.6
26	Spathulenol	33.3	1579	1577	0.9
27	Caryophyllene oxide	33.51	1584	1582	0.8
28	Globulol	33.87	1594	1590	1.2
29	*epi*-Cedrol	34.97	1623	1618	4.6
30	*14-hydroxy*-(*Z*)-Caryophyllene	36.62	1667	1666	0.8
31	Valeranone	36.82	1672	1671	**9.3**
32	Ishwarone	37.10	1680	1680	**6.0**
33	Germacra-4(15),5,10(14)-trien-1-α-ol	37.30	1685	1685	1.1
34	8-Cedren-13-ol	37.53	1691	1688	0.4
35	14-Hydroxy-α-humulene	38.54	1720	1713	1.7
36	β-(*Z*)-Curcumen-12-ol	39.77	1754	1754	1.2
37	Cyclocolorenone	39.97	1760	1759	0.7
38	β-Bisabolenal	40.32	1770	1768	2.4
39	(*Z*)-α-Santalol acetate	40.68	1780	1777	2.5
40	γ-Eudesmol acetate	40.85	1785	1783	0.2
41	(*E*)-Isovalencenol	41.21	1795	1793	0.4
42	(*Z*)-Nuciferol acetate	42.27	1826	1830	0.3
43	Eudesm-7(11)-en-4-ol acetate	42.77	1841	1839	1.8
44	Totarene	45.3	1916	1922	0.4
45	4-Hydroxy-stilbene	49.24	2040	2042	0.2
Total					92.5
Monoterpene hydrocarbons					0.7
Oxygenated monoterpenes					0.8
Sesquiterpene hydrocarbons					30.1
Oxygenated sesquiterpenes					60.3
Diterpene hydrocarbons					0.4
Non-terpenic compounds					0.2

Abbreviations: Rt, retention time; RI_E, retention indices relative to *n*-alkanes (C_7-C_{40}) on Equity-5 column; RI_L, retention indices from Adams[16] and the NIST standard database.[17]
[a]Bold: major compounds.

towards cancer cells MCF7 and HeLa with IC_{50} values of 63.2 and 41.4 μg/mL, respectively, but was inactive towards HepG2 ($IC_{50} > 256$ μg/mL). *Piper betle* f. *densum* leaf essential oil did not inhibit any of these 3 cancer cell lines ($IC_{50} > 256$ μg/mL). In

Table 2. Chemical Compounds in *Disepalum plagioneurum* Leaf Oil.[a]

No	Compounds	Rt	RI_E	RI_L	Concentration (%)
1	α-Pinene	6.17	935	932	2.3
2	Sabinene	7.17	974	969	1.8
3	β-Pinene	7.27	979	974	0.6
4	β-Myrcene	7.59	991	988	3.6
5	Limonene	8.67	1030	1024	0.6
6	1,8-Cineole	8.75	1033	1026	3.7
7	(*Z*)-β-Ocimene	8.89	1038	1037	**6.0**
8	(*E*)-β-Ocimene	9.19	1048	1049	**8.4**
9	γ-Terpinene	9.51	1060	1054	0.2
10	Linalool	10.66	1101	1095	0.7
11	Perillene	11.13	1117	1102	2.3
12	*allo*-Ocimene	11.51	1130	1128	0.4
13	Linalool acetate	15.09	1257	1254	1.0
14	*iso*-Dihydro carveol acetate	17.15	1332	1326	0.2
15	Bicycloelemene	17.41	1342	1338	2.9
16	α-Terpinyl acetate	17.70	1353	1346	1.7
17	α-Ylangene	18.35	1377	1373	0.2
18	α-Copaene	18.47	1382	1374	2.3
19	β-Elemene	18.87	1397	1389	1.4
20	α-Gurjunene	19.37	1417	1409	0.2
21	(*E*)-Caryophyllene	19.64	1428	1417	**12.7**
22	β-Copaene	19.86	1437	1430	0.2
23	γ-Elemene	19.93	1439	1434	0.3
24	Aromadendrene	20.13	1447	1439	1.1
25	*cis*-Muurola-3,5-diene	20.36	1456	1448	0.5
26	α-Humulene	20.50	1462	1452	2.0
27	*allo*-Aromadendrene	20.57	1465	1458	0.6
28	*dehydro*-Aromadendrane	20.69	1469	1460	0.2
29	α-Amorphene	21.04	1483	1483	0.2
30	Germacrene D	21.19	1489	1480	4.3
31	β-Selinene	21.33	1495	1489	1.2
32	Bicyclogermacrene	21.59	1506	1500	**26.8**
33	(*E,E*)-α-Farnesene	21.71	1510	1505	0.6
34	α-Bulnesene	21.80	1515	1509	0.5
35	δ-Amorphene	21.98	1522	1511	0.6
36	δ-Cadinene	22.18	1530	1522	1.0
37	Elemicin	22.87	1559	1555	0.3
38	(*E*)-Nerolidol	23.06	1567	1561	3.7
39	Spathulenol	23.54	1587	1577	1.0
40	Caryophyllene oxide	23.69	1594	1582	0.6
41	*epi*-α-Cadinol	24.97	1650	1638	0.7
42	Benzyl benzoate	27.88	1768	1759	0.3
Total					99.9
Monoterpene hydrocarbons					23.9
Oxygenated monoterpenes					9.6
Sesquiterpene hydrocarbons					59.8
Oxygenated sesquiterpenes					6.3
Non-terpenic compounds					0.3

Abbreviations: Rt, retention time; RI_E, retention indices relative to *n*-alkanes (C_7-C_{40}) on Equity-5 column; RI_L, retention indices from Adams[16] and the NIST standard database.[17]
[a]Bold: major compounds.

line with previous results, Vietnamese *Polyalthia* essential oils showed potential for cytotoxic treatments. *P*olyalthia *viridis* stem oil and *P suberosa* leaf essential oil prevented the proliferation of HepG2, MCF7, and A549 cells with IC_{50} values of 56.7 to 69.9 μg/mL.[13,19] Considering the role of the major compounds, bicyclogermacrence was mainly responsible for the cytotoxicity of *Nectandra leucantha* leaf essential oil against MCF7, HCT, U-87, and B16F10-Nex2 cancer cells.[21] The cytotoxicity of *Annona muricata* leaf essential oil against MCF7 cells (99.2% kill at 100 μg/mL) is likely due to the relatively high

Table 3. Antimicrobial Activity of the Studied Essential Oils.

Microbial strains		Minimum inhibitory concentration (MIC: μg/mL)				
		Piper betle f. *densum*	*Disepalum plagioneurum*	Streptomycin	Tetracycline	Nystatin
Gram (+)	*Bacillus subtilis*	>256	128	4		
	Staphylococcus aureus	>256	128	8		
	Clostridium sporogenes	64	>256	8		
Gram (–)	*Escherichia coli*	128	128		4	
	Pseudomonas aeruginosa	128	128		4	
Fungi	*Aspergillus brasiliensis*	>256	>256		8	
	Aspergillus niger	>256	64		8	
	Fusarium oxysporum	128	128		8	
Yeasts	*Candida albicans*	>256	>256			4
	Saccharomyces cerevisiae	>256	>256			8

concentration of (*E*)-caryophyllene (38.9%).[22] Hence, our current data provide new information for further research.

Conclusion

For the first time, the current research reports the chemical compositions of the essential oils of 2 Vietnamese plants, in which γ-elemene (12.7%), valeranone (9.3%), and ishwarone (6.0%) were the predominant compounds in *P betle* f. *densum* leaf essential oil, while *D plagioneurum* leaf essential oil was associated with the presence of the major compounds bicyclogermacrene (26.8%), (*E*)-caryophyllene (12.7%), (*E*)-*β*-ocimene (8.4%), and (*Z*)-*β*-ocimene (6.0%). The leaf essential oils of *P densum* and *D plagioneurum* exhibited strong antimicrobial activity against the Gram (+) bacterium *C sporogenes* and fungus *A niger* with the same MIC of 64 μg/mL, respectively. In addition, *D plagioneurum* leaf essential oil showed cytotoxicity toward cancer cells MCF7 and HeLa with IC_{50} values of 63.2 and 41.4 μg/mL, respectively.

Materials and Methods

Plant Materials

The fresh leaves of *Piper betle* f. *densum* (Blume) Fosberg and *Disepalum plagioneurum* (Diels) D. M. Johnson were collected from Pu Hoat and Pu Huong Natural Reserves, Nghean, Vietnam in March-2023, respectively. The plants were identified by our co-author Prof. Le Thi Huong. Two voucher specimens PD-2023 (*P betle* f. *densum* leaves) and DP-2023 (D plagioneurum leaves) were deposited at the faculty of Agriculture, Forestry and Fishery, Nghe An University of Economics.

Distillation

Each fresh powdered sample (1.5 kg) was submitted to hydrodistillation for 3.0 h. The extraction was performed using a Clevenger apparatus. The essential oils obtained by decantation were dried over Na_2SO_4 and then kept in sealed vials at −5 °C for further analysis.

GC-MS Analysis

GC-MS analysis was performed using a Shimadzu Technologies GCMS-QP2010 Plus (Shimadzu) chromatograph equipped with a fused silica Equity-5 capillary column (30 m 0.25 mm, film thickness 0.25 μm, Supelco).[23] The analytical settings were as follows: 1.5 mL/min of carrier helium, 280 °C injector and interface temperatures, and a temperature ramp from 60 °C (2 min hold) to 240 °C (10 min hold) at 3 °C/min, then to 280 °C at 5 °C/min for the column (40-min hold). A split ratio of 10:1 was used to inject the samples. The inlet pressure was 93.2 kPa, and the injection volume was 1.0 μL. The MS settings were ionization voltage 70 eV, detector voltage 0.82 kV, and acquisition scan mass range of 40 to 500 amu at a sampling rate of 0.5 scan/s. By co-injecting the constituents and comparing the results to a homologous series of *n*-alkanes (C7-C40), the retention indices (RI) of chemical constituents were calculated. Quantification was carried out on the basis of the relative area of the total ion chromatogram (TIC) peaks of volatile compounds. Chemical identification was carried out by comparison of RI values with those reported by Adams,[16] and the NIST standard databases.[17]

Antimicrobial Assay

The pathogenic ATCC (American Type Culture Collection) strains, including 3 Gram (+) bacteria, *Bacillus subtilis* ATCC 6633, *Staphylococcus aureus* ATCC 25923, and *C sporogenes* NCTC 12935, 2 Gram (–) bacteria, *Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 15442, 3 fungi, *Aspergillus brasiliensis* ATCC 16404, *A niger* ATCC 1015 and *Fusarium oxysporum* ATCC 46591, and 2 yeasts, *Candida albicans* ATCC 10231 and *Saccharomyces cerevisiae* ATCC 4098, were used in this study. All strains were cultured on Muller Hilton Agar (MHA, Merck) plates for one day at 37 °C.

The assay was described in previous work.[24-26] Briefly, the essential oil samples were dissolved in DMSO (5%) to reach concentrations of 4 to 256 μg/mL. A total of 180 μL of bacterial suspension with 10^6 CFU mL^{-1} in Muller Hilton Broth and 20 μL of essential oil were placed in each well (MHB, Merck).

The mixture was incubated at 37 °C, and the OD (optical density) was determined at 600 nm using an Elisa reader (RNE-9002, USA). The lowest concentration that showed no growth was identified as the MIC. The assays were performed 3 times. The same procedures were used for the negative control, which contained MHB and Tween, and the positive controls, which contained MHB and bacterial suspension without the tested sample. Streptomycin and tetracycline were used as reference compounds for the respective Gram (+) and Gram (–) bacteria, whereas nystatin was used for fungi and yeasts.

Cytotoxic Assay

The cytotoxicity of the 2 oil samples was tested on the proliferation of A549 (human lung cancer), HepG2 (human hepatocellular cancer), and MCF-7 (human breast cancer) cells using an MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) assay.[13,19] The cancer cells were cultured in RPMI 1640 medium supplemented with 10% fetal bovine serum (100 U/mL) and penicillin/streptomycin (100 g/mL) at 37 °C in a humidified 5% CO_2 atmosphere. The MTT assay was carried out as follows: human cancer cells (2.0×10^5 cells/mL) were treated for 72 h with either 256-32 µg/mL of the oil samples or the standard compound ellipticine. After incubation, MTT (0.1 mg) was added to each well. Cells were then incubated at 37 °C for 4 h. The plates were centrifuged at 1.000 rpm for 6 min at 25 °C, and the media was then aspirated. Continuously, DMSO (150 µL) was added to each well to dissolve the formazan crystals. The OD (optical density) was measured at 540 nm using an Accu-Tell Elisa Reader (ABER-2, inhibitory percentage that caused a reduction in the absorbance, compared with the untreated controls. The IC_{50} (50% inhibitory concentration) values were calculated using dose–response curves.

Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Funding

The author(s) received no financial support for the research, authorship, and/or publication of this article.

ORCID iDs

Do Ngoc Dai https://orcid.org/0000-0002-7741-9454
Ty Viet Pham https://orcid.org/0000-0002-6883-0823
Ninh The Son https://orcid.org/0000-0002-2413-7496

Supplemental Material

Supplemental material for this article is available online.

References

1. Mesquita KDSM, Feitosa BS, Cruz JN, et al. Chemical composition and preliminary toxicity evaluation of the essential oil from *Peperomia circinnata* Link var. *circinnata*. (*Piperaceae*) in *Artemia salina* leach. *Molecules*. 2021;26(23):7359.
2. Nayaka NMDR, Sasadara MMV, Sanjaya DA, et al. *Piper betle* (L): recent review of antibacterial and antifungal properties, safety profiles, and commercial applications. *Molecules*. 2021;26(8):2321.
3. Karak S, Acharya J, Begum S, Mazumdar I, Kundu R, De B. Essential oil of *Piper betle* L. Leaves: chemical composition, anti-acetylcholinesterase, anti-β-glucuronidase and cytotoxic properties. *J Appl Res Med Aromat Plants*. 2018;10:85-92.
4. Rawat AKS, Tripathi RD, Khan AJ, Balasurahmanyam VR. Essential oil components as markers for identification of *Piper betle* L. cultivars. *Biochem Syst Ecol*. 1989;17(1):35-38.
5. Cang MH, Nhi PTB, Linh NTC. Betel (*Piper betle* L.) leaf essential oil extraction using steam distillation. *Int J Adv Eng Res Sci*. 2020;7(6):166-169.
6. Santos ACD, Nogueira ML, Oliveira FP, Costa EV, Bezerra DP. Essential oils of *Duguetia* species A. St. Hill (Annonaceae): chemical diversity and pharmacological potential. *Biomolecules*. 2022;12(5):615.
7. Quang Hop N, The Son N. Botanical description, traditional uses, phytochemistry, and pharmacology of the genus *Artabotrys*: a review. *Chem Biodivers*. 2022;19(11):e202200725. doi:10.1002/cbdv.202200725
8. Cascaes MM, Carneiro ODS, Nascimento LDD, et al. Essential oils from Annonaceae species from Brazil: a systematic review of their phytochemistry, and biological activities. *Int J Mol Sci*. 2021;22(22):12140.
9. Toller S, Dodd G. *Fragrance: The Psychology and Biology of Perfume*. Elsevier; 1992: 268.
10. Tran TH, Ha LK, Nguyen DC, et al. The study on extraction process and analysis of components in essential oils of black pepper (*Piper nigrum* L.) seeds harvested in Gia Lai province, Vietnam. *Processes*. 2019;7(2):56.
11. Thin DB, Chinh HV, Luong NX, Hoi TM, Dai DN, Ogunwande IA. Chemical analysis of essential oils of *Piper laosanum* and *Piper acre* (Piperaceae) from Vietnam. *J Essent Oil-Bear Plants*. 2018;21(1):181-188.
12. Le NV, Sam LN, Huong LT, Ogunwande IA. Chemical compositions of essential oils and antimicrobial activity of *Piper albispicum* C. DC. from Vietnam. *J Essent Oil-Bear Plants*. 2022;25(1):82-92.
13. Son NT, Le TA, Thuy DTT, et al. Essential oils of the leaf and stem of *Polyalthia viridis* craib and their biological activities. *Nat Prod Commun*. 2021;16(9):1-6.
14. Ho PH. *An Illustrated Flora of Vietnam*. Vol. 1. 2nd ed. Tre Publishing House; 2000: 295.
15. Eparvier V, Nguyen VH, Thoison O, Martin MT, Sévenet T, Guéritte F. Cytotoxic monotetrahydrofuran acetogenins from *Disepalum plagioneurum*. *J Nat Prod*. 2006;69(9):1289-1294.
16. Adam RP. *Identification of Essential Oil Components by Gas Chromatography/Mass Spectrometry, Ed.4.1*. Allured Publ.; 2017.

17. Search for species data by chemical name. Accessed April 2023. https://webbook.nist.gov/chemistry/name-ser
18. Dai DN, Thang TD, Ogunwande IA. Chemical composition of essential oils from the leaves and stem barks of Vietnamese species of *Polyalthia harmandii, Polyalthia jucunda* and *Polyalthia thorelii*. *Nat Prod Res*. 2014;28(8):555-562.
19. The SN, Le Tuan A, Thu TDT, Dinh LN, Thi TT. Essential oils of *Polyalthia suberosa* leaf and twig and their cytotoxic and antimicrobial activities. *Chem Biodivers*. 2021;18(5):e2100020. doi:10.1002/cbdv.202100020
20. Dai DN, Huong LT, Le NV, Dung VT, Son NT. Essential oils of *Piper pendulispicum* C. DC. and *Piper hymenophyllum* Miq.: chemical composition and anti-microbial activity. *J Essent Oil-Bear Plants*. 2023;26(2):403-410.
21. Grecco SDS, Martins EGA, Girola N, et al. Chemical composition and in vitro cytotoxic effects of the essential oil from *Nectandra leucantha* leaves. *Pharm Biol*. 2015;53(1):133-137.
22. Owolabi MS, Ogundajo AL, Dosoky NS, Setzer WN. The cytotoxic activity of *Annona muricata* leaf oil from Badagary, Nigeria. *Am J Essent Oil Nat Prod*. 2013;1(1):1-3.
23. Tuan DQ, Dinh D, Pham TV, et al. Chemical composition of essential oil from the rhizomes and leaves of *Newmania sontraensis* H.Đ.Trân, Luu & Škornick (Zingiberaceae) from Vietnam. *J Essent Oil-Bear Plants*. 2021;24(6):1260-1268.
24. Son NT, Anh LT, Thuy DTT, Luyen ND, Tuyen TT. Essential oils of the *Asteraceae* plants *Blumea riparia* DC. and *Pluchea pteropoda* Hemsl. ex Hemsl. Growing in Vietnam. *Nat Prod Commun*. 2022;17(6):1-6.
25. Huong LT, Son NT, Sam LN, et al. Chemical compositions and antimicrobial activity of essential oils from the leaves of 4 Vietnamese Zingiberaceae species. *Nat Prod Commun*. 2022;17(12):1-6.
26. Son NT, Oda M, Hayashi N, et al Antimicrobial activity of the constituents of Dalbergia tonkinensis and structural-bioactive highlights. *Nat Prod Commun*. 2018;13(2):157-161.