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CHEMICAL COMPOSITION, ANTI-INFLAMMATORY AND CYTOTOXIC ACTIVITIES OF ESSENTIAL OILS FROM ZANTHOXYLUM MYRIACANTHUM WALL. EX HOOK. F. FRUITS, LEAVES AND STEMS COLLECTED IN LAM DONG PROVINCE

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Summary

Chemical Composition, Anti-Inflammatory and Cytotoxic Activities of Essential Oils from

***Zanthoxylum myriacanthum* Wall. ex Hook. f. Fruits, Leaves and Stems Collected in Lam Dong Province**

Essential oils extracted by hydrodistillation from fruits, leaves, and stems of *Z. myriacanthum* collected in Bidoup-Nui Ba National Park, Lam Dong province, Vietnam were investigated for their chemical composition and bioactivity. 51 compounds were identified in the fruit oil (ZMFR), of which *p*-cymen-8-ol (24.9%) and β -pinene (24.3%) were the most abundant. The leaf oil (ZML) contained 32 identified constituents, mainly *p*-methyl-acetophenone (29.9%), α -terpineol (17.0%), *ar*-curcumene (9.5%), (*E*)-carpacin (7.7%), 2-undecanone (6.6%) and 2-tridecanone (5.9%). The stem oil (ZMS) consisted of 23 compounds, the major ones being *ar*-curcumene (44.6%), terpinen-4-ol (13.2%), (1*R*,2*R*,4*R*)-(2-hydroxypropan-2-yl)-1-methylcyclohexane-1,2-diol (8.8%) and α -terpineol (8.4%). ZMFR exhibited moderate NO production inhibition in macrophage RAW 264.7 cells with an IC₅₀ value of 42.35 $\pm$ 2.63 μ g/mL. ZMFR and ZML showed moderate cytotoxic effects on SK-LU-1, MCF-7, and HepG2 cancer cell lines with the IC₅₀ values ranging from 50.97 $\pm$ 4.07 to 93.78 $\pm$ 3.67 μ g/mL. ZMS was cytotoxic against HepG2 (IC₅₀ = 69.84 $\pm$ 4.71 μ g/mL) and MCF-7 (IC₅₀ = 89.28 $\pm$ 5.50 μ g/mL), and showed minimal effect on SK-LU-1. The anti-inflammatory and cytotoxic activities of *Z. myriacanthum* essential oils were evaluated and reported for the first time.

Keywords: *Zanthoxylum myriacanthum*, Essential oil, GC-MS, Anti-microbial activity, NO inhibition, Cytotoxicity.

1. Introduction

Zanthoxylum myriacanthum Wall. ex Hook. f. belongs to the family Rutaceae, widely distributed in tropical areas of Asia such as South China, Southeast Asia, and India. In ethnic medicine, the seeds of *Z. myriacanthum* are used as a spice, and the roots and leaves are used to treat rheumatism, fractures, boils, fire burns, and snake bites [1]. The fruits, branches, and leaves of *Z. myriacanthum* all contain aromatic essential oils. The chemical composition and bioactivity of the essential oil in the fruits of this species have been the focus of much research [2],[3],[4],[5],[6]. The content of essential oil in the fruits is up to 4% by weight of the fresh fruits [5], and 14% by weight of the dry fruits [7]. The main constituents of *Z. myriacanthum* fruit essential oil from China were identified as limonene (67.06%), α -pinene (6.49%), β -myrcene (3.87%) and linalool (2.96%) [5]. Studies have also demonstrated antibacterial [5], anti-inflammatory [3], antiviral [6], renoprotective [3], and hepatoprotective [4] activities of the fruit essential oil. Furthermore, the essential oil extracted from *Z. myriacanthum* fruits showed insecticidal and repellent effects against three storage pests [8]. Recently, there was a study on the chemical composition of the essential oils from the leaves and branches of *Z. myriacanthum* from China and the main components of the leaf essential oil were identified as limonene (23.42%), caryophyllene (9.74%) and terpinen-4-ol (7.97%) and of the branches as bicyclo[3.1.0]hexane (20.65%) and terpinen-4-ol (13.34%) [9].

In Vietnam, the essential oil of dried fruits collected in Than Uyen, Lao Cai Province was investigated by Giang PM and Son PT. The main components in the essential oil were *p*-cymene (15.3%), limonene (27.1%), and β -phellandrene (11.9%) [7]. However, essential oils from the stems and leaves of this species have not been studied. In addition, the biological activity of essential oils from *Z. myriacanthum* is also largely unknown. Considering that this species is widely distributed in Lam Dong province, we conducted a study to elucidate and compare the chemical composition of essential oils hydrodistilled from fruits, leaves, and stems, as well as their anti-inflammatory and cytotoxic activities. Their inhibition of NO production in LPS-stimulated RAW 264.7 cells and cytotoxic activity against three cancer cell lines (SK-LU-1, MCF-7, and HepG2) were evaluated and reported for the first time.

2. Materials and methods

2.1. Plant materials

The fruits (fallen from the trees and naturally dried, 120 g), leaves (fresh, 1500 g), and stems (fresh, 1400 g) of *Zanthoxylum myriacanthum* Wall. ex Hook. f. were collected on October 15th 2022 from Bidoup-Nui Ba National Park, Lam Dong province, Vietnam at the coordinate 12°26'41" N, 108°43'28" E. The plants were identified by our research team member Dr. Luong Van Dung, Center for Climate Change and Biodiversity Research, Dalat University, Vietnam. Voucher specimen (DL221001) has been deposited in the Herbarium of Dalat University.

2.2. Chemicals, reagents, and other materials

Toluene, sodium sulfate, and distilled water were all of the analytical grades. *n*-Hexane (Sigma) was of GC grade. Lipopolysaccharide (LPS) from *Escherichia coli* was purchased from Sigma Chemical Co. (St. Louis, MO, USA). Dulbecco's Modified Eagle's Medium (DMEM) and fetal bovine serum (FBS) were purchased from Life Technologies, Inc. (Gaithersburg, MD, USA). Sodium nitrite, sulfanilamide, dimethyl sulphoxide (DMSO), N-1-napthylethylenediamine dihydrochloride, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide (MTT), sulforhodamine B (SRB), trichloroacetic acid (TCA), and 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) were purchased from Sigma Chemical Co. (USA). RAW 264.7 cell lines were a kind gift from Prof. Dr. Domenico Delfino, University of Perugia, Perugia, Italy. The cancer cell lines HepG2 (human hepatocellular carcinoma), MCF-7 (human breast carcinoma), and SK-LU-1 (human lung carcinoma) were provided by Dr. Jeanette Maier, University of Milan, Italy, and Dr. J M Pezzuto, University of Long Island, USA.

2.3. Obtaining of essential oils

The plant materials were cut and ground with an electric grinder. The hydrodistillation of essential oils was performed using a Clevenger apparatus according to the Vietnam Pharmacopoeia (2017), for three and a half hours. The obtained essential oils were filtered through sodium sulfate to remove water completely and stored in sealed glass vials at 4°C before being used for chemical analysis or bioassay. The essential oils from the fruits, leaves, and stems of *Z. myriacanthum* were designated as ZMFR, ZML, and ZMS, respectively. The water content of the materials was determined by the distillation method with solvent according to Appendix 12.13, Vietnam Pharmacopoeia (2017).

The yields of ZMFR, ZML, and ZMS obtained from fruits, leaves, and stems of *Z. myriacanthum* by hydrodistillation were 21.47%, 0.17%, and 0.08% (v/w), calculated on dry weight, respectively.

2.4. Analysis of essential oils

The essential oils were analyzed by gas chromatography coupled with mass spectrometry on a SCION 456-GC/SCION SQ (SCION Instruments, USA), equipped with a fused silica capillary Rxi-5MS column (30 m × 0.25 mm, film thickness 0.25 µm; 5% diphenyl/95% dimethyl polysiloxane; Restek, USA) in Center for Research Testing Pharmaceutical Chemistry, Institute of Applied Material Science, Ho Chi Minh City, Vietnam. The carrier gas used was helium at a rate of 1.0 mL/min; the oven temperature program started at 60°C, then gradually increased to 246°C at 3°C/min and increased from 246°C to 280°C at 20°C/min; the injector operated at 1:20 split ratio, injector temperature 250°C; the injected volume was 0.1 µL of 10% (v/v) solution of essential oil in GC grade *n*-hexane. Mass spectra were recorded in electronic impact (EI) mode at 70 eV, scanning the 50-500 *m/z* range. The ion source temperature was 250°C with 1 scan/sec cycle time. Linear retention indices (RIs) of the compounds were determined in relation to the homolog series of *n*-alkanes (C₉-C₂₈) ran under the same operating conditions according to the following formula: $RI(x) = 100 Pz + 100 [(RT(x) - RT(Pz)) / (RT(Pz+1) - RT(Pz))]$, where $RT(Pz) \leq RT(x) \leq RT(Pz+1)$ and P9 ... P28 were *n*-alkanes.

The identification of the compounds was based on the comparison of their mass spectra to those from the NIST mass spectra library (version 2.4, 2020), and the literature [10], as well as by the comparison of their RIs with those from the literature [10],[11]. The NIST software used the unknown spectrum collected by the MS detector and performed a spectrum search in the library. The mass spectral data was analyzed against all library spectra and the Match Factor was calculated to produce a list of compounds that NIST has identified as possible matches to the chemical structure. NIST's recommended general guidelines for Match Factor scores are: >900 is an excellent match, 800-900 is a good match, 700 - 800 is a fair match, and <600 is a poor match. Next, the compounds in the proposed list were searched for their RI index in the literature [10] and [11]. Compounds were identified if they had a Match Factor of > 700 and a retention index within seven units of

reported values. Relative percentages of the compounds were calculated based on the peak areas by GC software.

2.5. Bioassay for NO inhibition and cytotoxicity

Cell culture

All cell lines were cultured in DMEM medium with 1 mM sodium pyruvate, 2 mM L-glutamine, 10 mM HEPES, and 10% FBS in an incubator at 37°C, 5% CO₂. The cells were subcultured every three days at a 1:3 ratio.

NO inhibition assay

RAW 264.7 cells were seeded to a 96-well plate at a concentration of 2×10^5 cells/well. After incubation for 24 h, the cultured medium was refreshed by FBS-free DMEM. Then, the cells were treated with samples at different concentrations in the presence of LPS (1 µg/mL) for 24 h. Dexamethasone (Sigma) was used as the positive control and the cells treated with a diluted solution of DMSO (1.0%) were used as the negative control. Nitrite, an indicator of NO production, was determined by the Griess reagent system (Promega Corporation, WI, USA). Protocols supplied with the assay kit were used for the application of the assay procedure. The absorbance was measured at 540nm in a microplate reader (Bio-Rad, California, USA). The amount of nitrite in the media was calculated from the sodium nitrite (NaNO₂) standard curve. The ability to inhibit the nitric oxide (NO) was measured at doses of 100, 20, 4, and 0.8 µg/mL respectively, and IC₅₀ value was calculated using Table Curve Version 4.0 [12],[13].

MTT cell viability assay

RAW 264.7 cells were seeded in 96-well plates in the presence of various concentrations of essential oil similar to those of the NO experiment. After 24 h of incubation, MTT was added to a final concentration of 0.5 mg/mL, and the cells were incubated for 4 h at 37°C and 5% CO₂. Finally, the supernatant was removed and the formazan crystals were dissolved in dimethyl sulfoxide (DMSO). The absorbance was measured at 540 nm. The percentage of dead cells was determined relative to the control group [12],[13].

Cytotoxicity assay

SRB assay was used to determine the cytotoxic activity of the essential oils against three cancer cell lines (HepG2, MCF-7, and SK-LU-1) in a 96-well plate according to the method of Skehan et al. [14]. The assay measures cell density based on the total cellular protein content stained with SRB. Briefly, cells were incubated

with the sample for 48 h, fixed with TCA, and stained with SRB. The OD at 540 nm was measured using an ELISA plate reader. The inhibition percentage of cell growth was calculated using the following formula: (%) inhibition = $100\% - [(OD_{\text{sample}} - OD_{\text{day 0}}) / (OD_{\text{blank control}} - OD_{\text{day 0}})] \times 100$. Ellipticine was used as a positive control and the IC₅₀ value was determined using TableCurve 2Dv4 software.

2.6. Statistical analysis

Data were expressed as the mean of three replicate determinations $\pm$ standard deviation (SD). Statistical comparisons were made with the Student's test, p value < 0.05 was considered as significant difference.

3. Results and discussion

3.1. Chemical composition of *Z. myriacanthum* fruit, leaf and stem oils

The identification of the compounds was based on the comparison of their mass spectra and their RIs with those reported in the literature [10,11]. For example, to identify a compound at Rt 7.453 min (RI = 972), a list of compounds with suitable match factors was suggested by the software, and the RIs of these suggested compounds were found in the reference literature [10] and [11], such as α -phellandrene (RI = 1002), γ -terpinene (RI = 1054), α -pinene (932), β -pinene (974), α -thujene (925), sabinene (1124), p -mentha-1(7),8-diene (993). This compound was therefore identified as β -pinene. For compounds not included in Adams R.'s reference [10], RI data and MS spectra were found in the NIST Chemistry Webbook [11] for comparison. For example, the compound at Rt 19.275 min (RI = 1270) was identified as 10-undecenal based on its

MS match of 776 and RI according to documents compiled by the NIST Chemistry Webbook [11] of 1276 ± 2 . The RI values of the identified compounds in the essential oils and in the literature together with the MS Match factor are given in Table 1. The GC chromatograms of the essential oils and some identified names of the major peaks are shown in Fig. 1-3.

A total of 87 compounds were identified in the three investigated essential oils, including 51, 32, and 23 compounds in ZMFR, ZML, and ZMS, accounting for 97.9%, 96.7%, and 90.3% of the oils, respectively. Monoterpene compounds were predominant in the fruit essential oil with a total amount of 67.3%, including 26.1% monoterpene hydrocarbons and 41.2% oxygenated monoterpenes, of which the major component was identified as p -cymen-8-ol (24.9%), followed by β -pinene (24.3%) and α -terpineol (6.1%). Chemical compositions identified in ZML were quite diverse, including terpenes (a total of 43.1%), phenylethanoids (30.7%), phenylpropanoids (8.3%) and other compounds (14.6%) such as aliphatic ketones, alcohols, and esters. Its major constituents were p -methyl-acetophenone (phenyl ethanoid, 29.9%), α -terpineol (monoterpene, 17.0%), ar -curcumene (sesquiterpene, 9.5%), (E)-carpacin (phenylpropanoid, 7.7%), 2-undecanone (6.6%), and 2-tridecanone (5.9%). Terpene compounds were more predominant in ZMS than in ZML with a total amount of 81.8%, of which the main components were ar -curcumene (44.6%), terpinen-4-ol (13.2%), ($1R,2R,4R$)-4-(2-hydroxypropan-2-yl)-1-methylcyclohexane-1,2-diol (8.8%), and α -terpineol (8.4%).

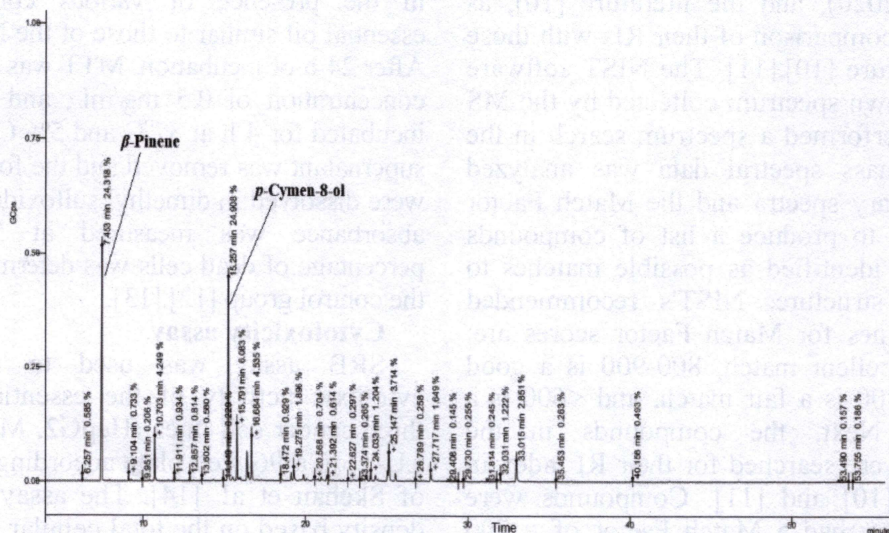


Fig. 1. GC chromatogram of ZMFR

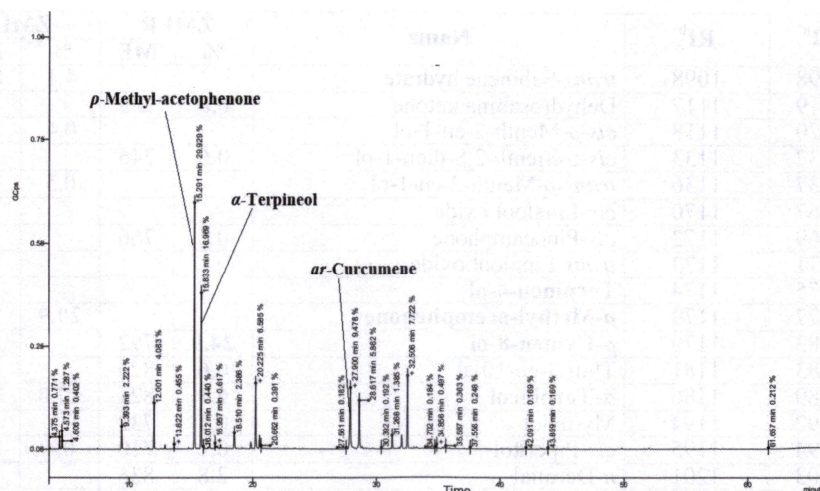


Fig. 2. GC chromatogram of ZML

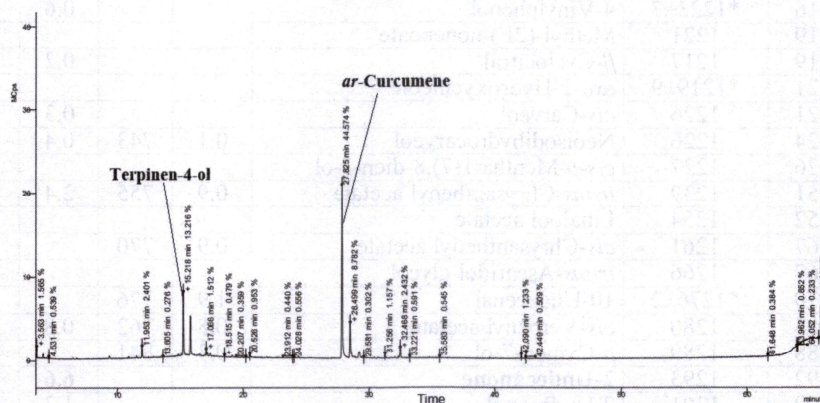


Fig. 3. GC chromatogram of ZMS

It can be seen that although fruits, leaves and stems are parts of the same plant, they have quite different essential oil compositions. Compared to the published research results on the chemical composition of the essential oil of *Z. myriacanthum* fruits, leaves, and stems, these results have in common that the essential oil belongs to the terpene type. However, the material collected from different locations contains different amounts of main components. For example, the main components of the fruit essential oil collected in China were limonene (67.06%), α -pinene (6.49%), β -myrcene (3.87%)

and linalool (2.96%) [5], while those collected in Lao Cai, Vietnam were limonene (27.1%), *p*-cymene (15.3%), β -phellandrene (11.9%) [7]. The situation is similar for essential oil from leaves and stems, where limonene (23.42%), caryophyllene (9.74%), and terpinen-4-ol (7.97%) were the major constituents of leaf oil, and bicyclo[3.1.0]hexane (20.65%) and terpinen-4-ol (13.34%) were the most abundant compounds of twig oil from samples in China [9]. These differences may be due to differences in geographical location, soil conditions, and climate, which affect the accumulation of substances in plants.

Table 1. Composition of the essential oils from fruits, leaves, and stems of *Z. myriacanthum*

No	Rt	RI ^a	RI ^b	Name	ZMFR		ZML		ZMS	
					%	MF	%	MF	%	MF
1	6.254	931	932	α -Pinene	0.6	852				
2	7.453	972	974	β -Pinene	24.3	877				
3	9.104	1022	1020	<i>o</i> -Cymene	0.7	924				
4	9.265	1026	1024	Limonene	0.2	801				
5	9.393	1030	1026	1,8-Cineole			2.2	823		
6	9.951	1044	1044	(<i>E</i>)- β -Ocimene	0.2	868				
7	10.378	1056	1054	γ -Terpinene	0.1	788				
8	10.703	1064	1064	<i>meta</i> -Tolualdehyde	4.2	799				
9	11.911	1096	1099	2,5-Dimethyl styrene	0.9	796				

No	Rt	RI ^a	RI ^b	Name	ZMFR		ZML		ZMS	
					%	MF	%	MF	%	MF
10	11.953	1098	1098	<i>trans</i> -Sabinene hydrate			4.1	804	2.4	737
11	12.857	1119	1117	Dehydrosabina ketone	0.8	788				
12	12.882	1120	1118	<i>cis-p</i> -Menth-2-en-1-ol			0.4	787		
13	13.602	1137	1133	<i>cis-p</i> -Menth-2,8-dien-1-ol	0.6	746				
14	13.605	1137	1136	<i>trans-p</i> -Menth-2-en-1-ol			0.5	744	0.3	719
15	14.854	1167	1170	<i>cis</i> -Linalool oxide					0.3	710
16	14.949	1169	1172	<i>cis</i> -Pinocamphone	0.2	766				
17	15.057	1171	1173	<i>trans</i> -Linalool oxide					0.4	761
18	15.218	1175	1174	Terpinen-4-ol					13.2	739
19	15.291	1177	1179	<i>p</i>-Methyl-acetophenone			29.9	790		
20	15.524	1183	1179	<i>p</i>-Cymen-8-ol	24.9	792				
21	15.530	1183	1181	Thuj-3-en-10-al	0.6	823				
22	15.791	1189	1186	α-Terpineol	6.1	821	17.0	824	8.4	819
23	15.91	1192	1194	Myrtenol	0.1	732				
24	15.989	1194	1195	<i>cis</i> -Piperitol	0.2	710	0.4	798		
25	16.406	1203	1201	<i>n</i> -Decanal	2.8	836				
26	16.51	1206	1207	<i>trans</i> -Piperitol	0.9	721	0.6	727		
27	16.684	1210	1211	Octanol acetate	4.5	802				
28	16.957	1216	*1223±7	4-Vinylphenol			0.6	872		
29	17.066	1219	1221	Methyl-(2E)-nonenoate					1.5	732
30	17.084	1219	1217	β -Cyclocitral			0.2	792		
31	17.16	1221	*1219±9	<i>exo</i> -2-Hydroxycineole					0.6	760
32	17.163	1221	1226	<i>cis</i> -Carveol			0.3	716		
33	17.278	1224	1226	Neoisodihydrocarveol	0.1	743	0.4	751		
34	17.379	1226	1227	<i>cis-p</i> -Mentha-1(7),8-dien-2-ol					0.5	152
35	18.472	1251	1239	<i>trans</i> -Chrysanthenyl acetate	0.9	755	2.4	769		
36	18.515	1252	1254	Linalool acetate					0.5	743
37	19.125	1267	1261	<i>cis</i> -Chrysanthenyl acetate	0.9	770				
38	19.135	1267	1266	<i>trans</i> -Ascaridol glycol					0.5	738
39	19.275	1270	*1276±2	10-Undecenal	1.9	776				
40	19.88	1284	1280	<i>cis</i> -Verbenyl acetate	0.8	762	0.8	784		
41	20.06	1288	1289	<i>p</i> -Cymen-7-ol	0.3	841				
42	20.207	1292	1293	2-Undecanone			6.6	883	0.4	740
43	20.526	1299	1301	2-Undecanol			1.3	801	1.0	746
44	20.568	1300	1298	<i>trans</i> -Pinocarvyl acetate	0.7	729				
45	20.778	1305	1303	(<i>E</i>)-Cinnamyl alcohol	0.3	729	0.4	723		
46	20.977	1310	1311	<i>cis</i> -Pinocarvyl acetate	0.6	742				
47	21.392	1320	1324	Myrtenyl acetate	0.6	746				
48	22.377	1342	1339	<i>trans</i> -Carvyl acetate	0.2	758				
49	22.627	1349	1348	α -Cubebene	0.3	793				
50	23.012	1358	1356	Neoisodihydrocarveol acetate	0.2	706				
51	23.374	1366	1365	<i>cis</i> -Carvyl acetate	0.3	702				
52	23.564	1371	1367	<i>n</i> -Undecanol	0.2	758				
53	23.74	1375	1373	α -Ylangene	0.8	834				
54	23.912	1379	1380	Ethyl-(4 <i>E</i>)-decenoate					0.4	731
55	24.028	1382	1381	Ethyl 2-nonynoate					0.6	700
56	24.033	1382	1379	Geranyl acetate	1.2	835				
57	24.325	1389	1387	β -Cubebene	0.9	735				
58	24.472	1393	1390	7- <i>epi</i> -Sesquithujene	0.2	728				
59	25.058	1407	1408	Dodecanal	1.1	888				
60	25.147	1409	1407	Decyl acetate	3.7	843				
61	25.552	1419	1417	(<i>E</i>)-Caryophyllene	1.1	864				
62	26.934	1453	1452	α -Humulene	0.1	784				
63	27.307	1462	1464	α -Acoradiene	0.2	713				
64	27.561	1468	1476	Allyl decanoate			0.2	802		
65	27.717	1472	1469	<i>n</i> -Dodecanol	1.6	861				
66	27.9	1477	1479	<i>ar</i>-Curcumene			9.5	766	44.6	750
67	28.499	1491	*1497	(1 <i>R</i> ,2 <i>R</i> ,4 <i>R</i>)-4-(2-Hydroxypropan-2-yl)-1-methylcyclohexane-1,2-diol					8.8	761
68	28.526	1492	1489	<i>cis</i> -Eudesma-6,11-diene			0.3	728		
69	28.597	1494	1495	2-Tridecanone			5.9	860	1.3	702
70	29.408	1514	1514	Cubebol	0.1	774				
71	29.73	1523	1522	δ -Cadinene	0.3	779				
72	30.392	1540	1540	(<i>Z</i>)-Carpacin			0.2	760		
73	31.256	1562	1561	(<i>E</i>)-Nerolidol			1.4	802	1.2	792

No	Rt	RI ^a	RI ^b	Name	ZMFR		ZML		ZMS	
					%	MF	%	MF	%	MF
74	31.814	1577	1577	Spathulenol	0.2	843				
75	32.031	1582	1582	Caryophyllene oxide	1.2	822	1.9	805	0.3	702
76	32.468	1593	1593	(E)-Carpacin			7.7	885	2.4	733
77	33.015	1608	1607	Dodecyl acetate	2.9	845				
78	33.037	1608	1608	β -Atlantol			0.2	777		
79	33.228	1613	1607*	2-Methoxy-4,5-methylenedioxybenzaldehyde			0.2	782		
80	34.702	1651	1652	α -Cadinol			0.2	756		
81	34.856	1658	1658	Selin-11-en-4- α -ol			0.5	843		
82	35.314	1670	1671	(3Z)-Butylidene phthalide			0.2	855		
83	35.453	1674	1671	n-Tetradecanol	0.3	882				
84	35.583	1678	*1695 $\pm$ 20	3-Oxo-7,8-dihydro- β -ionol					0.5	740
85	37.558	1733	1734	(Z)-Ligustilide			0.2	869		
86	40.158	1807	*1810 $\pm$ 1	1-Tetradecyl acetate	0.5	828				
87	61.648	2549	*2535 $\pm$ 16	Bis(2-ethylhexyl) phthalate			0.2	808	0.4	746
Monoterpenes					26.1					
Oxygenated monoterpenes					41.2		29.1		35.7	
Sesquiterpenes					3.9		9.8		44.6	
Oxygenated sesquiterpenes					1.6		4.2		1.4	
Phenylethanoid							30.7			
Phenylpropanoid							8.3		2.4	
Others					25.1		14.6		6.1	
Total					97.9		96.7		90.3	

Note: RI^a: Experimental linear retention indices on Rxi-5MS column relative to *n*-alkanes; RI^b: Linear retention indices in the literature [10],[11], those marked with * are from NIST Chemistry Webbook [11]; MF: MS Match Factor.

3.2. Inhibitory activity on NO production of ZMFR, ZML, and ZMS

RAW 264.7 cells activated by LPS produce large amounts of NO. Therefore, LPS-induced NO production in murine macrophage RAW 264.7 cells was used to evaluate the anti-inflammatory potential of the essential oils. The results presented in Table 2 showed that all three essential oils ZMFR, ZML, and ZMS inhibited LPS-induced NO production in macrophages and were safe against this cell type (> 80% cell survival at the concentration of 100 μ g/mL), among which ZMFR exhibited remarkable inhibitory activity on NO production

with an IC₅₀ value of 42.35 $\pm$ 2.63 μ g/mL. This result is consistent with published results on the NO production inhibitory activity of essential oil from *Z. myriacanthum* fruits collected in China [5]. However, this is the first time that essential oils from the leaves and stems of this plant have been evaluated for this activity. Overproduction of NO causes pathological problems associated with acute and chronic inflammation, apoptosis and necrosis, or neurodegenerative diseases [15]. Therefore, these findings support the use of *Z. myriacanthum* parts as native detoxification and pain relief remedies.

Table 2. NO inhibitory activity of ZMFR, ZML, and ZMS

C (μ g/mL)	ZMFR		ZML		ZMS		Dexamethasone	
	I (%)	CS (%)	I (%)	CS (%)	I (%)	CS (%)	I (%)	CS (%)
100	99.55	92.81	44.41	98.38	35.29	99.33	89.54	86.51
20	27.19	93.98	13.24	99.24	10.00	99.40	52.5	93.71
4	18.89		8.24		7.06		39.59	
0.8	5.07		4.41		2.06		28.24	
IC ₅₀	42.35 $\pm$ 2.63	-	>100		>100		14.20 $\pm$ 0.54	

Note: I: inhibition, CS: cell survival, NA: not active.

3.3. Cytotoxic activity of ZMFR, ZML, and ZMS on cancer cell lines

The cytotoxicity of ZMFR, ZML, and ZMS on the human lung cancer cell line (SK-LU-1), human breast cancer cell line (MCF-7), and human hepatocellular cancer cell line (HepG2)

was evaluated using SRB assay. As shown in Table 3, ZMFR and ZML exhibited cytotoxic effects on all three tested cell lines. ZMFR showed the best cytotoxic activity against SK-LU-1 with an IC₅₀ value of 50.97 $\pm$ 4.07 μ g/mL, while ZML exhibited the most potent effect

against HepG2 with an IC_{50} value of 58.86 ± 2.27 $\mu\text{g/mL}$. ZMS was more active against HepG2 ($IC_{50} = 69.84 \pm 4.71$ $\mu\text{g/mL}$) than MCF-7 ($IC_{50} = 89.28 \pm 5.50$ $\mu\text{g/mL}$) and showed only minimal effect on SK-LU-1. In previous studies, the essential oils from *Z. myriacanthum* fruits

demonstrated antibacterial [5], anti-inflammatory [3], antiviral [6], renoprotective [3], and hepatoprotective [4] activities. Our results add to the evidence for the anticancer potential of not only essential oils from the fruit but also essential oils from the leaves and stems.

Table 3. Half-maximal inhibitory concentration of ZMFR, ZML, and ZMS on three cancer cell lines SK-LU-1, MCF-7, and HepG2

Samples	IC_{50} ($\mu\text{g/mL}$)		
	SK-LU-1	MCF-7	HepG2
ZMFR	50.97 ± 4.07	85.25 ± 5.14	79.04 ± 4.46
ZML	93.78 ± 3.67	73.77 ± 4.97	58.86 ± 2.27
ZMS	>100	89.28 ± 5.50	69.84 ± 4.71
Ellipticine	0.51 ± 0.05	0.43 ± 0.03	0.32 ± 0.03

The compounds identified in essential oil constituents such as β -pinene and α -terpineol certainly contributed significantly to the anti-inflammatory and cytotoxic activities of the essential oils from *Z. myriacanthum*, as β -pinene [16] and α -terpineol [17] have been proven to possess related activities, and *ar*-curcumen [18] is also the major constituent occurred in several essential oils known to have cytotoxic activity [19]. In general, the results of the bioactivity of the essential oils in this paper have contributed to provide additional evidence that this is an important group of substances that create the folk medicinal effects of *Z. myriacanthum*.

4. Conclusions

The hydrodistillation of *Z. myriacanthum* fruits, leaves, and stems yielded essential oils with 51, 32, and 23 compounds identified, respectively. The major compounds in the fruit oil were *p*-cymen-8-ol

(24.9%) and β -pinene (24.3%). The leaf oil mainly contained *p*-methyl-acetophenone (29.9%) and α -terpineol (17.0%). The most abundant compounds in the stem oil were *ar*-curcumen (44.6%) and terpinen-4-ol (13.2%). The fruit oil exhibited moderate inhibitory activity on NO production in macrophage RAW 264.7 cells. The fruit and leaf oils showed moderate cytotoxic effects on SK-LU-1, MCF-7, and HepG2 cancer cell lines. The stem oil was active against HepG2 and MCF-7 but showed minimal effect on SK-LU-1. This is the first report on the inhibition of NO production and cytotoxicity of *Z. myriacanthum* essential oils.

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CHEMICAL CONSTITUENTS FROM TWIGS AND LEAVES OF *JASMINUM SUBTRIPLINERVE* BLUME.

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Summary

Chemical Constituents from Twigs and Leaves of *Jasminum subtripplinerve* Blume.

Seven compounds, including verbascoside (1), *p*-hydroxybenzoic acid (2), protocatechuic acid (3), vanillic acid (4), kaempferol (5), quercetin (6), and β -sitosterol (7) were isolated from the ethyl acetate extract of *Jasminum subtripplinerve* twigs and leaves. The structures of these compounds were identified using extensive nuclear magnetic resonance spectroscopy (NMR) data, mass spectrometry (MS), and comparison with published literature. Compounds 4 and 6 were reported for the first time from the *Jasminum* genus. Furthermore, the quantification of verbascoside (1) in *J. subtripplinerve* was performed using the HPLC-DAD method. The determined contents of verbascoside in the leaves, twigs, and combined twigs and leaves samples of *J. subtripplinerve* were 2.77%, 0.46%, and 0.74%, respectively. These results suggest that verbascoside might be used as a marker for standardizing the quality of *J. subtripplinerve*'s twigs and leaves.

Keywords: *Jasminum subtripplinerve* Blume., Phenylethanoid glycoside, Flavonoids, Phenolic acids, Verbascoside.

1. Introduction

Jasminum subtripplinerve Blume., a species of Oleaceae, is widely distributed worldwide, growing naturally in the mountains and midlands of countries such as India, Myanmar, Cambodia, Laos, and the southern provinces of China [1]. In Vietnam, *J. subtripplinerve* can be found in numerous provinces across mountains, midlands, and plains, including Lao Cai, Hoa Binh, Hanoi, Nghe An, Ha Tinh, Quang Tri, and Thua Thien Hue [2]. Traditional medicine employs *J. subtripplinerve* leaves, which are primarily utilized to manage irregular menstruation in postpartum women with high fever, lymphadenitis, mastitis, breast abscess, leukorrhea, rheumatism-induced bone and joint pain, scabies, impetigo, and pruritic skin

conditions [3]. The plant serves various medicinal purposes, such as supporting liver function, promoting bile secretion, aiding digestion, improving appetite, facilitating sleep, and demonstrating antibacterial and anti-inflammatory properties [4]. Investigations into chemical composition and biological activities reveal that *J. subtripplinerve* twigs and leaves contain phenylethanoid glycosides (e.g., verbascoside), terpenoids, flavonoids (e.g., kaempferol), phenolic acids (e.g., *p*-hydroxybenzoic acid, protocatechuic acid), and steroids (e.g., β -sitosterol) [5]. These constituents contribute to the plant's antibacterial, anti-inflammatory, antioxidant, liver-protective, cytotoxic, and antipyretic effects [1],[5],[6],[7],[8],[9].