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Chemical Constituents and Cytotoxic Activities of Essential Oils from the Flowers, Leaves and Stems of *Zingiber striolatum* Diels

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Abstract: The purpose of this study was to investigate chemical composition and cytotoxic activities of essential oils from *Zingiber striolatum* Diels flowers, leaves and stems. 73, 68 and 66 compounds representing 97.0%, 94.8% and 93.7% of flowers, leaves and stems essential oils were identified using GC-FID and GC-MS, respectively. The main constituents of the flowers oil were β-phellandrene (28.5%), α-humulene (14.7%), β-pinene (8.2%), β-elemene (5.5%), humulene oxide II (3.5%), cryptone (3.3%) and tricosane (3.2%). The predominant components in the leaves oil were hexahydrofarnesyl acetone (24.7%), α-humulene (12.2%), phytol (11.9%), humulene oxide II (6.3%), β-pinene (4.3%), sandaracopimaradiene (3.1%) and β-elemene (3.0%). The stems oil contained mainly α-humulene (15.6%), humulene oxide II (7.9%), hexahydrofarnesyl acetone (7.4%), phytol (7.2%), humulene oxide I (4.1%), β-elemene (3.8%) and 4-terpineol (3.2%). The cytotoxic activity against human leukemic (K562), prostatic carcinoma (PC-3) and lung cancer (A549) cell lines of essential oils was assessed using MTT assay. The essential oils of flowers, leaves and stems exhibited significant cytotoxicity against K562 (IC_{50}: 12.94–37.89 µg/mL), PC-3 (IC_{50}: 69.06–82.56 µg/mL) and A549 (IC_{50}: 45.73–66.12 µg/mL) cell lines. This is the first report on the chemical constituents and cytotoxic activities of *Z. striolatum* flowers, leaves and stems essential oils.

Keywords: *Zingiber striolatum* Diels; essential oils; GC-FID/MS; cytotoxic activity.

1. Plant Source

The flowers, leaves and stems of *Z. striolatum* were collected from Guizhou Province of China in September 2018. Identity of the species was confirmed by Prof. Shenghua Wei of Guizhou University of Chinese Medicine. The voucher specimen (NO.1936) was deposited at Guizhou Engineering Center for Innovative Traditional Chinese Medicine and Ethnic Medicine, Guizhou University.

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2. Previous Studies

The genus *Zingiber*, as an important source of essential oil, is widely used as food and traditional medicinal plant [1,2]. *Zingiber striolatum* Diels, a perennial plant of this genus widely cultivated in China, is a unique healthy vegetable and used as a traditional Chinese medicine for treatment of abdominal pain and diarrhea [3,4]. In previous studies, the ethanol extract of *Z. striolatum* was found to possess nematicidal activity [5] and hypoglycemic activity [6]. It was reported that the major constituents of *Z. striolatum* rhizome essential oil were *β*-phellandrene (23.96%), sabinene (17.34%), *β*-pinene (11.36%) and the essential oil demonstrated significant antimicrobial and anticancer properties [7]. To the best of our knowledge, the chemical composition and cytotoxic activities of *Z. striolatum* flowers, leaves and stems essential oils have not been reported.

3. Present Study

The dry *Z. striolatum* flowers, leaves and stems (800 g) were separately subjected to hydrodistillation for 5 h using a Clevenger-type apparatus to obtain the essential oils. The flowers, leaves and stems essential oils were separately dried over anhydrous Na_2SO_4 and stored in amber bottle at 4°C until further analysis.

Table 1. Chemical composition of essential oils from the flowers, leaves and stems of *Z. striolatum*

Compounds[a]	**RI[b]**	**RI[c]**	**% Area**			**Identification[d]**
			flowers	**leaves**	**stems**	
α-Pinene	933	931-939[a,b,c]	1.7	0.2	-	MS, RI
Sabinene	973	954-976[a,b,c,]	0.6	0.2	-	MS, RI
β-Pinene	977	973-980[a,b,c]	8.2	4.3	0.2	MS, RI
β-Myrcene	992	988-991[a,b,c]	1.1	0.1	-	MS, RI
α-Phellandrene	1006	1005[b,c]	1.0	0.1	0.1	MS, RI
α-Terpipene	1017	1017-1018[a,c]	0.1	0.1	-	MS, RI
p-Cymene	1025	1023-1026[a,b,c]	1.4	0.1	0.1	MS, RI
β-Phellandrene	1032	1031[c]	28.5	1.2	0.5	MS, RI
cis-Ocimene	1038	1038-1040[b,c]	0.2	-	-	MS, RI
trans-Ocimene	1048	1048-1050[b,c,d]	0.9	0.1	-	MS, RI
γ-Terpinene	1059	1051-1062[a,b,c]	1.1	0.1	-	MS, RI
Tetramethylpyrazine	1086	1089[c]	0.3	-	-	MS, RI
α-Terpinolene	1088	1082-1088[a,b,c]	0.2	-	-	MS, RI
Linalool	1102	1093-1098[a,b,c,d]	1.7	0.1	0.2	MS, RI
Nonanal	1104	1100-1102[c,d]	-	0.1	-	MS, RI
Fenchol	1115	1113[c]	0.2	0.1	0.2	MS, RI
p-menth-2-en-1-ol	1123	1126[c]	0.5	0.1	0.1	MS, RI
Nopinone	1136	1137[c]	0.1	0.2	0.2	MS, RI
trans-Pinocarveol	1139	1139[c]	0.2	0.8	0.4	MS, RI
trans-Verbenol	1146	1144[c]	0.1	0.2	0.1	MS, RI
Pinocarvone	1162	1162-1164[b,c]	0.2	0.2	0.2	MS, RI
Borneol	1167	1165-1167[b,c]	0.4	0.1	0.3	MS, RI
Nonanol	1172	1165-1173[c,d]	0.2	-	0.2	MS, RI
4-Terpineol	1179	1169-1177[a,b,c,]	1.3	0.7	3.2	MS, RI
Cryptone	1187	1184[c]	3.3	0.4	2.7	MS, RI
α-Terpineol	1193	1177-1189[a,b,c]	0.7	0.5	1.1	MS, RI
Myrtenal	1196	1193[b,c]	0.1	0.3	0.4	MS, RI
Myrtenol	1198	1994-1213[b,c]	0.5	1.7	1.3	MS, RI
Sabinol	1204	1143[c]	0.2	-	-	MS, RI
Decanal	1206	1206[c]	-	-	0.3	MS, RI
trans-Piperitol	1209	1208[c]	0.3	0.1	0.1	MS, RI
trans-Carveol	1221	1217[c]	0.1	0.2	0.3	MS, RI
Cuminic aldehyde	1241	1239[c]	0.2	-	0.1	MS, RI
Carvone	1244	1243[c]	-	-	0.1	MS, RI
Piperitone	1254	1253[c]	0.1	0.1	0.2	MS, RI
2-Phenylcrotonaldehyde	1273	1274[c]	0.1	0.1	-	MS, RI
Phellandral	1275	1273[c]	1.4	0.1	0.6	MS, RI
Borneol acetate	1286	1284-1285[b,c]	0.1	-	0.1	MS, RI

Cuminic alcohol	1293	1289[c]	0.2	-	0.2	MS, RI
2-Undecanone	1294	1291-1294[b,c]	0.3	-	0.2	MS, RI
4-Vinyl-2-methoxy-phenol	1316	1317[c]	-	0.4	-	MS, RI
Myrtenyl acetate	1326	1327[c]	0.1	0.4	0.2	MS, RI
α-Cubebene	1351	1351[c]	0.1	0.1	0.1	MS, RI
α-Copaene	1377	1376[b,c]	0.3	0.1	0.6	MS, RI
β-Bourbonene	1386	1384[c]	0.2	0.4	0.5	MS, RI
β-Elemene	1395	1391[b,c]	5.5	3.0	3.8	MS, RI
γ-Caryophyllene	1407	1404-1415[b,c]	-	-	1.8	MS, RI
α-Gurjunene	1411	1409[c]	0.1	0.1	0.1	MS, RI
β-Caryophyllene	1421	1418-1425[a,b,c]	2.5	2.5	1.0	MS, RI
α-Ionone	1429	1426-1427[c,d]	-	0.5	-	MS, RI
γ-Elemene	1435	1433[b,c]	0.2	0.1	2.3	MS, RI
Geranylacetone	1455	1452-1454[c,d]	-	2.5	-	MS, RI
α-Humulene	1458	1454[b,c]	14.7	12.2	15.6	MS, RI
Aromadendrene	1463	1469[c]	-	0.1	0.3	MS, RI
Germacrene D	1483	1456-1481[a,b,c]	0.1	-	-	MS, RI
β-Ionone	1487	1485[c,d]	-	0.8	0.4	MS, RI
β-Selinene	1488	1485-1486[b,c]	0.4	0.3	1.0	MS, RI
2-Tridecanone	1497	1494-1497[c,d]	-	-	1.1	MS, RI
Bicyclogermacrene	1497	1495-1500[a,c]	0.7	0.8	-	MS, RI
Pentadecane	1501	1500[c,d]	1.8	0.1	0.5	MS, RI
Germacrene A	1507	1504[c]	0.3	0.1	-	MS, RI
β-Farnesene	1510	1508[a,b,c]	0.3	0.6	1.3	MS, RI
β-Bisabolene	1510	1509[c]	0.6	0.5	1.6	MS, RI
β-Sesquiphellandrene	1526	1524[c]	0.4	-	-	MS, RI
Germacrene B	1559	1557[c]	0.5	0.2	-	MS, RI
Nerolidol	1566	1560-1564[b,c,d]	0.1	2.1	1.5	MS, RI
Spathulenol	1581	1572-1576[c,d]	0.8	1.9	2.8	MS, RI
Caryophyllene oxide	1585	1581[b,c]	0.7	2.2	2.3	MS, RI
Humulene oxide I	1597	1596[c]	0.3	0.2	4.1	MS, RI
Humulene oxide II	1615	1606[b,c]	3.5	6.3	7.9	MS, RI
α-Bisabolol	1690	1683-1685[b,c]	-	0.1	0.4	MS, RI
2-Pentadecanone	1700	1697-1698[c,d]	-	-	0.5	MS, RI
Pentadecanal	1715	1715[c]	0.2	-	0.8	MS, RI
Farnesol	1726	1722[c]	0.2	0.3	0.6	MS, RI
Neophytadiene	1843	1837[c]	-	0.5	0.5	MS, RI
Hexahydrofarnesyl acetone	1850	1844[c]	0.2	24.7	7.4	MS, RI
Farnesyl acetone	1925	1913-1919[c,d]	-	2.1	1.0	MS, RI
Methyl palmitate	1929	1926[c]	0.2	0.1	1.5	MS, RI
Sandaracopimaradiene	1984	1968-1975[c,d]	-	3.1	2.9	MS, RI
Ethyl palmitate	1996	1993[c]	0.1	-	2.9	MS, RI
Isokaurene	2015	1998[c]	-	0.2	-	MS, RI
Kaurene	2065	2042-2061[c,d]	-	0.2	1.0	MS, RI
Methyl linolelaidate	2098	1980[c]	0.1	-	0.5	MS, RI
Methyl linolenate	2105	2098[c]	0.1	-	-	MS, RI
Phytol	2119	2114[c]	0.1	11.9	7.2	MS, RI
Ethyl linoleate	2166	2160-2162[c,d]	0.1	0.2	1.5	MS, RI
Ethyl linolenate	2174	2169-2171[c,d]	0.2	-	0.2	MS, RI
1-Tricosene	2284	2278[c]	0.1	-	-	MS, RI
Tricosane	2302	2300[c,d]	3.2	0.2	0.3	MS, RI
Squalene	2841	2832[c,d]	0.2	0.1	-	MS, RI
Monoterpene hydrocarbons			**45.0**	**6.5**	**0.9**	
Oxygenated monoterpenes			**8.1**	**5.3**	**8.9**	
Sesquiterpene hydrocarbons			**26.9**	**21.1**	**30.0**	
Oxygenated sesquiterpenes			**5.6**	**15.2**	**20.6**	
Diterpenes			**0.3**	**40.6**	**18.1**	
Others			**11.1**	**6.1**	**15.2**	
Total			**97.0**	**94.8**	**93.7**	

[a]Compounds are listed in order of their elution from a FB-5MSi column; [b]RI: Retention index on FB-5MSi column, calculated using homologous series of C_9–C_{30} alkanes; [c]RI: Retention index of literature; a) [8], b) [9], c) NIST 14 and Wiley 275 databases, d) [10]; [d]Identification: MS, based on comparison with Wiley 275 and NIST 14 MS databases; RI, based on comparison of calculated RI with those reported in the literature, Wiley 275 and NIST 14 databases; - : not detected.

The yields of the hydrodistilled flowers, leaves and stems essential oils were 0.39% (w/w), 0.27% (w/w) and 0.18% (w/w) of dry weight, respectively. The essential oils were identified and quantified by GC-FID and GC-MS and the results were presented in Table 1. A total of 73, 68 and 66 compounds representing 97.0%, 94.8% and 93.7% of the total oil were identified in essential oils of flowers, leaves and stems, respectively. The chemical constituents of the essential oils were classified as monoterpene hydrocarbons (0.9-45.0%), oxygenated monoterpenes (5.3-8.9%), sesquiterpene hydrocarbons (21.1-30.0%), oxygenated sesquiterpenes (5.6-20.6%), diterpenes (0.3-40.6%) and others (6.1-15.2%). The main constituents of the flowers oil were *β*-phellandrene (28.5%), *α*-humulene (14.7%), *β*-pinene (8.2%), *β*-elemene (5.5%), humulene oxide II (3.5%), cryptone (3.3%) and tricosane (3.2%). The major components of the leaves oil were hexahydrofarnesyl acetone (24.7%), *α*-humulene (12.2%), phytol (11.9%), humulene oxide II (6.3%), *β*-pinene (4.3%), sandaracopimaradiene (3.1%) and *β*-elemene (3.0%). The stems oil contained mainly *α*-humulene (15.6%), humulene oxide II (7.9%), hexahydrofarnesyl acetone (7.4%), phytol (7.2%), humulene oxide I (4.1%), *β*-elemene (3.8%) and 4-terpineol (3.2%). In our previous study, the main components of the *Z. striolatum* rhizomes oil were *β*-phellandrene (23.96%), sabinene (17.34%), *β*-pinene (11.36%), geranyl linalool (8.56%), 4-terpineol (8.27%), *α*-pipene (5.56%) and crypton (4.49%) [7]. These compounds are present in different ratios in other *Zingiber* species. For example, *β*-phellandrene was the main compound of *Z. spectabilis* inflorescence oil, which was a key ingredient of cleaning, cosmetic and medical products [11,12]. *α*-Humulene was found to be the main constituent in *Z. zerumbet* rhizome essential oil, which was known for its anti-inflammatory property [13,14]. Hexahydrofarnesyl acetone and phytol have been reported to be the major constituents in *Z. chrysanthum* leaf hexane extract, and hexahydrofarnesyl acetone was the oxidation product of phytol [15,16]

In the previous studies, essential oils from genus *Zingiber* have been characterized. The major constituents in the essential oil from *Z. nimmonii* rhizome were *β*-caryophyllene (42.2%) and *α*-humulene (27.7%) [1]. The most abundant constituents in the essential oil from *Z. officinale* rhizome were reported as zingiberene and *ar*-curcumene [1]. Zerumbone was the predominant component in the rhizome oil of *Z. zerumbe*t, but the major components of its leaves and flowers oil were *(E)*-nerolidol, *β*-caryophyllene and linalool [9,13]. The main components of *Z. cassumunar* rhizome essential oil were terpinen-4-ol (40.5%) and sabinene (17.4%) [17]. The major components of the essential oil from *Z. spectabile* leaf were *β*-caryophyllene (21.3%) and *β*-elemene (12.5%), the main component in its rhizome oil was zerumbone (59.1%) [18]. The main constituents of the rhizome oil from *Z. anamalayanum* were *δ*-2-carene (52.83%), camphene (9.83%), endo-fenchol (9.42%) [19]. In the present study, the most abundant constituents in *Z. striolatum* flowers, leaves and stems essential oils were *β*-phellandrene (0.5-28.5%), *α*-humulene (12.2-15.6%), hexahydrofarnesyl acetone (0.2-24.7%) and phytol (0.1-11.9%). The chemical constituents of *Z. striolatum* essential oils varied according to different parts.

The cytotoxic activities of *Z. striolatum* flowers, leaves and stems essential oils were evaluated against human leukemic (K562), prostatic carcinoma (PC-3) and lung cancer (A549) cell lines using MTT assay. The results were presented in Table 2. The cytotoxic activities of the essential oils were compared with cisplatin as positive control. The essential oils of flowers, leaves and stems exhibited significant cytotoxicity against A549 (IC_{50}: 45.73–66.12 μg/mL), PC-3 (IC_{50}: 69.06–82.56 μg/mL) and K562 (IC_{50}: 12.94–37.89 μg/mL) cell lines in a concentration-dependent manner. The cytotoxic activities of the flowers, leaves and stems essential oils can be attributed to the high content of terpenoids, such as *α*-humulene, *β*-pinene, *β*-elemene and phytol, which have been reported to have cytotoxic activity [20-23]. In addition, *β*-caryophyllene, as a chemical component with less content in *Z. striolatum* essential oils, significantly potentiated the cytotoxic activity of *α*-humulene against human breast adenocarcinoma cell line (MCF-7) [24]. Therefore, the cytotoxic activities of *Z. striolatum* flowers, leaves and stems essential oils may be attributed to the specific chemical constituents, and/or synergies between various components.

Z. zerumbet rhizome oil exhibited significant cytotoxicity towards human cancer cell lines (A549, MDAMB-231, A431, K562, WRL-68, and HaCaT) with IC_{50} values (33.37–46.02 μg/mL) [13]. *Z. officinale* essential oil was found strong cytotoxic activity against human cancer cell lines (HO-8910 and Bel-7402) and IC_{50} values were 0.00643 and 0.00256% (v/v), respectively [25]. In the present study, the essential oils of *Z. striolatum* flowers, leaves and stems exhibited significant cytotoxicity against A549 (IC_{50}: 45.73–66.12 μg/mL), PC-3 (IC_{50}: 69.06–82.56 μg/mL) and K562 (IC_{50}: 12.94–37.89 μg/mL) cell lines. The cytotoxic activity of the essential oils against K562 cell line was found significantly higher than that against PC-3 and A549 cell lines ($p < 0.05$).

Table 2. Cytotoxic activity of essential oils from the flowers, leaves and stems of *Z. striolatum*

Treatment	**Cell line (IC_{50} μg/mL)[a]**		
	K562[b]	**PC-3[c]**	**A549[d]**
Flowers	26.75 ± 1.28	82.56 ± 2.83	52.65 ± 1.82
Leaves	37.89 ± 1.67	77.20 ± 1.87	45.73 ± 1.56
Stems	12.94 ± 1.38	69.06 ± 2.31	66.12 ± 2.34
Cisplatin	3.42 ± 0.28	10.26 ± 0.64	7.98 ± 0.23

[a]IC_{50}: The concentration of compound that affords a 50% reduction in cell growth (after 48 h of incubation); [b]Human leukemic cell line; [c]Human prostatic carcinoma cell line; [d]Human lung cancer cell line.

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Supporting Information

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