SHORT REPORT

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Chemical Composition, Antibacterial, Antioxidant and Cytotoxic Activities of the Essential Oil of *Dianella ensifolia*

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Abstract: The essential oil (EO) was isolated from aerial parts of *Dianella ensifolia* (L.) DC by hydro-distillation and its chemical constituents was determined by GC-FID and GC-MS. In total, sixty-three compounds comprising 97.2% of the EO were identified. The major compounds in *D. ensifolia* EO were found to be *allo*-aromadendrene (7.3%), geranylacetone (6.2%), hexahydrofarnesyl acetone (4.4%), longifolene (4.2%) and *β*-caryophyllene (4.0%). Besides, the essential oil was evaluated for its antibacterial activity by disc diffusion and broth microdilution method. The *D. ensifolia* EO exhibited a potential broad-spectrum *in vitro* antibacterial activity against both Gram-positive and Gram-negative bacteria. Also, antioxidant activities of the EO were examined by employing DPPH, ABTS as well as FRAP assays. A weak to moderate antioxidant activity of the EO was observed. Furthermore, *in vitro* cytotoxic activity evaluation against the cell lines HepG2 and MCF-7 by MTT method showed a potent cytotoxicity with IC_{50} values of 61.35 µg /mL and 56.53 µg /mL, respectively.

Keywords: *Dianella ensifolia* (L.) DC; essential oil; antibacterial activity; antioxidant activity; cytotoxic activity. © 2019 ACG Publications. All rights reserved.

1. Plant Source

The fresh aerial parts of *Dianella ensifolia* (L.) DC were collected in May 2018 from Guigang in Guangxi Province of China. The plant material (voucher specimen NO.0180036) was identified by Prof. Hong Zhao and deposited at the Laboratory of Botany of Marine College, Shandong University, China.

2. Previous Studies

Dianella ensifolia (L.) DC., an evergreen perennial herb belonging to the genus *Dianella* (Liliaceae family), is widely distributed in the south of China. In Chinese traditional medicine, *D. ensifolia* is widely used for treating lymphangitis, tinea and carbuncle sore abscess [1]. Previous phytochemical investigations conducted with various parts of *Dianella ensifolia* have resulted in the

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isolation and identification of triterpenoids [2] and flavans [3] from the roots, dihydronaphtaquinone and quinones from the leaves [4] and propanes from the methanol extract of the plant [5, 6].

3. Present Study

To the best of our knowledge, no study regarding *D. ensifolia* essential oil has been performed. Here we analyzed the chemical constituents of the essential oil extracted from the aerial parts of *D. ensifolia* and evaluated its antibacterial, antioxidant and cytotoxic activities (the experiments details were provided in Supporting Information). The air-dried aerial parts of *D. ensifolia* were isolated by hydrodistillation method and the EO was obtained in the yield of 0.28% (v/w). Sixty-three compounds accounting for 97.2% of the total essential oil were determined by GC-FID and GC-MS (Table 1). The predominant components of *D. ensifolia* EO were identified as *allo*-aromadendrene (7.3%), geranylacetone (6.2%), hexahydrofarnesyl acetone (4.4%), longifolene (4.2%) and *β*-caryophyllene (4.0%). It is interesting to note that these predominant compounds had been demonstrated to exhibit extensive biological activities. *Allo*-aromadendrene, the epimer of the tricyclic aromadendrene, has been demonstrated to significantly inhibit cell growth and proliferation in the highly malignant + SA mammary epithelial cells at a dose of 20 μM [7], and possessed a potent *in vivo* protective effect against juglone-induced oxidative stress in the *Caenorhabditis elegans* and prolonged its lifespan [8]. Also, Hexahydrofarnesyl acetone had been proven to exhibit a potent antimicrobial and cytotoxic activity [9, 10]. In addition, geranylacetone played an important role in the plant-insects interactions [11], and had a potential effect to inhibit the growth of Gram-positive bacteria [12].

Table 1. Chemical composition of the essential oil of *D. Ensifolia*

Compound[a]	**RI**[b]	**RI**[c]	**RI range**[d]	**%**
Cumene	921	924[e]	-	1.3
1-Octen-3-ol	978	974[e]	967-991	1.1
Furfuryl acetate	990	987[e]	-	1.4
Linalool	1098	1095[e]	1088-1109	2.2
(*E*)-Sabinol	1140	1137[e]	1134-1142	1.3
p-Methoxystyrene	1155	1158[f]	-	0.6
(*E*)-2-Nonenal	1158	1157[e]	1154-1173	0.5
α-Terpineol	1190	1186[e]	1178-1203	1.0
Thymol methyl ether	1235	1232[e]	-	0.7
(*E*)-2-Decenal	1261	1260[e]	1255-1276	0.3
Dihydroedulan	1293	1293[f]	-	0.6
Theaspirane	1304	1302[f]	-	0.3
Eugenol	1359	1356[e]	1345-1375	0.8
Silphiperfol-5,7(14)-diene	1361	1358[e]	-	0.8
Cyclosativene	1372	1369[e]	1360-1380	0.6
α-Copaene	1376	1374[e]	1363-1391	1.4
2-*epi*-*α*-Funebrene	1381	1380[e]	-	0.8
Iso-longifolene	1388	1389[e]	-	0.6
β-Elemene	1392	1389[e]	1374-1402	1.6
α-Gurjunene	1408	1409[e]	1394-1421	1.3
Longifolene	1410	1407[e]	1387-1434	4.2
β-Cedrene	1421	1419[e]	1415-1434	0.6
β-Caryophyllene	1420	1417[e]	1405-1440	4.0
Aromadendrene	1437	1439[e]	1419-1465	0.8
(*Z*)-*β*-Farnesene	1440	1440[e]	1438-1460	0.6
α-Himachalene	1447	1449[e]	1428-1453	0.5
Geranyl acetone	1452	1453[e]	1435-1461	6.2
allo-Aromadendrene	1458	1458[e]	1443-1477	7.3
Rotundene	1460	1457[e]	-	0.8

Compound[a]	RI[b]	RI[c]	RI range[d]	%
γ-Muurolene	1480	1478[e]	1461-1487	1.3
(*E*)-*β*-Ionone	1489	1487[e]	1470-1498	0.5
δ-Selinene	1494	1492[e]	-	1.5
α-Selinene	1501	1498[e]	1477-1510	1.5
β-Bisabolene	1507	1505[e]	1494-1525	1.1
δ-Cadinene	1523	1522[e]	1508-1539	1.7
Isoshyobunone	1533	1535[f]	-	0.5
(*Z*)-Sesquisabinene hydrate	1539	1542[e]	1524-1562	0.3
Selina-3,7(11)-diene	1543	1545[e]	1531-1546	1.8
Elemol	1549	1548[e]	1518-1555	0.8
β-Vetivenene	1558	1554[e]	-	1.0
(*E*)-Nerolidol	1562	1561[e]	1539-1570	1.5
Viridiflorol	1592	1592[e]	1569-1604	2.2
Cedrol	1604	1600[e]	1587-1616	1.9
Widdrol	1618	1617[f]	-	1.2
α-Cadinol	1649	1652[e]	1635-1664	1.6
Methyl jasmonate	1657	1655[f]		0.8
Neointermedeol	1661	1658[e]	-	2.6
(*E*)-2-Tetradecenal	1674	1673[f]	-	1.5
2-Pentadecanone	1694	1697[e]	1685-1716	1.0
Pentadecanal	1713	1715[f]	1703-1728	1.2
(2*E*,6*E*)-Farnesal	1739	1740[e]	-	0.5
Tetradecanoic acid	1756	1758[f]	1749-1782	1.2
Benzyl benzoate	1763	1759[e]	1735-1785	1.9
β-Costol	1767	1765[e]	-	1.7
α-Costol	1776	1773[e]	-	0.9
Neophytadiene	1838	1841[f]	-	2.4
Perhydrofarnesyl acetone	1844	1847[f]	-	4.4
(5*E*,9*E*)-Farnesyl acetone	1915	1913[e]	1918-1921	2.7
Isophytol	1946	1946[e]	1939-1951	1.1
(*E*)-15,16-Dinorlabda-8(17),11-dien-13-one	1994	1994[f]	-	1.0
Kaurene	2043	2042[e]	-	1.8
Oleic Acid	2141	2141[e]	2102-2161	2.1
(*E*)-Phytyl acetate	2214	2218[e]		3.8
Total identified				**97.2**

[a] Compounds are listed in order of their elution from a HP-5MS column; [b] **RI:** Linear retention index relative to C_8-C_{30} *n*-alkanes on HP-5MS column; [c] **RI:** Relative retention index that refers to Adams (2017) and/or Andriamaharavo (2014). e) [13], f) [14]; [d] **RI range:** 90% confidence retention index range reported by Babushok et al. (2011) [15].

The antibacterial property of the EO was estimated by disc diffusion [16] and micro dilution method [16] and the results are expressed as diameters of inhibition zones (DIZs), minimum inhibitory concentrations (MICs) and minimum bactericidal concentrations (MBCs) in Table 2. The *D. ensifolia* oil effectively inhibited the growth of all studied bacteria strains with the DIZ values ranged from 10.5 ± 1.3 to 16.3 ± 1.7 mm and the MIC values ranged from 0.16 to 0.31 mg/mL. The MBC/MIC ratio is no more than 2, which suggested that the *D. ensifolia* essential oil presented a good bactericidal effect for the tested bacterial strains. The good antibacterial activity of the *D. ensifolia* essential oil observed may be associated with the presence of geranylacetone, hexahydrofarnesyl acetone, longifolene and *β*-caryophyllene, which were reported to have antibacterial effects against many bacterial strains [9, 12, 17, 18]. However, synergistic effect between constituents should not be neglected, since it may cause a much more noticeable effect than single component [19].

Table 2. Antibacterial activity of *D. ensifolia* essential oil

Test strains	[a]Diameter of the inhibition zones (mm)		MIC (mg/mL)		MBC (mg/mL)	
	EO (10 µg/disk)	[a]Ch (0.1 µg/disk)	EO	Ch	EO	Ch
Gram positive						
Staphylococcus aureus ATCC 6538	15.5 ± 1.4	32.1 ± 1.2	0.16	0.004	0.16	0.16
Bacillus subtilis ATCC 6633	10.5 ± 1.3	36.5 ± 2.5	0.31	0.002	0.63	0.02
Gram negative						
Escherichia coli ATCC 25922	16.3 ± 1.7	35.8 ± 2.3	0.16	0.002	0.31	0.06
Pseudomonas aeruginosa ATCC 27853	11.1 ± 0.9	13.8 ± 1.4	0.31	0.125	0.63	2.50

[a] positive control (Ch, chloramphenicol)

The antioxidant capacity of the EO was examined using various methods, namely, 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging, 2,20-azinobis-3-ethylbenzothiazoline-6-sulphonate (ABTS) cation radical scavenging, and ferric reducing antioxidant potential (FRAP) assays [16, 20]. Trolox and BHT (butylated hydroxytoluene) were used as the positive controls. The results of DPPH assay demonstrated that the *D. ensifolia* essential oil showed a weak radical scavenging activity with IC_{50} of 1.37 mg/mL, which was much higher than the IC_{50} values of reference standards, BHT (IC_{50} of 0.005 mg/mL) and Trolox (IC_{50} of 0.036 mg/mL). Nevertheless, a moderate antioxidant activity was observed in the ABTS assay (IC_{50} value of 0.13 mg/mL). In FRAP assay, the ferric ion reducing capacity was expressed in TEAC (Trolox equivalent antioxidant concentration) units (µmol Trolox × g^{-1} EO). The result revealed a moderate reducing activity of the EO (TEAC = 134.70 µmol Trolox × g^{-1}). The most abundant compounds in the *D. ensifolia* essential oil, viz., *allo*-aromadendrene, geranylacetone, and *β*-caryophyllene have been reported to exhibit antioxidant activity [8, 11, 18].

Table 3. *In vitro* antioxidant activity (DPPH, ABTS and FRAP) of *D. ensifolia* essential oil

Sample	DPPH IC_{50} (mg/mL)	ABTS IC_{50} (mg/mL)	FRAP (µmol Trolox × g^{-1})
EO	1.37 ± 0.12	0.13 ± 0.02	134.70 ± 5.56
BHT	0.005 ± 0.001	0.003 ± 0.001	
Trolox	0.036 ± 0.005	0.038 ± 0.004	

The cytotoxicity of the EO was evaluated against HepG2 (liver hepatocellular cells) and MCF-7 (human breast adenocarcinoma cell line) cell lines using the MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] assay [16]. Doxorubicin was used as the positive control. The result is given in Table 4. The essential oil of *D. ensifolia* exerted a dose dependent cytotoxic effect on HepG2 and MCF-7 tumor cell lines with IC_{50} values of (61.35 ± 14.57) µg /mL and (56.53 ± 11.54) µg /mL, respectively. The interesting cytotoxic activity toward cell lines could be due to the main compounds in the essential oil. In addition to *allo*-aromadendrene and hexahydrofarnesyl acetone, literature reported that *β*-caryophyllene also showed potential ability to inhibit tumour motility, cell invasion and tumour aggregation [18].

Table 4. Cytotoxicity of *D. ensifolia* essential oil against HepG2 and MCF-7 cells

	HepG2 IC_{50} (µg/mL)		MCF-7 IC_{50} (µg/mL)	
	EO	Doxorubicin	EO	Doxorubicin
24h	110.33 ± 13.64	1.43 ± 0.24	93.68 ± 6.32	0.61 ± 0.23
48h	92.13 ± 6.65	0.48 ± 0.01	78.63 ± 6.68	0.18 ± 0.07
72h	61.35 ± 14.57	0.27 ± 0.04	56.53 ± 11.54	0.07 ± 0.02

Supporting Information

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