SHORT REPORT

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Chemical Composition, Antibacterial, Synergistic Antibacterial and Cytotoxic Properties of the Essential Oil from *Gelsemium elegans* (Gardner & Champ.) Benth.

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Abstract: This study aimed to analyze the chemical composition of the essential oil (GE-EO) isolated from *Gelsemium elegans* (Gardner & Champ.) Benth. aerial parts by GC/FID and GC/MS, and to evaluate its antibacterial, cytotoxic, and synergistic antibacterial properties. A total of 40 compounds were characterized, representing 95.1% of the total oil. The major constituents were identified as *α*-terpineol (18.8%), *n*-pentadecanal (11.5%), methyl hexadecanoate (7.2%), *n*-tetradecanol (5.2%) and linalool (4.1%). In microbroth dilution tests, GE-EO demonstrated antibacterial activities against *Staphylococcus aureus*, *Bacillus subtilis*, and *Escherichia coli* with minimum inhibitory concentrations (MICs) ranging from 0.16 to 0.32 mg/mL. In addition, significant synergistic effects were observed in both combinations of GE-EO with chloramphenicol and streptomycin. Based on the MTT assay, GE-EO was found to have broad-spectrum cytotoxicities against the A-549, MCF-7, HepG2, HCT-116, and HL-7702 cell lines with IC_{50} values ranging from 60.51 ± 1.08 to 159.56 ± 9.13 μg/mL.

Keywords: *Gelsemium elegans*; essential oil; antibacterial; synergistic; cytotoxic. © 2023 ACG Publications. All rights reserved.

1. Plant Source

The aerial parts of *Gelsemium elegans* (Gardner & Champ.) Benth. were harvested in Rong County, Guangxi Province, China in June 2021. The plant was identified by Dr. Hong Zhao and a voucher specimen was deposited in the herbarium of Institute of Botany, Chinese Academy of Sciences (PE02064381).

2. Previous Studies

The genus *Gelsemium* (family Loganiaceae) comprises three species, of which *Gelsemium elegans* (Gardner & Chapm.) Benth. is a poisonous liana native to China and Southeast Asia [1]. In Chinese folk medicine, it is used for the treatment of pain, spasticity, ulcers, inflammation, and

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gastrointestinal cancer [2]. Alkaloids, the primary active compounds in the *Gelsemium elegans*, have been extensively investigated for their biological properties in a variety of pharmaceutical fields, such as analgesic, anti-inflammatory [3], and anti-tumor activities [4, 5]. To the best of our knowledge, this is the first report on the chemical composition, antibacterial and cytotoxic activities of GE-EO, as well as the synergistic interactions of GE-EO with commercial antibiotics.

3. Present Study

In the present study, hydrodistillation of the aerial parts of *G. elegans* produced a pale-yellow oil, with a yield of 0.12% (w/w, based on the dry weight). The constituents of GE-EO were analyzed by GC/FID and GC/MS. Forty compounds were identified in the essential oil of *G. elegans*, accounting for 95.1% of the total content of GE-EO (Table 1). Oxygenated monoterpenes (36%), and oxygenated sesquiterpenes (16.8%) were dominant in the essential oil. The major components in GE-EO were identified as *α*-terpineol (18.8%), *n*-pentadecanal (11.5%), methyl hexadecanoate (7.2%), *n*-tetradecanol (5.2%) and linalool (4.1%). *α*-Terpineol, the most abundant compound among the identified constituents, is a natural monocyclic monoterpene tertiary alcohol that possesses a broad range of biological properties including antimicrobial [6], antioxidant, anti-inflammatory, anti-nociceptive, and anticancer activities [7].

Table 1. Chemical composition of GE-EO

Compounds	**RI** [a]	**RI$_{lit}$** [b]	**RI range** [c]	**%**
Linalool	1098	1095[d]	1088–1109	4.1
α-Terpineol	1190	1186[d]	1178–1203	18.8
2-Hydroxycineol	1223	1229[e]	1218-1252	3.2
(2*E*,4*Z*)-Decadienal	1292	1292[f]	1287–1310	1.2
Methyl geranate	1316	1322[d]	1316–1331	0.8
Sobrerol	1378	1388[e]	1388[e]	1.3
(3*Z*)-Hexenyl-(3Z)-hexenoate	1383	1383[d]	1389[f]	1.3
(*E*)-Caryophyllene	1414	1417[f]	1405–1440	2.4
Carvone hydrate	1425	1424[f]	1424[f]	2.1
Aromadendrene	1441	1439[e]	1419–1465	1.6
α-Terpinyl isobutanoate	1471	1471[d]	1467[f]	0.8
Dehydro-*β*-ionone	1482	1485[d]	1466-1492	1.7
(*E*)-*β*-Ionone	1486	1487[d]	1470–1498	2.0
(*Z*)-*α*-Bisabolene	1507	1506[f]	1495–1509	1.5
cis-Calamenene	1530	1528[e]	1511–1541	0.9
Dihydroactinidiolide	1534	1535[e]	1489-1540	1.2
(*E*)-Nerolidol	1562	1561[d]	1539–1570	1.2
(3*Z*)-Hexenyl benzoate	1571	1565[d]	1552–1588	1.3
Ledol	1574	1571[f]	1549–1599	1.3
Caryophyllene oxide	1587	1582[d]	1563–1595	2.0
Viridiflorol	1595	1592[d]	1569–1604	1.0
Tetradecanal	1608	1611[d]	1605–1623	2.3
Isospathulenol	1614	1630[f]	1621–1641	1.4
Ledene oxide-(II)	1629	1631[f]	1630-1673	1.3
τ-Muurolol	1644	1640[d]	1623–1654	1.1
Neointermedeol	1659	1658[d]	1654–1677	2.1
n-Tetradecanol	1672	1671[d]	1668–1686	5.2
Cadalene	1679	1675[d]	1652–1680	1.9
trans-Calamenen-10-ol	1687	1676[e]	1678[f]	2.0
n-Pentadecanal	1710	1715[e]	1703–1728	11.5
Hexahydrofarnesyl acetone	1840	1847[e]	1831–1855	1.8
Benzyl salicylate	1870	1864[d]	1857–1881	1.5
Methyl hexadecanoate	1920	1921[d]	1910–1931	7.2
Isophytol	1943	1946[d]	1939–1951	0.5

Compounds	RI [a]	RI_{lit} [b]	RI range [c]	%
Methyl linolenate	2089	2098[f]	2069-2108	0.8
Phytol	2108	2114[e]	2104–2136	0.8
Methyl octadecanoate	2120	2124[d]	2110–2139	0.5
Linoleic acid	2137	2132[d]	2097–2158	0.7
Gamolenic acid	2147	2144[f]	2144[f]	0.3
Ethyl linolenate	2172	2173[f]	1088–1109	0.5
Total identified				**95.1**

[a]Retention index calculated from n-alkanes (C_7-C_{30}) on HP-5MS column; [b]Linear retention indices from literature: [d][8]; [e][9]; [f][10]; [c]RI range: range of retention indices [10, 11].

Antibacterial Activity of GE-EO: The GE-EO was evaluated for antibacterial activity by the microbroth dilution method against four bacterial strains: *Bacillus subtilis* (ATCC 6633), *Staphylococcus aureus* (ATCC 6538), *Escherichia coli* (ATCC 25922), and *Pseudomonas aeruginosa* (ATCC 27853) [12]. The results in Table 2 showed that the GE-EO displayed strong growth inhibition activities against *S. aureus*, *B. subtilis*, and *E. coli* with MIC values ranging from 0.156 to 0.320 mg/mL, and MBC values from 0.320 to 0.640 mg/mL, and moderate activity against *P. aeruginosa*. The antibacterial activity may be due to the presence of abundant volatile terpenoids such as α-terpineol and linalool, which have been extensively studied for antibacterial activities [6, 13-15]. Linalool has previously been reported to inhibit bacterial growth by disrupting the cell membrane [16], and α-terpineol showed antibacterial activity against *E. coli* by inducing morphostructural changes directly in *E. coli*. [6].

Table 2. Antibacterial activity of GE-EO

Test strains	MIC (mg /mL)		MBC (mg /mL)	
	GE-EO	Ch [a]	GE-EO	Ch [a]
Gram-positive				
Staphylococcus aureus (ATCC 6538)	0.160	0.004	0.320	0.008
Bacillus subtilis (ATCC 6633)	0.320	0.004	0.320	0.016
Gram-negative				
Escherichia coli (ATCC 25922)	0.320	0.004	0.640	0.008
Pseudomonas aeruginosa (ATCC 27853)	0.640	0.032	1.280	0.256

[a] Positive control: Chloramphenicol.

Synergistic Effect of GE-EO with Conventional Antibiotics: The synergistic interactions of GE-EO with the antibiotics chloramphenicol and streptomycin against four pathogens were tested using the checkerboard method [17]. The FICI (Fraction Inhibition Concentration Index) of GE-EO with chloramphenicol or streptomycin are shown in Tables 3 and 4, respectively. The results showed that GE-EO combined with both chloramphenicol and streptomycin exhibited significant synergistic effects on all tested bacteria strains, with FICI values of 0.25-0.50 mg/mL. Additionally, the results of the checkerboard test also demonstrated that the combinations of GE-EO and conventional antibiotics effectively optimize the antibacterial effect of both. Therefore, the strategy of using GE-EO in combination with traditional antibiotics has the potential to treat infections and reverse bacterial resistance.

Table 3. FICI values of GE-EO and chloramphenicol combinations

Microorganism		MIC_a, μg/mL	MIC_c, μg/mL	FICI
Staphylococcus aureus	GE-EO	160.00	40.00	0.31 (S)
ATCC 6538	Ch	4.00	0.25	
Bacillus subtilis	GE-EO	320.00	40.00	0.25 (S)
ATCC 6633	Ch	4.00	0.50	
Escherichia coli	GE-EO	320.00	80.00	0.50 (S)
ATCC 25922	Ch	4.00	1.00	
Pseudomonas aeruginosa	GE-EO	640.00	80.00	0.25 (S)
ATCC 27853	Ch	32.00	4.00	

MICa: MIC alone; MICc: MIC combined; Chl: chloramphenicol. S, synergy (FICI ≤ 0.5).

Table 4. FICI values of GE-EO and Streptomycin combinations

Microorganism		MIC_a, μg/mL	MIC_c, μg/mL	FICI
Staphylococcus aureus	GE-EO	160.00	40.00	0.50 (S)
ATCC 6538	SM	2.00	0.50	
Bacillus subtilis	GE-EO	320.00	80.00	0.38 (S)
ATCC 6633	SM	4.00	0.50	
Escherichia coli	GE-EO	320.00	80.00	0.38 (S)
ATCC 25922	SM	4.00	0.50	
Pseudomonas aeruginosa	GE-EO	640.00	40.00	0.31 (S)
ATCC 27853	SM	8.00	2.00	

SM: streptomycin.

Cytotoxic Activity of GE-EO: MTT assay was used to evaluate the potential cytotoxic activity of GE-EO on four human cancer cells (HepG2 liver cancer cells, MCF-7 breast cancer cells, A-549 lung cancer cells, and HCT-116 colon cancer cells) and one non-cancerous cell (human normal liver cells HL-7702) [17]. Doxorubicin was used as a positive control. As shown in Figure 1 and Table 5, GE-EO exerted a dose-dependent cytotoxic effect on all of the cell lines used in the experiment. The most susceptible to the action of GE-EO were HCT-116 cancer cell line with an IC_{50} value of 60.51±1.08 μg/mL after 48 h treatment, followed by the cell lines HL-7702 (IC_{50} =70.04 ± 3.76 μg/mL), MCF-7 (IC_{50} =105.35 ± 4.76 μg/mL), HepG2 (IC_{50} =112.99 ± 6.26 μg/mL) and A-549 (IC_{50} =159.56 ± 9.13 μg/mL). The cytotoxic activities of GE-EO could be mainly attributed to the major compounds of the essential oil such as *α*-terpineol and linalool, the cytotoxic activities of which have already been investigated previously [7, 18-20], as well as the interactions of the individual constituents. Previous studies have shown that linalool exerts cytotoxic effects by inducing cell apoptosis and cell death, inducing cancer-specific oxidative stress, and activating antitumor immunity [18, 19]. Hassan et al. reported that *α*-terpineol inhibited growth and induced cell death in various tumor cells by blocking NF-kB expression [20].

Table 5. Cytotoxicity (IC_{50} μg/mL) of GE-EO

	GE-EO	Doxorubicin
HepG2	112.99 ± 6.26	0.46 ± 0.02
MCF-7	105.35 ± 4.76	0.70 ± 0.05
HL-7702	70.04 ± 3.76	0.60 ± 0.13
A-549	159.56 ± 9.13	0.48 ± 0.01
HCT-116	60.51 ± 1.08	0.57 ± 0.03

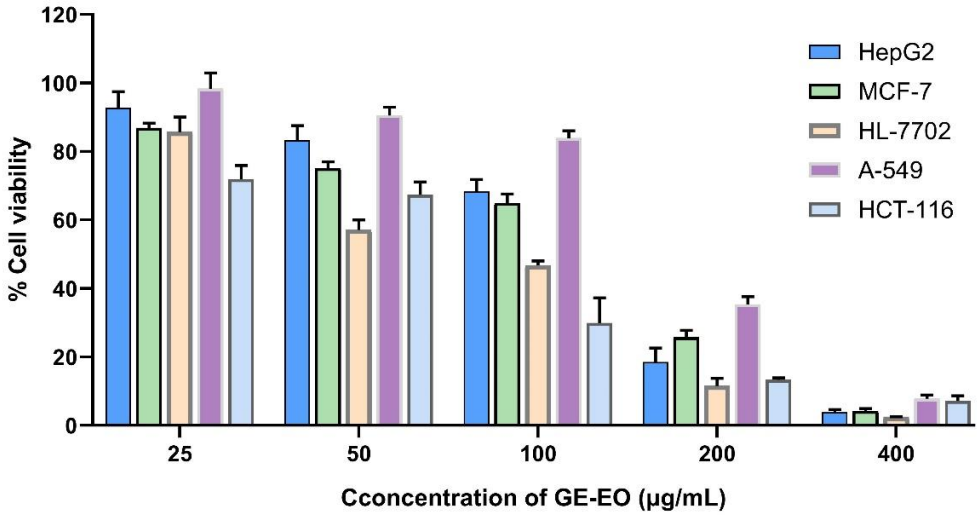

Figure 1. Cytotoxic activity of GE-EO ($P < 0.05$).

In conclusion, the major components of the essential oil distilled from the aerial parts of *Gelsemium elegans* were determined to be *α*-terpineol (18.8%), n-pentadecanal (11.5%), methyl hexadecanoate (7.2%), n-tetradecanol (5.2%), and linalool (4.1%). The essential oil of *Gelsemium elegans* displayed potential antibacterial activities against *S. aureus*, *B. subtilis,* and *E. coli* with MICs ranging from 0.16 to 0.32 mg/mL. Furthermore, synergistic antibacterial effects were observed when *Gelsemium elegans* essential oil was combined with the antibiotics chloramphenicol or streptomycin. Moreover, the cytotoxic activity evaluation demonstrated that the *Gelsemium elegans* essential oil showed moderate cytotoxicity against cancer cell lines HCT-116, HepG2, MCF-7, and A-549. Although further *in vivo* experiments are needed, these findings showed that the essential oil obtained from *Gelsemium elegans* was a potential natural source of antibacterial and cytotoxic products.

Supporting Information

Supporting Information accompanies this paper on http://www.acgpubs.org/journal/records-of-natural-products

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