

Essential Oil from *Hedyotis chrysotricha*: Chemical Composition, Cytotoxic, Antibacterial Properties and Synergistic Effects with Streptomycin

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Abstract: *Hedyotis chrysotricha* (Palib.) Merr. is a traditional Chinese herb that has been used to treat multiple ailments. In this study, we extracted and characterized the essential oil of *H. chrysotricha* for the first time and assessed its *in vitro* cytotoxic, antibacterial activities and the synergistic interaction with Streptomycin. Chemical constituents were determined using GC-FID/MS, which showed 37 components constituted 94.7% of the total oil; borneol (32.7%), linalool (7.5%), isobornyl formate (5.4%) and α -terpineol (4.8%) were the dominant components. The essential oil of *H. chrysotricha* inhibited strongly the growth of Gram-positive bacteria and possessed a significant cytotoxic activity towards LO2, HepG2 and MCF-7 cells. Besides, the checkerboard assay revealed that the *H. chrysotricha* essential oil exhibited synergistic potential with Streptomycin. Based on these findings, it can be concluded that the *H. chrysotricha* essential oil exhibits interesting bioactivities, and could be used as a source of natural antibacterial and anti-tumor compounds.

Keywords: *Hedyotis chrysotricha* (Palib.) Merr; essential oil; antibacterial activity; cytotoxic activity; synergistic effects. © 2022 ACG Publications. All rights reserved.

1. Plant Source

The aerial parts of *Hedyotis chrysotricha* (Palib.) Merr. were collected in Zhejiang Province, China, in August 2020. The plants were identified and authenticated by Prof. Hong Zhao. The voucher specimen (NO.020015) has been deposited at the Laboratory of Botany, Shandong University, China.

2. Previous Studies

Hedyotis chrysotricha (Palib.) Merr. [synonym: *Oldenlandia chrysotricha* (Palib.) Chun.], is a perennial herb belonging to the family Rubiaceae with a wide distribution throughout Southern China [1]. As a plant ingredient for Chinese traditional medicine, *H. chrysotricha* is extensively utilized for the treatment of rheumatic arthritis, acute nephritis, hepatitis and cancer [2]. Pharmacological studies have demonstrated that extracts of the whole plant of *H. chrysotricha* possessed inhibitory activities against the growth of SK-HEP-1 human hepatocellular carcinoma cells and HL-60 human leukemia cells [3, 4]. Several alkaloids and iridoid glucosides had been reported from *H. chrysotricha* in the

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previous phytochemical studies [2-4]. Additionally, a trace alkaloid chrysotricine was isolated from the alcoholic extracts of *H. chrysotricha* which exhibits an *in vitro* cytotoxic activity against HL-60 cells [4]. However, a search of the literature revealed no previous study has investigated the essential oil of *H. chrysotricha*. Accordingly, the present study was undertaken to describe the composition of the essential oil of *H. chrysotricha* (HC-EO) and to evaluate its biological activities.

3. Present Study

The aerial parts of *H. chrysotricha* were hydrodistilled to give an essential oil in a yield of 0.15 ± 0.03% (w/w), calculated on a dry weight basis. The compounds are listed in Table 1 according to the elution on an HP-5 MS capillary column.

Table 1. Percentage compositions identified in the essential oil of aerial parts of *H. chrysotricha*

Compounds ^a	RI ^b	RI _{lit} ^c	RI range ^d	%
Cumene	926	924 ^e		1.2
Lavender lactone	1038	1034 ^e		2.4
<i>cis</i> -Linalool Oxide	1070	1067 ^e	1061–1090	2.5
<i>trans</i> -Linalool oxide	1086	1084 ^e	1070–1097	2.2
Linalool	1099	1095 ^e	1088–1109	7.5
<i>trans</i> -Sabinene hydrate	1110	1107 ^f	1084–1115	1.5
Nopinone	1139	1135 ^e	1120–1145	1.8
Camphor	1145	1141 ^e	1127–1155	3.7
Borneol	1169	1165 ^e	1152–1177	32.7
α -Terpineol	1192	1186 ^e	1178–1203	4.8
2-Methylisoborneol	1197	1197 ^g		1.7
Isobornyl formate	1232	1235 ^e		5.4
Bornyl acetate	1288	1289 ^e	1264–1297	1.9
(<i>E,E</i>)-2,4-Decadienal	1317	1315 ^e	1305–1334	0.3
Dehydro-ar-ionene	1359	1354 ^g		0.2
1-Tetradecene	1389	1388 ^e		0.5
Hexahydropseudoionone	1403	1408 ^g		0.2
(<i>E</i>)- α -Ionone	1430	1428 ^e	1403–1435	0.5
β -Barbatene	1452	1452 ^f		0.5
Dehydro- β -ionone	1486	1485 ^g		0.3
(<i>E</i>)- β -Ionone	1489	1487 ^e	1470–1498	1.3
Caryophyllene oxide	1585	1582 ^e	1563–1595	0.6
Tetradecanal	1609	1611 ^e	1605–1623	0.6
Junenol	1618	1618 ^e		0.3
(<i>Z</i>)-6,7-Dihydrofarnesol	1674	1673 ^f		1.0
2-Pentadecanone	1694	1697 ^e	1685–1716	0.9
Pentadecanal	1711	1715 ^f	1703–1728	2.9
Octadecene	1787	1789 ^e		0.6
Neophytadiene	1838	1841 ^f		2.4
Hexahydrofarnesyl acetone	1844	1847 ^f		3.9
(<i>Z</i>)-9-Hexadecen-1-ol	1877	1863 ^g		0.8
Methyl hexadecanoate	1921	1921 ^e	1910–1931	1.5
Isophytol	1945	1946 ^e	1939–1951	1.5
Hexadecanoic acid	1955	1959 ^e	1939–1996	0.3
Ethyl hexadecanoate	1988	1992 ^e	1975–2000	0.3
Eicosene	1990	1987 ^e		0.2
Oleic acid	2141	2141 ^e	2102–2161	3.8
Total identified				94.7

^a Compounds are listed in order of their elution on the HP-5MS column; ^b **RI**: Retention index on the HP-5MS column relative to C₇–C₃₀ n-alkanes; ^c **RI_{lit}**: Retention indices data from the literature e) [16], f) [17] and g) [18]; ^d **RI range**: range of Kováts retention indices (90% confidence) reported by Babushok et al. (2011) [19].

Using GC-FID and GC-MS, a total of 37 compounds, representing 94.7% of the total essential oil content were identified (The experiments' details were provided in Supporting Information).

The major constituents of the HC-EO were borneol (32.7%), linalool (7.5%), isobornyl formate (5.4%), α -terpineol (4.8%), hexahydrofarnesyl acetone (3.9%) and oleic acid (3.8%). Borneol is a naturally occurring bicyclic monoterpene, widely used in Chinese folk medicine for the treatment of sore throat, treating burns, wound healing, and skin infections [5]. It was also reported to exhibit a variety of pharmacological activities, such as antioxidant, anti-inflammation [6], antinociceptive [7] and antiepileptogenic activities [8]. Linalool is a monoterpene alcohol with various pharmacological activities, including antimicrobial [9], neuroprotective [10], antinociceptive [11], anti-inflammatory [12], antiproliferative and anticancer effects [13]. Another monoterpene alcohol, α -terpineol, has also been demonstrated to possess a broad spectrum of pharmacological properties, such as anticonvulsant, antidiarrheal, antimicrobial, gastroprotective [14], anti-inflammatory [15].

Antibacterial Activity of the Essential Oil: The antibacterial activity of the essential oil was tested by micro-broth dilution method against four bacterial strains, belonging to both Gram-positive and Gram-negative bacteria — namely *Bacillus subtilis* (ATCC 6633) and *Staphylococcus aureus* (ATCC 6538) represent Gram-positive; and *Escherichia coli* (ATCC 25922) and *Pseudomonas aeruginosa* (ATCC 27853) represent Gram-negative bacteria [20]. The minimal inhibitory concentrations (MICs) and Minimal bactericidal concentration (MBCs) of the oil were listed in Table 2. Chloramphenicol was used as the positive control. The results revealed that the essential oil of *H. chrysotricha* showed significant antibacterial activity on Gram-positive bacteria *S. aureus* and *B. subtilis* with MICs of 0.039 mg/mL and 0.078 mg/mL, and a moderate activity on Gram-negative bacteria *E. coli* and *P. aeruginosa* (MICs = 0.625 mg/mL). Accordingly, it could be observed that Gram-positive bacteria were more sensitive to the essential oil of *H. chrysotricha* than Gram-negative bacteria.

Table 2. Antibacterial activity of *H. chrysotricha* essential oil

Bacteria	MIC ¹		MBC ²	
	EO (mg/mL)	Ch (mg/mL)	EO (mg/mL)	Ch (mg/mL)
Gram-positive				
<i>Bacillus subtilis</i> ATCC 6633	0.078	0.016	0.078	0.031
<i>Staphylococcus aureus</i> ATCC 6538	0.039	0.008	0.078	0.031
Gram-negative				
<i>Escherichia coli</i> ATCC 25922	0.625	0.031	0.625	0.125
<i>Pseudomonas aeruginosa</i> ATCC 27853	0.625	0.062	1.250	0.125

¹ Minimal inhibitory concentration; ² Minimal bactericidal concentration; Ch, chloramphenicol.

Synergistic Effects Screening: The checkerboard assay was conducted to estimate the synergistic interactions between the essential oil and antimicrobial agent Streptomycin [20]. The study showed that HC-EO presented a marked synergistic effect in combination with Streptomycin against reference strains (the FICI values ranged from 0.19 to 0.38). The detailed results are summarized in Table 3.

Biological activities of essential oil from *Hedyotis chrysotricha***Table 3.** The antibacterial activity of essential oils in combination with Streptomycin

Bacteria		MIC _a (µg/mL)	MIC _c (µg/mL)	FICI
<i>Bacillus subtilis</i> ATCC 6633	EO	78.13	19.53	0.31 (S)
	SM	2.50	0.16	
<i>Staphylococcus aureus</i> ATCC 6538	EO	39.06	9.77	0.31 (S)
	SM	5.00	0.31	
<i>Escherichia coli</i> ATCC 25922	EO	625.00	78.13	0.38 (S)
	SM	5.00	1.25	
<i>Pseudomonas aeruginosa</i> ATCC 27853	EO	625.00	39.06	0.19 (S)
	SM	5.00	0.63	

MIC_a: MIC of an individual sample; MIC_c: MIC at the most effective combination; FICI: fractional inhibitory concentration index; SM: Streptomycin; S, synergy (FICI ≤ 0.5).

In vitro Cytotoxic Activity Evaluation: The potential cytotoxic activity of *H. chrysotricha* essential oil was carried out by MTT assay *in vitro* and measured in four human cancer cell lines, namely HepG2 liver cancer cells, MCF-7 breast cancer cells, A-549 lung cancer cells, HCT-116 colon cancer cells, and a normal human hepatocyte cell line LO2 [21]. Doxorubicin was used as a reference drug. All cells were exposed to increasing concentrations of HC-EO or Doxorubicin for 24 h, 48 h and 72 h, respectively. As shown in Table 4, the essential oil of *H. chrysotricha* revealed strong cytotoxic activities towards LO2, HepG2, and MCF-7 with IC₅₀ values range from 12.55 ± 0.76 to 19.52 ± 0.49 µg/mL for 72 h. Moderate cytotoxic effects towards HCT-116 and A-549 cells with IC₅₀ values of 20.87 ± 0.41 and 42.45 ± 1.58 µg/mL for 72 h were also observed.

Table 4. Cytotoxicity (IC₅₀ µg/mL) of the essential oil from *H. chrysotricha*

Sample		24h	48h	72h
HepG2	EO	42.56 ± 3.39	30.76 ± 12.23	14.43 ± 2.74
	Doxorubicin	1.46 ± 0.08	1.09 ± 0.08	0.43 ± 0.07
MCF-7	EO	34.37 ± 3.48	24.43 ± 4.16	19.52 ± 0.49
	Doxorubicin	1.56 ± 0.03	0.79 ± 0.02	0.39 ± 0.06
LO2	EO	24.89 ± 3.94	16.19 ± 5.11	12.55 ± 0.76
	Doxorubicin	1.80 ± 0.29	0.46 ± 0.02	0.55 ± 0.12
A-549	EO	62.01 ± 4.08	48.11 ± 2.92	42.45 ± 1.58
	Doxorubicin	1.04 ± 0.07	0.85 ± 0.05	0.33 ± 0.01
HCT-116	EO	46.77 ± 3.71	32.08 ± 5.13	20.87 ± 0.41
	Doxorubicin	1.33 ± 0.15	0.57 ± 0.02	0.48 ± 0.06

The bioactivities of essential oils could be attributed to their constituents. Some predominance components of *H. chrysotricha* essential oil have been reported for their antibacterial and cytotoxic activities, such as borneol [22], linalool [9,13], α -terpineol [9, 23], hexahydrofarnesyl acetone [24] and oleic acid [25]. Besides, the possible synergistic effects among all of the components in essential oil may be also responsible for these activities.

Overall, the present study has, for the first time, identified the composition of *H. chrysotricha* essential oil, and demonstrated the HC-EO exhibit a potential cytotoxic activity towards LO2, HepG2 and MCF-7 cells, and a significant antibacterial activity against gram-positive bacteria strains, as well as a remarkable synergistic interaction with Streptomycin. Accordingly, the essential oil of *H. chrysotricha* could be used as a source of natural antibacterial and anti-tumor compounds. However, further *in vivo* experimentation is required to provide more evidence for being used as a potential treatment of anti-tumor and control of pathogenic bacteria.

Supporting Information

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

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