SHORT REPORT

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Chemical Composition and Evaluation of the Antibacterial, Synergistic Antibacterial, Antioxidant and Cytotoxic Activities of the Essential Oil of *Macrothelypteris torresiana* (Gaudich.) Ching

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Abstract: In this study, we evaluated the chemical composition, antioxidant, cytotoxic, and antibacterial activities of the essential oil extracted from the aerial parts of *Macrothelypteris torresiana* (Gaudich.) Ching (MT-EO), as well as its synergistic antibacterial effect in combination with commercial antibiotics. Fifty-seven compounds were identified in MT-EO, representing 97.9% of the total oil content. The compounds bicyclogermacrene (12.9%), spathulenol (11.9%), *β*-elemene (5.7%), and hexyl hexanoate (5.0%) were detected as the main constituents. The microdilution and checkerboard assays were used to evaluate the antibacterial and synergistic properties of the essential oil. It was found that MT-EO possessed bactericidal activity against all tested bacteria, with MIC values between 0.625 to 1.250 mg/mL, which was the same as MBCs. Additionally, synergistic effects were detected in both *M. torresiana* essential oil -chloramphenicol and -streptomycin combinations. Besides, according to the MTT test, MT-EO possessed broad-spectrum cytotoxicities on various cell lines with IC_{50} values ranging from 15.12 ± 0.96 to 47.07 ± 1.96 μg/mL, including the MCF-7, A-549, HCT-116, HepG2, and LO2 cell lines. Furthermore, MT-EO showed moderate antioxidant activities in DPPH, ABTS, and FRAP assays, with IC_{50} values of 434.5 ± 9.6 and 98.1 ± 1.1 μg/mL, and Trolox equivalent of 97.11 ± 3.37 μmol Trolox × g^{-1}, respectively.

Keywords: *Macrothelypteris torresiana*; essential oil; antibacterial; synergistic; antioxidant; cytotoxic.

1. Plant Source

The aerial parts of *Macrothelypteris torresiana* (Gaudich.) Ching were collected in August 2022 from Jieyang, Guangdong Province, China. The botanical identification was conducted by Prof. Hong Zhao, Shandong University, China. A herbarium specimen of the plant was stored at the herbarium of the Institute of Botany, Chinese Academy of Sciences (PE 01768725).

2. Previous Studies

Macrothelypteris torresiana is a perennial fern of the Thelypteridaceae family, native to the W. Indian Ocean, tropical and subtropical regions of Asia, and Pacific Islands [1]. The aerial part of *M. torresiana* is used to treat fever, pain, and granulation in Pakistan, India, and China [2]. Additionally, it is employed in traditional Chinese medicine to alleviate edema in individuals suffering from renal disorders [3]. Previous studies have demonstrated the renoprotective potential of the total polyphenols fraction

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derived from *Macrothelypteris torresiana* through ameliorating oxidative stress and proinflammatory cytokines [3]. Furthermore, research on phytochemistry indicated *M. torresiana* contains various constituents, including flavonoids, terpenoids, and glycosides [4-6], and the potential biological properties have been examined, such as antitumor, hepatoprotective, anti-inflammatory, and antimicrobial activities [7, 8]. However, there have been no reported studies on the essential oil of *M. torresiana*.

3. Present Study

The aerial parts of *M. torresiana* were subjected to hydrodistillation to extract the essential oil. The yield of essential oil was 0.15 ± 0.03% (w/w) based on dry weight. The chemical composition of MT-EO was analyzed using GC/FID and GC/MS. As shown in Figure S1 and Table 1, a total of fifty-seven components were identified, which account for 97.9% of the overall MT-EO. Sesquiterpenes are the most abundant chemical class in MT-EO, and the relative amounts were 40.6% in the volatile fractions. The second major chemical class was oxygenated sesquiterpenes with 37.2%, followed by oxygenated monoterpenes with 4.5%. The major compounds were identified as bicyclogermacrene (12.9%), spathulenol (11.9%), *β*-elemene (5.7%), hexyl hexanoate (5.0%), *δ*-elemene (3.9%), isospathulenol (3.5%), globulol (3.4%), and epi-*α*-cadinol (3.3%). Spathulenol, one of the major constituents of MT-EO, has been found to possess a broad spectrum of biological activities, including antioxidant, anti-inflammatory, antiproliferative, antimycobacterial, and anti-*M. tuberculosis* capabilities [9, 10]. *β*-Elemene, known for its anticancer properties against a variety of cell lines, has been demonstrated to possess anti-proliferative effects via triggering apoptosis [11] and antibacterial activity against *Mycobacterium tuberculosis* strain H37Ra [12]. Besides, bicyclogermacrene exhibited significant cytotoxic potential against HL-60 cells [13].

Table 1. The essential oil composition of *M. torresiana*

No.	Compounds	RI [a]	RI_{lit} [b]	RI range	%	Identification Method
1	1-Octen-3-ol	972	974[c]	967-991[f]	0.5	MS, RI
2	Hexyl acetate	1011	1007[c]	999-1020[f]	0.4	MS, RI
3	2-Nonanol	1093	1097[c]	1076-1120[f]	0.3	MS, RI
4	Linalool	1099	1097[g]	1098-1101[g]	0.3	MS, RI
5	*n*-Nonanal	1103	1100[c]	1093-1118[f]	1.5	MS, RI
6	4-Ethylbenzaldehyde	1161	1169[e]	1144-1197[e]	0.3	MS, RI
7	*β*-Cyclocitral	1219	1217[c]	1205-1225[f]	0.3	MS, RI
8	*δ*-Elemene	1336	1335[c]	1327-1344[f]	3.9	MS, RI
9	Cyclosativene	1372	1369[c]	1360-1380[f]	1.4	MS, RI
10	Hexyl hexanoate	1383	1382[c]	1371-1399[e]	5.0	MS, RI
11	isoLongifolene	1387	1389[c]	1373-1425[e]	1.3	MS, RI
12	*β*-Elemene	1391	1389[c]	1374-1402[f]	5.7	MS, RI
13	(2*E*)-Hexenyl caproate	1395	1385[c]	1385[c]	0.8	MS, RI
14	*α*-Gurjunene	1408	1409[c]	1394-1421[f]	2.8	MS, RI
15	Dihydrodehydro-β-ionone	1415	1424[e]	1424[e]	1.0	MS, RI
16	(*E*)-*α*-Ionone	1426	1428[c]	1403-1435[f]	0.4	MS, RI
17	2-Butyldecahydro-naphthalene	1429	1432[c]	1424-1450[f]	0.7	MS, RI
18	Aromadendrene	1438	1439[c]	1419-1465[f]	1.8	MS, RI
19	*α*-Humulene	1452	1455[g]	1450-1454[g]	2.5	MS, RI
20	allo-Aromadendrene	1460	1458[c]	1443-1477[f]	0.9	MS, RI
21	Precocene I	1464	1461[c]	1461[c]	0.5	MS, RI
22	*γ*-Gurjunene	1471	1475[c]	1455-1485[f]	1.3	MS, RI
23	Germacrene D	1480	1481[g]	1478-1488[g]	0.8	MS, RI
24	(*E*)-β-Ionone	1485	1487[c]	1470-1498[f]	2.4	MS, RI
25	*γ*-Amorphene	1491	1495[c]	1474-1485[f]	0.6	MS, RI
26	Bicyclogermacrene	1495	1500[c]	1477-1503[f]	12.9	MS, RI
27	*γ*-Cadinene	1513	1510[g]	1511–1521[g]	2.5	MS, RI
28	*cis*-Calamenene	1522	1528[c]	1511-1541[f]	1.6	MS, RI
29	Elemol	1552	1548[c]	1534-1557[f]	0.4	MS, RI
30	(*E*)-Nerolidol	1563	1561[c]	1539-1570[f]	0.3	MS, RI

No.	Compounds	RI [a]	RI_{lit} [b]	RI range	%	Identification Method
31	Palustrol	1566	1567[c]	1561-1571[f]	0.7	MS, RI
32	Spathulenol	1578	1566[g]	1568-1590[g]	11.9	MS, RI
33	Globulol	1583	1590[c]	1568-1592[f]	3.4	MS, RI
34	Viridiflorol	1591	1592[c]	1569-1604[f]	2.7	MS, RI
35	Isoaromadendrene epoxide	1599	1594[e]	1572-1618[e]	0.5	MS, RI
36	Guaiol	1601	1600[c]	1585-1615[f]	0.8	MS, RI
37	Geranyl isovalerate	1609	1606[c]	1582-1613[f]	1.1	MS, RI
38	1,10-di-epi-Cubenol	1614	1618[c]	1591-1623[f]	1.1	MS, RI
39	1-*epi*-Cubenol	1628	1627[c]	1611-1631[f]	0.5	MS, RI
40	Isospathulenol	1638	1640[d]	1621-1641[f]	3.5	MS, RI
41	*epi*-*α*-Cadinol	1641	1638[c]	1624-1648[f]	3.3	MS, RI
42	*epi*-*α*-Muurolol	1644	1640[c]	1623-1654[f]	0.8	MS, RI
43	*α*-Cadinol	1654	1652[c]	1635-1664[f]	0.9	MS, RI
44	Ylangenol	1663	1666[d]	1666[d]	0.8	MS, RI
45	Elemol acetate	1669	1675[d]	1680[d]	0.7	MS, RI
46	*n*-Pentadecanal	1713	1715[d]	1703-1728[f]	2.1	MS, RI
47	*β*-Santalol	1717	1715[c]	1676-1736[e]	1.0	MS, RI
48	*iso*-Longifolol	1731	1728[c]	1728[c]	0.4	MS, RI
49	8*α*-hydroxy-Eremophila-1,11-dien-9-one	1780	1777[d]	1777[d]	0.5	MS, RI
50	Saussurea lactone	1793	1806[d]	1806[d]	0.4	MS, RI
51	Dehydrosaussurea lactone	1831	1838[d]	1838[d]	0.5	MS, RI
52	Neophytadiene	1836	1841[d]	1804-1857[e]	0.3	MS, RI
53	Hexahydrofarnesyl acetone	1843	1847[d]	1831-1855[f]	0.8	MS, RI
54	Valerenic acid	1868	1877[d]	1877[d]	0.5	MS, RI
55	(*E*)-2-Hexadecenal	1881	1878[d]	1813-1880[e]	1.9	MS
56	Gazaniolide	1891	1894[d]	-	0.7	MS
57	Hexadecanoic acid	1961	1959[c]	1939-1996[f]	1.0	MS, RI
	Oxygenated monoterpenes				4.5	
	Sesquiterpene hydrocarbons				40.6	
	Oxygenated sesquiterpenes				37.2	
	Total identification				**97.9**	

[a]Retention index calculated from n-alkanes (C_7-C_{30}) on HP-5MS column; [b]Linear retention indices from literature: [c][14], [d][15], [e][16], [f][17], [g][18].

The MT-EO was evaluated for possible antibacterial activity against selected Gram-positive and Gram-negative pathogenic bacterial strains by using a broth microdilution assay [19]. The positive control utilized was chloramphenicol.

Table 2. Antibacterial activity of MT-EO

Bacterial strains	MIC, µg/mL		MBC, µg/mL	
	MT-EO	**Ch**	**MT-EO**	**Ch**
Gram-positive				
Bacillus subtilis ATCC 6633	625.0	2.5	625.0	20.0
Staphylococcus aureus ATCC 6538	625.0	5.0	625.0	40.0
Gram-negative				
Escherichia coli ATCC 25922	625.0	2.5	625.0	40.0
Pseudomonas aeruginosa ATCC 27853	1250.0	40.0	1250.0	156.3

Table 2 shows the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) values of MT-EO against the strains tested. As presented in Table 2, MT-EO exhibits broad-spectrum antibacterial activity against all bacteria strains tested, with MIC values ranging from 0.625 to 1.250 mg/ml, which were the same as MBCs, indicating the potent bactericidal activity of MT-EO. MT-EO exhibited weaker antibacterial activities compared to the synthetic antibiotic Chloramphenicol. The main constituents of MT-EO are sesquiterpenoids and oxygenated sesquiterpenes, which are known to have remarkable antibacterial properties and are considered to be responsible for antibacterial activity [20].

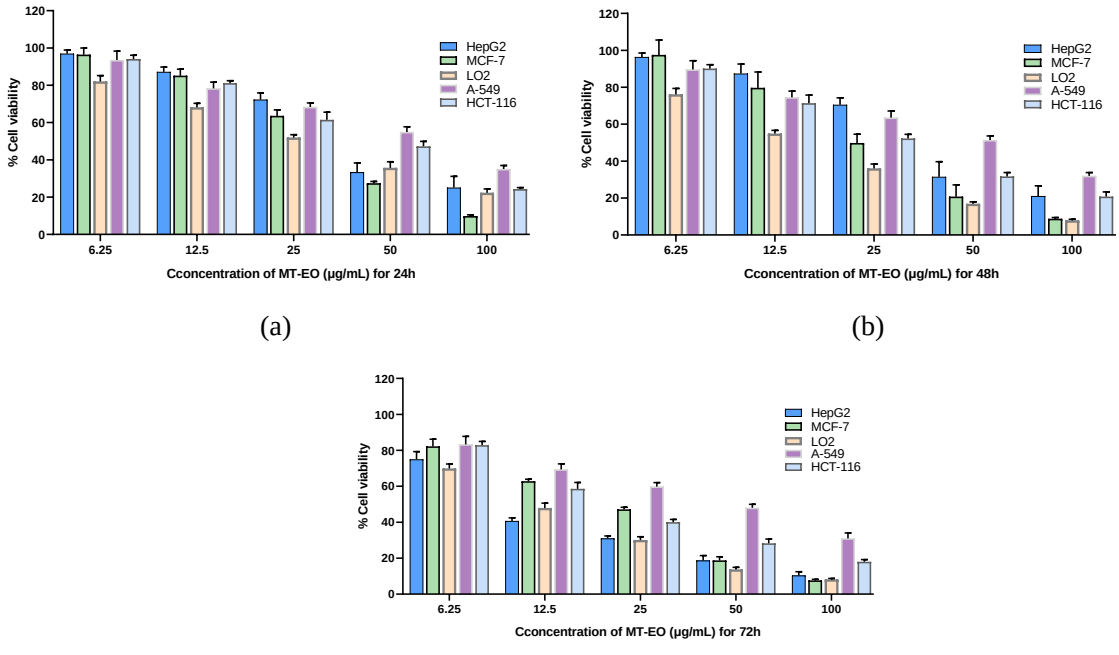
The interaction between MT-EO and conventional antibiotics (chloramphenicol and streptomycin) was assessed using the checkerboard microdilution method [21] based on determined MIC values. The results presented in Table 3 revealed the remarkable synergistic effects of MT-EO combined with antibiotics on all tested bacterial strains, with FICI ranging from 0.15 to 0.37. It is noteworthy that the MIC_c values of chloramphenicol and streptomycin were observed to be 4-32 and 6-12 times lower than those normally required for direct inhibition of bacterial growth. The results suggested that combining MT-EO with traditional synthetic antibiotics could be an effective way to enhance antibiotic antimicrobial efficacy, expanding the range of antimicrobial activity, preventing resistance, and reducing harmful or undesired side effects [22].

Table 3. Effect of combination of MT-EO with Chloramphenicol and Streptomycin

Strains	Sample	MICa	MICc	FICI	sample	MICa	MICc	FICI
B. subtilis ATCC 6633	MT-EO Ch	625.0 2.4	156.2 0.3	0.37 (S)	MT-EO Sm	625.0 0.6	39.1 0.1	0.23 (S)
S. aureus ATCC 6538	MT-EO Ch	625.0 4.9	39.1 1.2	0.31 (S)	MT-EO Sm	625.0 1.2	78.1 0.1	0.21 (S)
E. coli ATCC 25922	MT-EO Ch	625.0 2.4	78.1 0.3	0.25 (S)	MT-EO Sm	625.0 2.4	78.1 0.3	0.25 (S)
P. aeruginosa ATCC 27853	MT-EO Ch	1250.0 19.5	312.5 0.6	0.28 (S)	MT-EO Sm	1250.0 2.4	156.5 0.3	0.15 (S)

MICa: MIC of EO or antibiotic alone; MICc (µg/mL): MIC of EO or antibiotic in the most effective combination (µg/mL); (S): synergy (FICI ≤ 0.5).

The cytotoxic efficacy of MT-EO was evaluated via the MTT assay on four human cancer cell lines: hepatocellular carcinoma (HepG2), breast cancer (MCF7) cells, lung adenocarcinoma (A-549), and colorectal carcinoma (HCT-116), alongside the non-cancerous human liver cell line LO2 [23]. Doxorubicin was employed as a positive control. The results of the 24, 48, and 72-hour exposures (Table 4 and Figure 1) showed that MT-EO had significant cytotoxic activity against all tested cell lines and exhibited dose- and time-dependent cytotoxic effects. The selectivity index of MT-EO was calculated in the range of 0.32 to 0.60 (48 h). The main compounds present in the essential oils may be responsible for the interesting cytotoxic activity, such as spathulenol [9], *β*-elemene [11], *δ*-elemene [24], and bicyclogermacrene [13], which has been demonstrated to exhibit cytotoxic effects on multiple tumor cell lines. However, MT-EO exhibited weaker activity in comparison with positive control Doxorubicin.



(a) (b)

(c)

Figure 1. Cytotoxic activity of MT-EO for 24 h (a); 48 h (b); 72 h (c). ($P < 0.05$)

Table 4. Cytotoxic activity (IC_{50}, μg/mL) of MT-EO

	Sample	24h	48h	72h
HepG2	EO	40.20 ± 2.05	34.19 ± 0.51	12.41 ± 1.24
	Doxorubicin	1.46 ± 0.08	1.09 ± 0.08	0.43 ± 0.07
MCF-7	EO	31.73 ± 2.17	25.20 ± 2.28	19.26 ± 0.70
	Doxorubicin	1.56 ± 0.032	0.79 ± 0.02	0.39 ± 0.06
LO2	EO	27.67 ± 1.95	15.12 ± 0.96	12.11 ± 1.02
	Doxorubicin	1.80 ± 0.29	0.46 ± 0.02	0.55 ± 0.12
A-549	EO	55.56 ± 1.96	47.07 ± 1.96	40.21 ± 2.96
	Doxorubicin	1.04 ± 0.07	0.85 ± 0.05	0.33 ± 0.01
HCT-116	EO	41.50 ± 2.43	28.89 ± 2.19	20.28 ± 1.30
	Doxorubicin	1.33 ± 0.15	0.57 ± 0.02	0.48 ± 0.06

The antioxidant activities of MT-EO were evaluated using three antioxidant models: DPPH, ABTS, and FRAP [25]. The results are shown in Table 5. The study confirms that MT-EO demonstrates moderate antioxidant activity in DPPH and ABTS assays, with IC_{50} values of 434.5 ± 9.6 and 98.1 ± 1.1 μg/mL, respectively. Compared to the standard antioxidants BHT and Trolox, MT-EO showed a mild free radical scavenging activity. In addition, it has a moderate activity for reducing ferric ions, with a trolox equivalent of 97.11 ± 3.37 mol Trolox·g^{-1}.

Table 5. Results of antioxidant activity *in vitro* (DPPH, ABTS and FRAP) of MT-EO

Test Sample	DPPH IC_{50} (μg/mL) [a]	ABTS IC_{50} (μg/mL) [a]	FRAP (μmol Trolox × g^{-1})
MT-EO	434.5 ± 9.6	98.1 ± 1.1	97.11 ± 3.37
BHT [b]	5.3 ± 0.3	2.6 ± 0.1	
Trolox [b]	6.1 ± 0.4	5.3 ± 0.3	

[a] IC_{50} = The sample concentration for a 50% reduction in the assay; [b] Positive control used.

Supporting Information

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

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