ORIGINAL ARTICLE

A C G publications

Rec. Nat. Prod. 19:1 (2025) 84-94

records of natural products

Phytochemical Constituents, Antibacterial, Cytotoxic and Antioxidant Activities of the Essential Oil of Rhizomes of *Davallia bullata* Wall. ex Hook.

Fan Xu [1], Xiran Chen [1], Jinyi Zhang [1], Chunlei Qu [1], Ling Gu [2] and Xiang Xing [2*]

[1] *Sdu-Anu Joint Science College, Shandong University, Weihai, 264209, China*
[2] *Marine College, Shandong University, Weihai 264209, China*

(Received January 14, 2025; Revised February 18, 2025; Accepted February 19, 2025)

Abstract: In this study, the phytochemical constituents of *Davallia bullata* rhizomes essential oil (DBEO) and its biological activities were investigated. According to the GC-FID and GC-MS analysis, the major compounds of DBEO were found to be *β*-barbatene (17.2%), *n*-hexadecanoic acid (6.6%), oleic acid (5.7%), nonanal (5.5%), and 1-octen-3-ol (4.9%). For biological activities, DBEO was tested against gram-positive bacteria, *B. subtilis* and *S. aureus*, and gram-negative bacteria, *E. coli* and *P. aeruginosa*, in order to determine its potential antibacterial activity. The results revealed minimal inhibitory concentration values in the range of 0.16–0.64 mg/mL, while minimum bactericidal concentration values ranged from 0.16 to 1.28 mg/mL. Interestingly, DBEO possessed remarkable synergistic effects when combined with chloramphenicol and streptomycin, with the fractional inhibitory concentration indexes (FICI) varying from 0.13 to 0.50, as determined by the Checkerboard method. Furthermore, DBEO exhibited a moderate level of cytotoxicity against MCF-7, HepG2, HCT-116, and A-549 cells with IC_{50} values varying from 58.38 ± 0.19 to 94.37 ± 5.09 µg/mL and weak cytotoxicity against non-cancerous HL-7702 cells (177.05 ± 1.34 µg/mL), and the antioxidant capacity of DBEO was evaluated and reported herein.

Keywords: *Davallia bullata;* essential oil; antibacterial; synergistic; cytotoxic; antioxidant.

1. Introduction

Essential oil (EO) consists of volatile secondary metabolites produced by aromatic plants, demonstrating a broad spectrum of pharmacological properties, including neuroprotection, antimutagenic, antioxidant, antiviral, antibacterial, antitumor, and anti-inflammatory activities. The complexity of the chemical composition gives rise to the characteristic fragrances of EOs and is related to their medicinal use [1]. Due to their natural origins and therapeutic properties, essential oils have gained immense application and interest during the last few decades.

Fern species are an important ecosystem component and produce various bioactive compounds with multiple functions utilized in traditional medicine [2, 3]. Unlike vascular plants, ferns have unique secondary metabolites, which may help explain their potential medicinal uses [2]. Recent studies have demonstrated the presence of essential oils in fern species [4-8]. These oils primarily comprise terpenes and other aromatic compounds, known for their diverse pharmacological properties, such as antioxidant, antiproliferative, anti-inflammatory, and antimicrobial activities [4-8]. Accordingly, fern essential oils

* Corresponding author: E-Mail: xingxiang@email.sdu.edu.cn

The article was published by ACG Publications
http://www.acgpubs.org/journal/records-of-natural-products January-February 2025 EISSN:1307-6167
DOI: http://doi.org/10.25135/rnp.498.2501-3404
Available online: February 26, 2025

may represent promising substitutes in pharmacology and holistic health practices as further research reveals their chemical profiles and health benefits.

Davallia is a genus of ferns belonging to the Davalliaceae family, consisting of around 40 species. Various species from this genus have been utilized in Gusuibu, a well-known traditional Chinese folk medicine, to cure bone injuries, inflammation, cancer, and osteoporosis [3]. Isolation and characterization of secondary metabolites of some *Davallia* species, including triterpenoids, sesquiterpenoids, flavanones, and cyanogenic glycosides have been reported [9-13]. *Davallia bullata* Wall. ex Hook. is a perennial fern belonging to the genus *Davallia*, primarily found in China, Korea, and Japan [14]. Its rhizomes are utilized in traditional folk medicine for various ailments, including the common cold, rheumatism, stomach cancer, lumbago, toothache, neuralgia, and tinnitus [15, 16]. In addition, it is consumed to help with conditions such as inflammation, osteoporosis, traumatic brain injury, and arteriosclerosis [17].

Since phytochemical constituents and potential biological activities of the essential oil of *D. bullata* have not been explored enough yet, thus, full characterization of chemical constituents and evaluation of its antibacterial and cytotoxic activities together with the antioxidant capacity of the essential oil that is obtained from the rhizomes of *D. bullata* (DBEO) was aimed herein.

2. Materials and Methods

2.1. Plant Materials Collection

The rhizomes of *Davallia bullata* were collected in August 2022 from Zhejiang Province, China. The plant specimen (NO.022063) was authenticated by botanist Prof. Hong Zhao (Shandong University) and deposited in the Herbarium of Shandong University, China. The specimen was identified as consistent with voucher specimen NAS00158424, which is deposited in the Herbarium of the Institute of Botany, Jiangsu Province and Chinese Academy of Sciences.

2.2. Obtaining the Essential Oil from the Rhizomes of Davallia bullata

1000 grams of *Davallia bullata* rhizomes were ground into powder in a mill and subjected to hydrodistillation for 3.5 hours using a Clevenger-type apparatus. The obtained essential oil was dried over anhydrous Na_2SO_4 and stored at 4 °C until the measurement. The yields were determined as 0.11% (w/w) on a dry weight basis.

2.3. Phytochemical Analyses

The analyses of DBEO were conducted using an Agilent Technologies gas chromatograph 6890 equipped with a capillary column HP-5MS (30 m × 0.25 mm i.d.; 0.25 μm film thickness) and a flame ionization detector (FID). The oven was set to rise by 8°C/min from its starting temperature of 60°C (1 minute) to 230°C (maintained for 14 minutes). Injector and detector temperatures were set at 250°C and 260°C, respectively. Helium, with a 1.2 mL/min flow rate, was the carrier gas used in this experiment.

GC/MS was used to characterize the chemical composition using a Hewlett Packard 6890 gas chromatograph (Agilent) equipped with a Hewlett Packard 5975C mass selective detector and an HP-5MS fused silica column. As previously indicated, the GC parameters were identical to those specified above for GC/FID. The electron impact ionization voltage was 70 eV. The mass spectra were acquired in the range of *m/z* 50-550.

Through computer-aided matching of mass spectra fragmentation patterns with those of the NIST and WILEY libraries and a comparison of retention indices (with reference to C_7-C_{30} n-alkanes, under identical GC/FID experimental conditions) against those reported in the existing literature [18, 19], DBEO components were identified. The mass spectral data of unidentified compounds are provided in the Supporting Information (SI).

2.4. Determination of DIZ, MIC, and MBC

The American Type Culture Collection (ATCC) was selected for the evaluated bacterial species, which are *Bacillus subtilis* ATCC 6633, *Staphylococcus aureus* ATCC 6538, *Pseudomonas aeruginosa* ATCC 27853, and *Escherichia coli* ATCC 25922, representing Gram-positive and Gram-negative bacteria, respectively. The preliminary evaluation of the antibacterial activity of DBEO was carried out utilizing the disc agar diffusion method as prescribed by the Clinical and Laboratory Standards Institute (CLSI) [20]. Blank sterile antibiotic disks (6 mm diameter) were soaked with 10 µL of the essential oil (10 mg/mL) or positive control chloramphenicol (1 mg/mL) and then aseptically placed on the inoculated MHA plates. After incubating at 37°C for 24 hours, the antibacterial property was determined by measuring the diameter of the inhibitory zones (DIZ) where there was a visible absence of bacteria growth, including the 6 mm filter paper disc.

The assessment of minimum inhibitory concentration (MIC) values was performed utilizing the microdilution test [21]. The DBEO was serially two-fold diluted and dispensed into individual wells, with a volume of 100 µL per well. Chloramphenicol served as the positive control in this study. A volume of 100 µL of the inoculum was introduced into each well to reach a final concentration of 5×10^5 CFU/mL. The negative control contained the medium containing the microorganism, while the growth control contained only the medium. Following incubation at 37°C for 24 hours, for staining of viable organisms, 20 µL of 1% 2,3,5-triphenyl tetrazolium chloride solution was added to all the plates and employed as an indicator to assess microbial growth. The last no-colored well was determined as the minimal inhibitory concentration (MIC, mg/mL) [22]. To determine the minimum bactericidal concentration (MBC), a 100 µL sample from each well with no color change was subsequently subcultured onto Mueller Hinton agar plates and incubated for a duration of 18–24 hours at 37°C, corresponding to an overnight incubation period. The lowest concentration at which no bacterial growth is observed is the MBC. The experiments were conducted in duplicate to minimize experimental error.

2.5. Synergistic Antibacterial Test

The checkerboard method was utilized to assess the efficacy of the interaction between DBEO and synthetic drugs [23]. In summary, eight 50 µL volumes of serial two-fold dilutions of essential oils were added to the columns of the 96-well plate, and eight 50 µL volumes of antibiotics were added to the rows similarly. Subsequently, 100 µL of bacterial suspension (10^6 CFU/mL) was added to each well. The MIC of EO and antibiotics, both alone (MICa) and in combination (MICc), was evaluated following a 24-hour incubation period at 37°C. The fraction inhibitory concentration index (FICI) value was determined using the following formula to assess the combination effect: FICI = (MICc of EO) / (MICa of EO) + (MICc of antibiotic) / (MICa of antibiotic). The following criteria were employed to interpret the interaction: $FICI \leq 0.5$, synergy; $0.5 < FICI \leq 1$, additive; $1 < FICI \leq 4$, indifference; and $FICI > 4$, antagonism [24].

2.6. Cytotoxic Activity Assay

A human normal cell line (HL-7702) and four cancer cell lines (A-549, MCF-7, HCT-116, and HepG2) were acquired from the Shanghai Institute for Biological Sciences for the present study. These cell lines were cultivated in RPMI 1640 medium, supplemented with 2 mM glutamine, 10% fetal bovine serum, 100 µg/mL streptomycin, and 100 U/mL penicillin. The cells were cultured under standard conditions of 37°C temperature and 5% CO_2 concentration.

The cell viability was assessed using the MTT test. The experimental procedure followed the methodology outlined in a previous publication [25]. The cells were seeded in 96-well plates at a density of 5×10^3 cells per well. A volume of 200 µL of culture media was used for each well. The cells were then incubated for 24 hours to facilitate cell attachment. The DBEO was dissolved in dimethyl sulfoxide and subsequently diluted with a culture medium for its intended application. The DBEO dilutions ranging from 0.016 to 2 mg/mL were introduced into the wells, except for the negative control wells, which received only culture media. Doxorubicin was included as a positive control in the experiment. The experimental procedure involved conducting each sample in triplicate. The microplates underwent incubation for 48 hours. Following the incubation period, a volume of 20 µL of MTT (5 mg/mL

dissolved in PBS) was introduced into each well. After incubating for 4 hours, the formazan crystals were solubilized in 100 µL of dimethyl sulfoxide. The measurement of optical density was conducted at a wavelength of 570 nm with an ELISA reader. The percentages representing the cell growth were determined by the following calculation method:

$$\text{Cell growth (\%)} = [\text{A (sample)} / \text{A (control)}] \times 100\%$$

The cytotoxic action was quantified by determining the concentration of the DBEO that resulted in a 50% suppression of cell growth, known as the IC_{50}.

2.7. Antioxidant Effect Evaluation

In the present work, the scavenging abilities of DBEO on 2,2-diphenyl-1-picrylhydrazyl (DPPH) and 2,2′-azinobis-3-ethylbenzothiazoline-6-sulphonate (ABTS) cation radicals were evaluated by a previously described method [26]. The IC_{50} value was determined and reported, representing the concentration at which 50% of the free radicals are effectively scavenged. The assessment of reducing ability was conducted by Ferric Reducing Antioxidant Power (FRAP) test, following a previously established methodology with minor adjustments [27]. TPTZ (10 mM in HCl (40 mM)), $FeCl_3$ (20 mM in distilled water), and acetate buffer (300 mM) were combined in a volumetric ratio of 10:1:1 to prepare the FRAP reagent, which was then incubated at 37°C for 10 minutes before use. For the analysis, 20 µL of sample solution and 180 µL of FRAP reagent were combined in a 96-well plate, incubated for 20 minutes at 37°C, and then the absorbance at 593 nm was measured. A calibration curve was established using Trolox standards within the concentration range of 20–200 mg/L.

2.8. Statistical Analysis

The data was analyzed using IBM SPSS version 29.0 and GraphPad Prism 9.0. The statistical significance of the results was determined using the Student's t-test, with a $p < 0.05$ considered to be statistically significant.

3. Results and Discussion

3.1. Compositions of DBEO

The essential oil obtained from the rhizomes of *D. bullata* by hydrodistillation gave a yield of 0.11% (w/w) on a dry weight base. A comprehensive analysis of the *D. bullata* oil resulted in the identification of 40 compounds, collectively accounting for 96.1% of the overall oil composition (Table 1). The chromatogram profiles of DBEO are shown in Figure S1 (in Supporting Information). The predominant constituents of the DBEO were found to be carbonylic compounds, comprising 26.4% of the total composition, followed by sesquiterpenes (25.3%), fatty acids and derivatives (21.5%), and oxygenated sesquiterpenes (9.2%). The main chemical components identified in DBEO were *β*-barbatene (17.2%), *n*-hexadecanoic acid (6.6%), oleic acid (5.7%), nonanal (5.5%), 1-octen-3-ol (4.9%), 2-butyl-2-octenal (4.5%), benzene acetaldehyde (4.3%), and *β*-bisabolol (4.3%). Previous studies on metabolite characterization of some other *Davallia* species (*Davallia denticulata*, *Davallia heterophylla*, and *Davallia solida*) have been reported, which found that the constituents were 26.09% fatty acids, 17.39% terpenoids, 13.4% phytosterols, 13.04% hydrocarbons, and 13.04% fatty acid ethyl esters [28].

3.2. Antibacterial Activity

Initially, the antibacterial activities of the essential oil and standard antibiotic chloramphenicol (Chl) were determined by *in vitro* disc agar diffusion and microdilution methods against four selected bacterial strains. The antibacterial activity was quantified in terms of the DIZ, MIC, and MBC values listed in Table 2. Accordingly, the DBEO demonstrated strong antibacterial properties specifically against the tested Gram-positive bacteria *S. aureus* (DIZ = 18.4 ± 3.6 mm, MIC = MBC = 0.16 mg/mL) and *B. subtilis* (DIZ = 17.2 ± 2.3 mm, MIC = 0.32 mg/mL, MBC = 0.64 mg/mL).

Table 1. Chemical composition of DBEO

No.	Compound	RI [a]	RI.[b]	RI range	%	Identification
1	1-Octen-3-ol	979	974[c]	967-991[e]	4.9	MS, RI
2	Benzene acetaldehyde	1042	1036[c]	1032-1063[e]	4.3	MS, RI
3	Linalool oxide	1070	1067[c]	1060-1091[f]	2.2	MS, RI
4	Nonanal	1102	1100[c]	1093-1118[e]	5.5	MS, RI
5	(*Z*)-3-Nonen-1-ol	1152	1152[c]	1127-1168[g]	0.8	MS, RI
6	(*E*)-2-Nonenal	1157	1157[c]	1154-1173[e]	1.1	MS, RI
7	Menthol	1171	1171[c]	1169-1194[e]	0.7	MS, RI
8	*α*-Terpineol	1190	1186[c]	1189-1194[h]	1.3	MS, RI
9	Decanal	1203	1201[c]	1195-1217[e]	1.4	MS, RI
10	Undecanal	1304	1305[c]	1295-1319[e]	0.6	MS, RI
11	(*E*,*E*)-2,4-Decadienal	1314	1315[c]	1305-1334[e]	3.7	MS, RI
12	(*E*)-2-Undecenal	1361	1357[c]	1351-1379[e]	0.4	MS, RI
13	2-Butyl-2-octenal	1371	1372[d]	1354-1392[g]	4.5	MS, RI
14	Hexyl hexanoate	1382	1382[c]	1356-1386[i,j]	3.3	MS, RI
15	Longifolene	1409	1407[c]	1387-1434[e]	2.5	MS, RI
16	*β*-Caryophyllene	1419	1417[c]	1384-1430[k]	1.3	MS, RI
17	Widdrene	1435	1429[c]	1424-1450[e]	0.5	MS, RI
18	*γ*-Elemene	1440	1434[c]	1410-1486[e]	0.3	MS, RI
19	*β*-Barbatene	1448	1452[c]	1435-1470[e]	17.2	MS, RI
20	2,6,10-Trimethyltridecane	1458	1465[d]	1440-1466[g]	0.5	MS, RI
21	*β*-Chamigrene	1482	1476[c]	1471-1496[e]	2.5	MS, RI
22	Cuparene	1510	1504[c]	1498-1523[e]	1.1	MS, RI
23	Isoshyobunone	1513	1514[c]	1514-1521[c, l, m]	1.8	MS, RI
24	*δ*-Cadinene	1525	1522[c]	1515-1547[n]	0.5	MS, RI
25	cis-Sesquisabinene hydrate	1536	1542[c]	1524-1562[e]	1.0	MS, RI
26	Dodecanoic acid	1559	1565[c]	1557-1587[e]	0.4	MS, RI
27	Tetradecanal	1608	1611[c]	1605-1623[e]	2.1	MS, RI
28	*β*-Bisabolol	1673	1674[c]	1662-1684[e]	4.3	MS, RI
29	*n*-Pentadecanal	1710	1715[d]	1703-1728[e]	1.7	MS, RI
30	Tetradecanoic acid	1756	1758[d]	1749-1782[e]	0.3	MS, RI
31	Isopropyl tetradecanoate	1820	1828[c]	1812-1837[g]	0.9	MS, RI
32	Hexahydrofarnesyl acetone	1840	1747[d]	1831-1855[e]	2.1	MS, RI
33	(*E*)-2-Hexadecenal	1875	1878[d]	1845-1880[o,p]	1.1	MS, RI
34	Methyl hexadecanoate	1920	1921[c]	1910-1931[e]	0.7	MS, RI
35	9-Hexadecenoic acid	1935	1942[c]	1903-1967[g]	0.7	MS, RI
36	Pimaradiene	1950	1961[c]	1923-1981[g]	0.2	MS, RI
37	*n*-Hexadecanoic acid	1959	1959[c]	1939-1996[e]	6.6	MS, RI
38	Phytol	2108	2114[d]	2104-2136[e]	2.5	MS, RI
39	Oleic Acid	2137	2141[c]	2102-2161[e]	5.7	MS, RI
40	Hexyl hexadecanoate	2370	2381[d]	2364-2381[d,q]	2.9	MS, RI
	Oxygenated monoterpenes				4.2	
	Sesquiterpene hydrocarbons				25.3	
	Oxygenated sesquiterpenes				9.2	
	Carbonylic compounds				26.4	
	Fatty acids and derivatives				21.5	
	Total identified				**96.1**	

[a] Linear retention index calculated relative to *n*-alkanes (C_7-C_{30}) on HP-5MS column; [b] Retention indices reported in the literature: c [18], d [19], e-q [29-41].

In contrast, DBEO possessed lower activity against the Gram-negative bacteria *P. aeruginosa* (DIZ = 13.3 ± 1.1 mm, MIC = 0.64 mg/mL, MBC = 1.28 mg/mL) and *E. coli* (DIZ = 11.5 ± 1.6 mm, MIC = MBC = 0.64 mg/mL).This observation aligns with previous research indicating that the structural differences in bacterial cell walls contribute to their varying susceptibility to essential oils. The existence of a hydrophilic outer layer has been found to be connected with the relative tolerance of Gram-negative bacteria to EOs [42]. This is due to the presence of hydrophilic lipopolysaccharides (LPS) in the outer membrane of Gram-negative bacteria, which act as a defence shield, effectively preventing the penetration of hydrophobic substances. Gram-positive bacteria lack this additional complex membrane; instead, they have a peptidoglycan wall that is too thin to withstand tiny antibiotic molecules, allowing the cell membrane to be accessed [43-45]. Therefore, it may be observed that Gram-negative bacteria exhibit a greater level of resistance toward essential oils [46]. Furthermore, the promising antibacterial activity of essential oils might be dependent on factors like chemical composition and solubility [47]. Previous reports have demonstrated the antibacterial properties of the predominant chemicals found in DBEO, specifically 1-octen-3-ol [48], *β*-bisabolol [49], hexadecanoic acid [50], and oleic acid [51]. Minor components, alongside main substances, also enhance the antibacterial efficacy of essential oils. This may result from the synergistic interaction of prominent and minor characteristics that increase the overall efficacy of the EO [52].

Table 2. DIZ, MIC and MBC values of *D. bullata* EO

Bacterial strains	DIZ, mm		MIC, mg/mL		MBC, mg/mL	
	DBEO	Chl	DBEO	Chl	DBEO	Chl
Gram-positive						
B. subtilis ATCC 6633	17.2 ± 2.3	25.6 ± 3.3	0.32	0.002	0.64	0.016
S. aureus ATCC 6538	18.4 ± 3.6	26.9 ± 2.7	0.16	0.002	0.16	0.002
Gram-negative						
E. coli ATCC 25922	11.5 ± 1.6	24.8 ± 2.7	0.64	0.002	0.64	0.004
P. aeruginosa ATCC 27853	13.3 ± 1.1	19.7 ± 2.9	0.64	0.064	1.28	0.128

3.3. Synergistic Antibacterial Effect

The results obtained for the synergistic effects of DBEO with standard antimicrobial drugs chloramphenicol (Chl) and streptomycin (SM) against four pathogens are displayed in Table 3 and Table 4. The tested combinations showed significant synergistic interaction (FICI ranges from 0.13 to 0.50) against all tested strains with a substantial reduction in the MIC of antibiotics. This study showed that combining DBEO and chloramphenicol or streptomycin enhanced the efficiency of the two routinely used antibiotic agents against all tested strains, thereby providing promising treatment for infections and the challenges posed by antibiotic resistance. These synergistic effects are attributed to various mechanisms, such as the disruption of cell structure, increased permeability of bacterial membranes, inhibition of biofilm formation, disruption of existing biofilms, competition with antibiotics for binding sites on bacterial targets, and multiple antibacterial mechanisms of EOs [53]. The synergistic antibacterial effects observed between essential oils and antibiotics underscore the potential for integrating natural products into antibiotic therapy.

Table 3. Combination interaction of DBEO with chloramphenicol (Chl).

Bacterial strains	sample	MICa (μg/mL)	MICc (μg/mL)	FICI
Bacillus subtilis ATCC 6633	DBEO	320.00	20.00	0.31 (S)
	Chl	2.00	0.50	
Staphylococcus aureus ATCC 6538	DBEO	160.00	40.00	0.50 (S)
	Chl	2.00	0.50	
Escherichia coli ATCC 25922	DBEO	640.00	160.00	0.38 (S)
	Chl	2.00	0.25	
Pseudomonas aeruginosa ATCC 27853	DBEO	640.00	80.00	0.25 (S)
	Chl	64.00	8.00	

Table 4. Combination interaction of DBEO with streptomycin (SM).

Bacterial strains	sample	MICa (µg/mL)	MICc (µg/mL)	FICI
Bacillus subtilis ATCC 6633	DBEO	320.00	40.00	0.25 (S)
	SM	1.00	0.13	
Staphylococcus aureus ATCC 6538	DBEO	160.00	10.00	0.31 (S)
	SM	1.00	0.25	
Escherichia coli ATCC 25922	DBEO	640.00	160.00	0.38 (S)
	SM	2.00	0.25	
Pseudomonas aeruginosa ATCC 27853	DBEO	640.00	40.00	0.13 (S)
	SM	4.00	0.25	

3.4. Cytotoxic Activity of D. bullata Essential Oil

An *in vitro* cytotoxic analysis was conducted using the MTT colorimetric assay to investigate the potential cytotoxic effects of DBEO. This analysis involved four human cancer cell lines, namely lung adenocarcinoma A-549 cells, breast cancer MCF7 cells, hepatocellular carcinoma HepG2 cells, and colorectal carcinoma HCT-116 cells. Additionally, a non-cancerous cell, HL-7702 human liver cells, was included in the analysis. Doxorubicin was used as a positive control. Each of the five cells was treated with a series of different concentrations of essential oils. The results are presented in Table 5. The IC_{50} values for the cytotoxic effects of DBEO on HepG2, MCF-7, A-549, HCT-116, and HL-7702 cells were determined to be 83.27 ± 4.91 µg/mL, 58.38 ± 0.19 µg/mL, 94.37 ± 5.09 µg/mL, 85.62 ± 5.89 µg/mL, and 177.05 ± 1.34 µg/mL for 48 hours of exposure, respectively. Besides, the Selectivity Index (SI = IC_{50} (normal cells)/IC_{50} (cancer cells)) values of DBEO range from 1.88 to 3.03. In addition, as shown in Figure 1, the essential oil of *D. bullata* possessed cytotoxic effects on the corresponding cell lines in a dose-dependent manner. The cytotoxicity of DBEO may be due to the significant cytotoxic effects of the major components of DBEO, such as n-hexadecanoic acid [54], nonanal [55], and 1-octen-3-ol [56, 57]. However, DBEO demonstrated moderate cytotoxicity compared to the positive control doxorubicin.

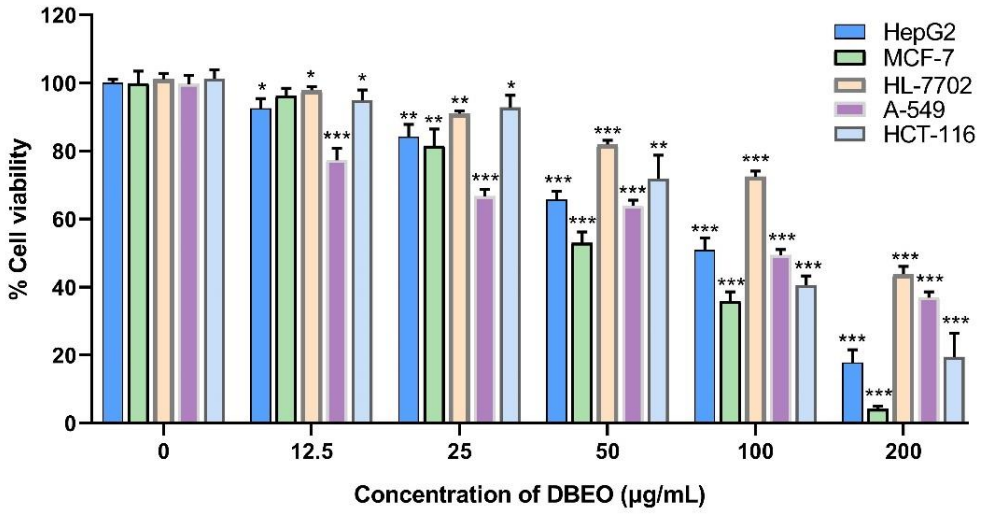

Figure 1. The cell viability percentage at various concentrations of DBEO treatment over a 48-hour period. P-values vs. untreated control of < 0.05 (*), p < 0.01 (**), and p < 0.001 (***) were considered statistically significant differences.

Table 5. Cytotoxicity of *D. bullata* EO (IC_{50}, μg/mL)

Cell lines	Essential oil	Doxorubicin	Selectivity indices of DBEO
HepG2	83.27 ± 4.91	0.56 ± 0.03	2.13
MCF-7	58.38 ± 0.19	0.79 ± 0.05	3.03
A-549	94.37 ± 5.09	0.59 ± 0.02	1.88
HCT-116	85.62 ± 5.89	0.65 ± 0.04	2.07
HL-7702	177.05 ± 1.34	0.67 ± 0.13	-

3.5. Antioxidant Activity of D. bullata Essential Oil

Three antioxidant capacity assay models were performed to assess the antioxidant capacity of the DBEO, namely DPPH and ABTS radical cation scavenging activity and FRAP ferric reducing antioxidant activity. The results of the antioxidant capacity of DBEO are shown in Table 6. The DBEO exhibited a weak level of activity in scavenging radicals compared to synthetic standard antioxidants BHT and Trolox, with IC_{50} values of 675.5 ± 53.3 μg/mL for DPPH and 523.3 ± 28.6 μg/mL for ABTS. Based on the FRAP assay results, the DBEO revealed a moderate ability to reduce ferric ions, as shown by a TEAC value of 107.4 ± 7.5 μmol Trolox × g^{-1}. The antioxidant activity of essential oils (EOs) is primarily attributed to specific chemical components that possess the ability to scavenge free radicals and inhibit oxidative processes. Among the major compounds, studies report IC_{50} values for oleic acid's DPPH radical scavenging activity ranging from 73.213 μM to 104.178 μM, indicating its effectiveness as an antioxidant [58], which may be responsible for the activity of DBEO.

Table 6. Antioxidant potential of *D. bullata* EO

Sample	DPPH IC_{50} (μg /mL)	ABTS IC_{50} (μg /mL)	FRAP (μmol Trolox × g^{-1})
DBEO	675.5 ± 53.3	523.3 ± 28.6	107.4 ± 7.5
BHT	32.5 ± 3.6	9.3 ± 1.5	
Trolox	23.7 ± 2.3	11.6 ± 2.6	

In conclusion, the characterization of the chemical composition of the essential oil obtained by hydrodistillation from *Davallia bullata* rhizomes and the evaluation of its antibacterial, cytotoxic, and antioxidant capacities are presented for the first time herein. While DBEO showed weak antioxidant activity, it was found that the DBEO exhibited significant antibacterial activity against the gram-positive strains. Furthermore, the study revealed the presence of synergistic effects when used together with chloramphenicol or streptomycin, resulting in increased susceptibility of the tested strains to these antibiotics. DBEO exhibited a moderate level of cytotoxicity against HCT-116, HepG2, A-549, and MCF7 cell lines. In contrast, DBEO was observed to show limited cytotoxicity against non-cancerous HL-7702 cell lines. Based on the present findings, DBEO has the potential to be a source of bioactive substances of significant commercial value in pharmaceuticals.

Supporting Information

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

ORCID
Fan Xu: 0009-0009-5502-6316
Xiran Chen: 0009-0001-2503-2055
Jinyi Zhang: 0009-0009-9921-3615
Chunlei Qu: 0009-0007-0999-2260
Ling Gu: 0009-0008-0979-9580
Xiang Xing: 0000-0003-0951-5708

References

[1] H.A.E. Shaaban, A.H. El-Ghorab and T. Shibamoto (2012). Bioactivity of essential oils and their volatile aroma components: Review, *J. Essent. Oil Res.* **24**, 203-212.

[2] H. Cao, T.T. Chai, X. Wang, M.F.B. Morais-Braga, J.H. Yang, F.C. Wong, R. Wang, H. Yao, J. Cao, L. Cornara, et al. (2017). Phytochemicals from fern species: potential for medicine applications, *Phytochem. Rev*. **16**, 379-440.

[3] H.C. Chang, G.J. Hung, D.C. Agrawal, C.L. Kuo, C.R. Wu and H. S. Tsay (2007). Antioxidant activities and polyphenol contents of six folk medicinal ferns used as" Gusuibu", *Bot. Stud*. **48**, 397-406.

[4] M.G. Santos, C.P. Fernandes, L.A.C. Tietbohl, R. Garrett, J.F.R. Lobo, A. Kelecom and L. Rocha (2013). Chemical composition of essential oils from two fern species of *Anemia*, *Am. Fern J*. **103**, 215-224.

[5] S. Ajikumaran Nair, S.R. Rajani Kurup, S.S. Lal, R. Raju, R. Antony and S. Baby (2023). Chemical composition and antiproliferative activity of rhizome and frond essential oils of *Anemia schimperiana* subsp. *wightiana*, a rare fern endemic to South India, *J. Biol. Act. Prod. Nat*. **13**, 105-117.

[6] E. Talebi, I. Nasrollahi and Z. Bashardoost (2022). Phytochemical compounds and bioactivity properties of the whole plant of maidenhair fern (*Adiantum capillus-veneris* L.) essential oil, *Safe Future Agric. Res. J*. **1**, 1-10.

[7] S. Hammami, A. Snène, R. El Mokni, K. Faidi, D. Falconieri, H. Dhaouadi, A. Piras, Z. Mighri and S. Porcedda (2016). Essential oil constituents and antioxidant activity of *Asplenium* ferns, *J. Chromatogr. Sci*. **54**, 1341-1345.

[8] X. Li, S. Qiu, S. Song and P. Lai (2024). Chemical composition and evaluation of the antibacterial, synergistic antibacterial, antioxidant and cytotoxic activities of the essential oil of *Macrothelypteris torresiana* (Gaudich.) Ching, *Rec. Nat. Prod*. **18**, 538-543.

[9] Y.H. Chen, F.R. Chang, Y.J. Lin, P.W. Hsieh, M.J. Wu and Y.C. Wu (2008). Identification of antioxidants from rhizome of *Davallia solida*, *Food Chem*. **107**, 684-691.

[10] C.B. Cui, Y. Tezuka, T. Kikuchi, H. Nakano, T. Tamaoki and J.H. Park (1990). Constituents of a fern, *Davallia mariesii* Moore. I. Isolation and structures of davallialactone and a new flavanone glucuronide, *Chem. Pharm. Bull*. **38**, 3218-3225.

[11] Y.J. Ko, J.B. Wu, H.Y. Ho and W.C. Lin (2012). Antiosteoporotic activity of *Davallia formosana*, *J. Ethnopharmacol*. **139**, 558-565.

[12] A.S. Cheng, W.C. Chang, Y.H. Cheng, K.Y. Chen, K.H. Chen and T.L. Chang (2012). The effects of Davallic acid from *Davallia divaricata* Blume on apoptosis induction in A549 lung cancer cells, *Molecules* **17**, 12938-12949.

[13] J. Cao, X. Xia, X. Dai, Q. Wang and J. Xiao (2014). Chemical composition and bioactivities of flavonoids-rich extract from *Davallia cylindrica* Ching, *Environ. Toxicol. Pharmacol*. **37**, 571-579.

[14] D.J. Yu (1999). Flora of China, Science Press, Beijing. **6** (**1**), p. 300.

[15] L.J. Ni, N.N. Wang, L.G. Zhang, Y.Z. Guo and W.Z. Shi (2016). Evaluation of the effects of active fractions of Chinese medicine formulas on IL-1β, IL-6, and TNF-α release from ANA-1 murine macrophages, *J. Ethnopharmacol*. **179**, 420-431.

[16] Y.T. Lin, S.W. Peng, Z. Imtiyaz, C.W. Ho, W.F. Chiou and M.H. Lee (2021). *In vivo* and *in vitro* evaluation of the osteogenic potential of *Davallia mariesii* T. Moore ex Baker, *J. Ethnopharmacol*. **264**, 113126.

[17] R. Ho, T. Teai, J.P. Bianchini, R. Lafont and P. Raharivelomanana (2011). Ferns: from traditional uses to pharmaceutical development, chemical identification of active principles. In: A. Kumar, H. Fernández, M. Revilla (eds) Working with ferns. Springer, New York, pp. 321-346.

[18] R.P. Adams (2017). Identification of essential oil components by gas chromatography/mass spectrometry. Texensis Publishing. Gruver, Texas.

[19] N.R. Andriamaharavo (2014). Retention Data NIST Mass Spectrometry Data Center, NIST Mass Spectrometry Data Center.

[20] CLSI, Performance Standards for Antimicrobial Disk Susceptibility Tests, Approved Standard, 7th ed., CLSI document M02-A11. Clinical and Laboratory Standards Institute, 950 West Valley Road, Suite 2500, Wayne, Pennsylvania 19087, USA, 2012.

[21] Clinical and Laboratory Standards Institute (CLSI) Performance Standards for Antimicrobial Susceptibility Testing. 28th ed. Clinical and Laboratory Standards Institute; Wayne, PA, USA: 2018. CLSI Supplement M100.

[22] J.M. Andrews (2001). Determination of minimum inhibitory concentrations, *J. Antimicrob. Chemother*. **48**, 5-16.

[23] P. Bellio, L. Fagnani, L. Nazzicone and G. Celenza (2021). New and simplified method for drug combination studies by checkerboard assay, *MethodsX* **8**, 101543.

[24] Z. Schelz, J. Molnar and J. Hohmann (2006). Antimicrobial and antiplasmid activities of essential oils, *Fitoterapia* **77**, 279-285.

[25] T. Mosmann (1983). Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays, *J. Immunol. Methods* **65**, 55-63.

[26] P. Lai, H. Rao and Y. Gao (2018). Chemical composition, cytotoxic, antimicrobial and antioxidant activities of essential oil from *Anthriscus caucalis* M. Bieb grown in China, *Rec. Nat. Prod.* **12**, 290-294.

[27] J. Rumpf, R. Burger and M. Schulze (2023). Statistical evaluation of DPPH, ABTS, FRAP, and Folin-Ciocalteu assays to assess the antioxidant capacity of lignins, *Int. J. Biol. Macromol*. **233**, 123470.

[28] M. Mildawati, S. Sobir, S. Sulistijorini and T. Chikmawati (2022) Metabolite profiling of *Davallia* in The Mentawai Islands, West Sumatra, Indonesia. In: Proceedings of the 7th International Conference on Biological Science (ICBS 2021). *Adv. Biol. Sci. Res*. **22**, 66-72.

[29] V.I. Babushok, P.J. Linstrom and I.G. Zenkevich (2011). Retention indices for frequently reported compounds of plant essential oils, *J. Phys. Chem. Ref. Data*. **40**, 1-47.

[30] Y. Wang, Y. Zheng, Q. Lyu, S. Li, L. Wu, D. Yu and S. Du (2024). Chemical profiles and bioactivities of the essential oils from four Lauraceae plants for controlling *Tribolium castaneum* Herbst, *Rec. Nat. Prod*. **18**, 114-124.

[31] P.J. Linstrom and W.G. Mallard (2014). NIST Chemistry WebBook, NIST Standard Reference Database Number 69. (http://webbook.nist.gov).

[32] M. Kulić, D. Drakul, D. Sokolović, J. Kordić-Bojinović, S. Milovanović and D. Blagojević (2023). Essential oil of *Satureja montana* L. from Herzegovina: assessment of composition, antispasmodic, and antidiarrheal effects, *Rec. Nat. Prod*. **17**, 536-548.

[33] J. Adedeji, T.G. Hartman, R.T. Rosen and C.T. Ho (1991). Free and glycosidically bound aroma compounds in hog plum (*Spondias mombins* L.), *J. Agric. Food Chem*. **39**, 1494-1497.

[34] D. Fraternale, D. Ricci, G. Flamini and G. Giomaro (2011). Volatile profile of red apple from Marche region (Italy), *Rec. Nat. Prod*. **5**, 202-207.

[35] L.D. Herrera-Sanabria, A.K. Villagómez-Guzmán, J. Herrera-Camacho, E.A. Estrella-Parra, J.G. Avila-Acevedo, R.E. del Río, G. Rodríguez-García., T. Hernández-Delgado and M.A. Gómez-Hurtado (2024). Essential oil composition and antibacterial activity of *Trixis michuacana* Lex. var. *michuacana* and *Trixis michuacana* var. *longifolia*, *Rec. Nat. Prod*. **18**, 629-642.

[36] M. Gonny, P. Bradesi and J. Casanova (2004). Identification of the components of the essential oil from wild Corsican *Daucus carota* L. using ^{13}C-NMR spectroscopy. *Flavour Fragr. J*. **19**, 424-433.

[37] J. Paolini, P. Tomi, A.F. Bernardini, P. Bradesi, J. Casanova and J. Kaloustian (2008). Detailed analysis of the essential oil from *Cistus albidus* L. by combination of GC/RI, GC/MS and ^{13}C-NMR spectroscopy. *N.Z. J. Agric. Res*. **22**, 1270-1278.

[38] S. Ugras, P.G. Rasgele, S. Temizce, Z. Emire and T. Dirmenci (2024). Protective effects of *Origanum onites* and its components on lead-nitrate induced genotoxicity in root cells of *Allium cepa* L. *Rec. Nat. Prod.* **18**, 143-154.

[39] M.G. Chisholm, J.A. Jell and D.M. Cass (2003). Characterization of the major odorants found in the peel oil of *Citrus reticulata* Blanco cv. Clementine using gas chromatography-olfactometry, *Flavour Fragr. J*. **18**, 275-281.

[40] F.A. Marques, J.S. McElfresh and J.G. Millar (2000). Kováts retention indexes of monounsaturated C12, C14, and C16 alcohols, acetates and aldehydes commonly found in lepidopteran pheromone blends, *J. Braz. Chem. Soc*. **11**, 592-599.

[41] T. Okumura (1991). Retention indices of environmental chemicals on methyl silicone capillary column, *J. Environ. Chem*. **1**, 333-358.

[42] D. Kalemba and A. Kunicka (2003). Antibacterial and antifungal properties of essential oils, *Curr. Med. Chem.* **10**, 813-829.

[43] S. Chouhan, K. Sharma and S. Guleria (2017). Antimicrobial activity of some essential oils—present status and future perspectives, *Medicines* **4**, 58.

[44] P. Kwiatkowski, Ł. Łopusiewicz, M. Kostek, E. Drozłowska, A. Pruss, B. Wojciuk, M. Sienkiewicz, H. Zielińska-Bliźniewska and B. Dołęgowska (2019). The antibacterial activity of lavender essential oil alone and in combination with octenidine dihydrochloride against MRSA strains, *Molecules* **25**, 95.

[45] M. Hyldgaard, T. Mygind and R.L. Meyer (2012). Essential oils in food preservation: mode of action, synergies, and interactions with food matrix components, *Front. Microbiol*. **3**, 12.

[46] M. Hashemi, A. Ehsani, N.H. Jazani, J. Aliakbarlu and R. Mahmoudi (2013). Chemical composition and *in vitro* antibacterial activity of essential oil and methanol extract of *Echinophora platyloba* DC against some of food-borne pathogenic bacteria, *Vet. Res. Forum*. **4**, 123-127.

[47] Q.N. Okechukwu, F.U. Ugwuona, C.E. Ofoedu, S. Juchniewicz and C.O.R. Okpala (2022). Chemical composition, antibacterial efficacy, and antioxidant capacity of essential oil and oleoresin from *Monodora myristica* and *Tetrapleura tetraptera* in Southeast Nigeria, *Sci. Rep*. **12**, 19861.

[48] C. Xiong, Q. Li, S. Li, C. Chen, Z. Chen and W. Huang (2017). *In vitro* antimicrobial activities and mechanism of 1-octen-3-ol against food-related bacteria and pathogenic fungi, *J. Oleo Sci*. **66**, 1041-1049.

[49] D.R. Bukvicki, A.K. Tyagi, D.G. Gottardi, M.M. Veljic, S.M. Jankovic, M.E. Guerzoni and P.D. Marin (2013). Assessment of the chemical composition and *in vitro* antimicrobial potential of extracts of the liverwort *Scapania aspera*, *Nat. Prod. Commun*. **8**, 1313–1316.

[50] G. Casillas-Vargas, C. Ocasio-Malavé, S. Medina, C. Morales-Guzmán, R.G. Del Valle, N.M. Carballeira and D.J. Sanabria-Ríos (2021). Antibacterial fatty acids: An update of possible mechanisms of action and implications in the development of the next-generation of antibacterial agents, *Prog. Lipid Res*. **82**, 101093.

[51] F. Dilika, P. Bremner and J. Meyer (2000). Antibacterial activity of linoleic and oleic acids isolated from *Helichrysum pedunculatum*: a plant used during circumcision rites, *Fitoterapia* **71**, 450-452.

[52] N. Sharma, Z.N. Sheikh, S. Alamri, B. Singh, M.S. Kesawat and S. Guleria (2023). Chemical composition, antibacterial and combinatorial effects of the essential oils from *Cymbopogon spp*. and *Mentha arvensis* with conventional antibiotics, *Agronomy* **13**, 1091.

[53] M. Fadli, A. Saad, S. Sayadi, J. Chevalier, N.E. Mezrioui, J.M. Pagès and L. Hassani (2012). Antibacterial activity of *Thymus maroccanus* and *Thymus broussonetii* essential oils against nosocomial infection–bacteria and their synergistic potential with antibiotics, *Phytomedicine* **19**, 464-471.

[54] L. Ravi and K. Krishnan (2017). Research article cytotoxic potential of n-hexadecanoic acid extracted from *Kigelia pinnata* leaves, *Asian J. Cell Biol*. **12**, 20-27.

[55] N. Awad, D. Weidinger, L. Greune, J. Kronsbein, N. Heinen, S. Westhoven, S. Pfaender, C. Taube, S. Reuter and M. Peters (2024). Functional characterization of OR51B5 and OR1G1 in human lung epithelial cells as potential drug targets for non-type 2 lung diseases, *Cell Biol. Toxicol*. **40**, 96.

[56] B. Köksal (2015). Cytotoxic and neurotoxic effects of 1-octen-3-ol on learning and memory functions of brain, *Adv. Biol. Res*. **9**, 444-448.

[57] L. Kreja and H.J. Seidel (2002). On the cytotoxicity of some microbial volatile organic compounds as studied in the human lung cell line A549, *Chemosphere* **49**, 105-110.

[58] A.M.A.A. Ramadan, S.A.H. Zidan, R.M. Shehata, H.H. El-Sheikh, F. Ameen, S.L. Stephenson and O.A.-H.M. Al-Bedak (2024). Antioxidant, antibacterial, and molecular docking of methyl ferulate and oleic acid produced by *Aspergillus pseudodeflectus* AUMC 15761 utilizing wheat bran, *Sci. Rep*. **14**, 3183.