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Chemical Composition, Cytotoxic, Antimicrobial and Antioxidant Activities of Essential oil from *Anthriscus caucalis* M. Bieb Grown in China

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Abstract: The essential oil of the aerial parts of *Anthriscus caucalis* M. Bieb was obtained by hydrodistillation and its components were analyzed by using GC and GC-MS. Forty-six compounds representing 97.2% of the total oil were identified. The main constituents in the oil were identified as *β*-Bisabolene (28.4%), Germacrene D (18.9%), (*Z, E*)-*α*-Farnesene (16.8%) and *γ*-Muurolene (7.3%). *In vitro* cytotoxicity evaluation against two cell lines of HepG2 (liver hepatocellular cells) and MCF-7 (human breast adenocarcinoma) cells showed a potent cytotoxic activity with the IC_{50} values of 67.50 µg/mL and 55.83 µg/mL according to the MTT assay. Furthermore, the essential oil exhibited a considerable activity against *Bacillus subtilis* and *Escherichia coli* with the MIC values of 0.095 mg/mL and 0.105 mg/mL tested by micro-dilution method. The antioxidant activity was evaluated by DPPH and FRAP methods, and the essential oil gave an IC_{50} value of 0.451 mg/mL and a Trolox equivalent antioxidant concentration of 191.7 ± 11.3 µmol Trolox × g^{-1} in DPPH and FRAP, respectively. The results indicated that the essential oil was relatively active and may be useful in food and pharmaceuticals after more detailed study.

Keywords: *Anthriscus caucalis* M. Bieb; essential oil; antioxidant activity; antibacterial activity; cytotoxic activity.

1. Plant Source

The aerial parts from *Anthriscus caucalis* M. Bieb were collected from southern hills of Xifeng County in Guizhou province of China, during September 2016. The plant material was identified by Associate Prof. Hong Zhao of the Marine College, Shandong University (Weihai). A voucher specimen (NO.10678) has been deposited at the Laboratory of Botany of Marine College, Shandong University.

2. Previous Studies

Anthriscus caucalis M. Bieb, an annual plant in the Apiaceae family, is a newly naturalized plant of Chinese Mainland and is native to and common in parts of Europe and Asia [1]. The

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composition of the essential oil from *Anthriscus caucalis* M. Bieb (Ac-EO) collected in the urban area of Vienna has been studied, where the main compounds of all oils were *cis*-chrysanthenyl acetate (up to 74%) and *cis*-chrysanthenol (up to 16 %) [2]. However, it is noteworthy that these two compounds were not found in present study.

3. Present Study

To the best of our knowledge, the composition of the essential oil of *Anthriscus caucalis* from China have not been investigated before, and there was no published study on the biological activities of essential oil of species. For this reason, we extracted essential oil of species by hydrodistillation and the chemical composition is identified by GC-FID and GC-MS (Gas Chromatography-Mass Spectrometer). Furthermore, we evaluated the antibacterial, cytotoxic and antioxidant activities of the studied oil. (See Supporting Information for the details of the experiment [3])

Table 1. Chemical composition of essential oil of *A. caucalis* M.Bieb

Compound [a]	RI [b]	RI [c]	% Area[d]	Ref.	Compound [a]	RI [b]	RI [c]	% Area[d]	Ref.
(*Z*)-*β*-Ocimene	1038	1037	0.1	[4]	(Z,E)-α-Farnesene	1494	1495	16.7	[4]
γ-Terpinene	1056	1056	0.1	[5]	Germacrene D	1503	1500	18.9	[7]
Terpinolene	1092	1092	t	[5]	*α*-Farnesene	1509	1509	3.0	[5]
Undecane	1100	1100	0.2	[5]	*β*-Bisabolene	1520	1519	28.4	[5]
Linalool	1103	1100	1.3	[6]	Bicyclogermacrene	1528	1533	1.0	[5]
3-Octyl acetate	1118	1117	t	[5]	*δ*-Cadinene	1537	1539	2.5	[7]
Geijerene	1147	1147	0.1	[5]	*α*-Muurolene	1543	1540	0.2	[5]
p-Mentha-1,5-dien-8-ol	1154	1156	0.4	[5]	*γ*-Cadinene	1554	1553	0.2	[5]
Thymol methyl ether	1237	1237	0.1	[5]	Nerolidol	1569	1567	0.2	[5]
Tridecane	1300	1300	0.2	[5]	Viridiflorol	1587	1587	0.2	[5]
α-Cubebene	1361	1361	t	[7]	*β*-Elemenone	1600	1605	0.6	[5]
α-Copaene	1389	1390	1.2	[7]	Caryophyllene oxide	1612	1611	0.1	[5]
β-Cubebene	1394	1392	0.9	[5]	Zingiberenol	1618	1616	0.1	[5]
β-Elemene	1404	1404	0.9	[6]	*τ*-Muurolol	1633	1632	0.2	[5]
α-Bergamotene	1427	1428	0.1	[5]	Cubenol	1651	1650	0.4	[5]
Isogermacrene D	1439	1439	0.3	[5]	*τ*-Cadinol	1667	1665	0.6	[5]
α-Himachalene	1443	1444	2.6	[5]	*α*-Cadinol	1680	1680	0.8	[5]
β-Caryophyllene	1446	1441	2.9	[6]	*α*-Bisabolol	1688	1689	0.2	[4]
(E)-*β*-Farnesene	1453	1453	0.5	[5]	*cis*-Farnesol	1704	1699	0.2	[5]
(Z)-*β*-Farnesene	1457	1458	1.8	[6]	3-Oxo-7,8-dihydro-α-ionol	1719	1710	0.4	[5]
9-*epi*-*β*-Caryophyllene	1465	1465	0.1	[5]	Neophytadiene	1833	1832	0.4	[6]
cis-Muurola-4(14),5-diene	1471	1470	0.1	[5]	Hexadecanoic acid	1960	1960	0.5	[5]
γ-Muurolene	1482	1481	7.3	[4]	Phytol	2113	2113	0.2	[5]
					Monoterpene hydrocarbons			0.3	
					Oxygenated monoterpenes			0.9	
					Sesquiterpene hydrocarbons			89.8	
					Oxygenated sesquiterpenes			3.5	
					Diterpenes hydrocarbons			0.4	
					Oxygenated diterpenes			0.2	
					Total identified			97.2	

[a] are listed in order of their elution from a HP-5MS column; [b] (retention index): RI-non-isothermal Kovats retention indices on a HP-5MS column relative to C_{10}-C_{30} n-alkanes; [c] linear retention indices according to the literature and NIST Chemistry WebBook on a HP-5MS column; [d] The content (%) of the individual components was calculated based on the peak area (FID response); t: trace (<0.1%).

The essential oil was yellow with a strong earthy odor. The GC-MS analyses revealed 46 compounds representing 97.2% of the oil. The identified compounds with their RI are listed in Table 1 (See Supporting Information). *β*-Bisabolene (28.4%), germacrene D (18.9%), (Z,E)-*α*-Farnesene (16.8%), *γ*-Muurolene (7.3%), α-Farnesene (3.0%), *β*-Caryophyllene (2.9%), α-Himachalene (2.6%) and δ-Cadinene (2.5%) were identified and found to be the predominant compounds in the essential oil. These compounds are encountered in other *Anthriscus* species in different proportions. For example, Germacrene D, was found to be one of the main compounds of *A. cerefolium* and *A. nemorosa* [3]. Moreover, the presence of sesquiterpenoids as the main components is in accordance with previous studies on species of the *Anthriscus* genus [8,9].

Cytotoxic activity test: The Cytotoxic activity of the essential oil was estimated using the MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] assay against the HepG2 (liver hepatocellular cells) and MCF-7 (human breast adenocarcinoma cell line) cancer cell lines [10,11]. The result of cytotoxic activity test is given in Table 1.

According to the cytotoxic activity test, the growth of both cell lines was inhibited by essential oil of *A. caucalis* in a concentration-dependent manner. Likewise, cytotoxicity of the oil was increased as a function of duration of exposure, suggesting a time-dependent effect of the oil. The IC_{50} values for the cytotoxic effects of essential oil of *A. caucalis* on HepG2 and MCF-7 cells for 72 h were calculated to be (67.50 ± 17.70) µg /mL and (55.83 ± 8.34) µg /mL, respectively.

The cytotoxic activity of essential oil of *A. caucalis* against the tested cells could be attributed to the presence of significant amounts of *β*-Bisabolene and *β*-Caryophyllene. β-Bisabolene, an intermediate in the biosynthesis of many other natural chemical compounds, exhibited potent anti-tumour properties against B16-F10, HepG2, HL-60 and MCF-7 cells lines [12,13]. Besides, β-Caryophyllene also has been reported to potentiate the cytotoxic activity of paclitaxel against MCF-7, DLD-1 and L-929 tumor cell lines [14].

Antibacterial Activity test: The antibacterial activity of essential oil of *A. caucalis* was qualitatively and quantitatively assessed by the presence or absence of inhibition zone diameters and MIC values.

As presented in Table 3, essential oil of species showed a strong antibacterial activity against tested Gram-positive and Gram-negative bacteria strains. The best activities were observed against *B. subtilis* with an MIC value of 0.095 mg/mL followed by *E. coli* (MIC=0.105 mg/mL), *S. aureus* (MIC=0.150 mg/mL), *P. aeruginosa* (MIC=0.320 mg/mL).

Table 2. Cytotoxic activity of essential oil of *A. caucalis* against HepG2 and MCF-7 cells

	IC_{50} (µg /mL)			
	HepG-2		**MCF-7**	
	Ac-EO	**Doxorubicin**	**Ac-EO**	**Doxorubicin**
24h	114.53 ± 14.74	2.64 ± 0.44	95.88 ± 6.12	1.12 ± 0.42
48h	91.03 ± 6.54	0.88 ± 0.02	79.65 ± 6.58	0.34 ± 0.12
72h	67.50 ± 17.70	0.49 ± 0.07	55.83 ± 8.34	0.13 ± 0.04

IC_{50}: the concentration of compound that affords a 50% reduction in cell growth (after 24, 48, and 72 h of incubation). Doxorubicin was tested as a reference. Expressed as the mean ± SD of triplicate experiments.

The strong antibacterial activity of essential oil against the tested bacteria could be attributed to the presence of high concentration of β-Bisabolene, Germacrene D, (*Z,E*)-α-Farnesene and β-Caryophyllene. β-Bisabolene has been reported to exhibit the potential to restore the effectiveness of ampicillin against resistant *S. aureus* [15]. Likewise, some studies demonstrated that Germacrene D, (*Z,E*)-α-Farnesene and β-Caryophyllene have significant antibacterial and antifungal activities [16-18].

Table 3. Antibacterial activity of essential oil of *A. caucalis*

Test strains	Ac-EO		chloramphenicol.	
	ZI[a] (mm)	MIC[b] (mg/mL)	ZI (mm)	MIC (mg/mL)
Gram positive				
Staphylococcus aureus ATCC 6538	21.1±0.5	0.150	27.8±0.9	0.025
Bacillus subtilis ATCC 6633	22.4±0.8	0.095	30.2±1.0	0.013
Gram negative				
Escherichia coli ATCC 25922	21.5±0.4	0.105	32.5±0.5	0.125
Pseudomonas aeruginosa ATCC 27853	12.4±0.4	0.320	15.5±0.4	0.100

ZI [a] = The diameter of the inhibition zones (mm), including the disc diameter (6 mm), are given as the mean ± SD of triplicate experiments and MIC [b] = minimum inhibitory concentration

Antioxidant activity test: A single assay does not accurately account for all of the groups of antioxidant compounds, particularly in a complex system. Therefore, essential oil of *A. caucalis* was subjected to screening for the possible antioxidant activity by two methods namely DPPH (2,2-diphenyl-1-picrylhydrazyl) radical-scavenging [19] and ferric reducing antioxidant power (FRAP) assays [20]. The results are presented in Table 4.

It was observed that essential oil of *A. caucalis* exhibited a weak DPPH radical-scavenging activity with an IC_{50} value of 0.451 mg/mL compared with the standard, BHT (IC_{50} value of 0.043 mg/mL). In view of the results of FRAP assay, the essential oil showed a moderate ferric ion reducing activity (Trolox equivalent antioxidant concentration = 191.7 ± 11.3 μmol Trolox × g^{-1}).

Table 4. Results of antioxidant activity *in vitro* (DPPH, FRAP) of essential oil of *A. caucalis*

Test Sample	DPPH IC_{50} (mg/ml)[a]	FRAP (μmol Trolox × g^{-1})
Ac-EO	0.451 ± 0.032	191.7 ± 11.3
BHT [b]	0.043 ± 0.001	

Note:Each value is presented as mean ± standard deviation (n = 3).
[a] IC_{50} = The concentration of compound that affords a 50% reduction in the assay.
[b] Positive control used.

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Supporting Information

Supporting Information accompanies this paper on http://www.acgpubs.org/RNP

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