SUPPLEMENTARY MATERIAL

Chemical composition, in vitro antioxidant activity and α -glucosidase inhibitory effects of the essential oil and methanolic extract of *Elsholtzia densa* Benth.

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Abstract

Elsholtzia densa Benth. is a traditional aromatic herb used in the pharmaceutical and flavoring industries. To analyze and compare the chemical composition, the oils and nonvolatile compounds in *E. densa* and *Mosla chinensis* Maxim. were extracted via hydrodistillation, solvent extraction or ultrasound-assisted extraction. 73 volatile compounds in the volatile oil ($0.35\pm0.06\%$) obtained by *E.densa* via hydrodistillation were investigated by Gas Chromatography-Mass Spectrometer and compared based on different parameters. Also, the antioxidant activity and α -glucosidase inhibitory effects of the five sub-fractions of the methanolic extract were studied and the ethyl acetate sub-fraction ($EC_{50}=7.9\mu\text{g/mL}$) and petroleum ether sub-fraction ($EC_{50}=0.0955\text{mg/mL}$) showed the strongest activity respectively. This study has provided a scientific basis for scientific collection, effective development, use of *Elsholtzia densa* Benth., and suggested that it can be used as a potential source of antioxidants in food and a potential candidate for the management of type 2 diabetes mellitus.

Keywords: *Elsholtzia densa* Benth.; comparisons of the chemical composition; antioxidant activity; α -glucosidase inhibitory effects

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Experimental

1. Plant materials

Samples of *E. densa* were collected from the Qinling mountains (Shaanxi, China) in June 2014 and were dried at 30 °C. Samples of dried *M. chinensis* were purchased from the Shaanxi Guangji Pharmacy. Species identification was confirmed by Professor Guo Zengjun of the School of Pharmacy, Xi'an Jiaotong University. The voucher specimen was kept at the Pharmacy Department of the Medical School of Xi'an Jiaotong University for future reference.

2. Preparation of the methanol extracts

Methanol extract of dried plant (50g) was refluxed at a temperature of 70°C. The resulting extracts were filtered and concentrated in vacuum at 40°C using a rotary evaporator. The residues obtained was suspended in water using an ultrasonic bath and successively partitioned with petroleum ether, chloroform, ethyl acetate and butanol to obtain five sub-fractions. Then the solvents were removed by a rotary evaporator, and the dried residues were kept in a dark place at -4°C until the test.

3. Extraction of the essential oil

Hydrodistillation: The dried *E. densa* samples were subjected to HD with a Clevenger-type apparatus for 4 h, and the essential oil was collected. The aerial parts and roots of the dried *E. densa*, as well as the dried *M. chinensis* samples, were similarly treated. The obtained essential oils were dried with anhydrous sodium sulfate and stored at 4 °C until further use. The nonvolatile components were dissolved in water and fractionated by ethyl acetate; the samples were stored at 4 °C until further use.

Solvent Extraction: The dried plant samples were weighed and quantitatively transferred into a conical flask, before the chemical components were extracted with ethyl acetate for 48 h at room temperature. After extraction, the samples were stored at 4 °C until further use.

Ultrasound-assistant Extraction: The dried plant samples were weighed and quantitatively transferred into a conical flask before these samples were treated with a fixed ultrasound power of 150 W for 2 h at room temperature. After extraction, the samples were stored at 4 °C until further use.

4. Analysis of the essential oil

GC/MS was performed on a Shimadzu GCMS-QP2010 Plus detector coupled to a Shimadzu GC-2010 apparatus with a Rtx-5MS capillary column (5% diphenyl, 95% dimethyl polysiloxane; length, 30 m; inner diameter, 0.25 mm; film thickness, 0.25 μ m). The injector, GC–MS interface, and ion source were maintained at 240, 280, and 200 °C, respectively. The following temperature program was employed: 60 °C (3 min), 60–260 °C (10 °C min), and holding time at 260 °C (30 min). All the compounds were identified by comparing their EI-mass spectra and their retention times with the National Institute of Standards and Technologies Mass Spectra Library.

5. Antioxidant capacity assays

DPPH radical scavenging activity was measured according to the method of Blois (1958) with minor modifications. Briefly, methanol-diluted sample was mixed with same volume of 0.1mmol/L DPPH solution dissolved in methanol. Following 30min incubation at 37°C in the dark, absorbance was measured at 517 nm and DPPH radical scavenging activity was calculated using the following formula: Inhibition% = $[(A_{\text{control}} - A_{\text{sample}})/A_{\text{control}}] \times 100$. The five sub-fractions of the methanol extract were successively determined and vitamin C was used as the standard.

6. α -glucosidase inhibition assay

Inhibitory activity against α -glucosidase was determined as described by Tibbot and Skadsen (1996). Briefly, the reaction mixture containing 20 μ L of diluted extract sample, 10 μ L 10U/mL α -glucosidase solution (25mM PBS buffer; pH 6.86) and 1.5mL 25mM PBS buffer (pH 6.86) was pre-incubated at 37°C for 10 min. Then, 20 μ L of 2.5mmol/L pNPG (25mM PBS buffer; pH 6.86) was added to the mixture, incubated in a water bath at 37°C for 20 min, and the reaction was stopped by 0.5mL 1mol/L Na₂CO₃ solution. The absorbance was measured at 405 nm. AGI activity was calculated as: Inhibition% = $[(A_{\text{control}} - A_{\text{sample}})/A_{\text{control}}] \times 100$. The five sub-fractions of the methanol extract were successively determined and acarbose was used as the standard.

Table S1 Chemical composition of oils obtained from the whole plants, aerial parts and roots of *E. densa*

Constituents	RI	MS%		
		Whole Plants	Aerial Parts	Roots
Toluene	669.6	0.10		3.47
Hexanal	687.5	0.43	0.66	4.06
Camphene	786.4	0.18	0.40	
1-Octen-3-ol	803.9	2.90	tr	
5-Hepten-2-one, 6-methyl-	807.9	6.50	tr	
3-Octanone	813.2	1.68	8.68	
3-Octanol	821.4	tr	5.52	
Eucalyptol	839.3	1.38	1.69	2.22
1,3,6-Octatriene, 3,7-dimethyl-, (Z)-	848.2	5.06	6.20	
Acetophenone	864.1	6.22	9.31	1.89
Octen-1-ol, acetate	889.3	1.99		2.26
Cyclobutanecarboxylic acid, octyl ester	896.1	1.46	1.66	
2,6-Octadienal, 3,7-dimethyl-, (Z)-	975.5	7.62	3.63	
2,6-Octadienal, 3,7-dimethyl-, (E)-	992.8	7.78	4.93	
2,6-Octadien-1-ol, 3,7-dimethyl-, acetate, (Z)-	1004.5	1.28	2.00	
Di-epi-. α .-cedrene	1055.2	tr		3.91
Benzene, 2-(1,1-dimethylethyl)-1,4-dimethoxy-	1070.2	tr		2.46
Caryophyllene	1075.2	7.85	8.54	0.68
Cedrene	1077.3	tr	0.74	6.21
1,6-Cyclodecadiene,1-methyl-5-methylene-8-(1-methylethyl)-, [s-(E,E)]-	1079.0	2.44	4.22	
2-Methyl-2-octene	1084.6	2.20	2.29	2.39
α -Caryophyllene	1093.4	14.34		9.16
Benzene, 1-(1,5-dimethyl-4-hexenyl)-4-methyl-	1100.7	3.68		6.60
α -Farnesene	1105.6	0.92	1.60	
1,6,10-Dodecatrien-3-ol, 3,7,11-trimethyl-, (E)-	1137.5	1.68	2.88	
1,5,5,8-Tetramethyl-12-oxabicyclo[9.1.0]dodeca-3,7-diene	1166.7	2.43	3.50	2.77
.tau.-Muurolol	1183.9	2.08	2.81	
α -Cadinol	1186.0	2.21	2.66	
1-Heptatriacotanol	1194.4	tr		3.16
1H-Indene, 2,3-dihydro-1,1,3-trimethyl-3-phenyl-	1223.6	0.21		2.59
2-Pentadecanone, 6,10,14-trimethyl-	1249.8	tr	0.79	3.01
Geranyl benzoate	1280.2	tr		14.75
Pregnenolone	1283.4	1.65	1.04	3.15
(E,E,E)-3,7,11,15-Tetramethylhexadeca-1,3,6,10,14-pentaene	1298.9	tr		3.4
Bicyclo[4.1.0]heptan-2-one,3,4,4-trimethyl-3-(3-methyl-1,3-butadienyl)-	1546.9	tr		7.09

Table S2 Nonvolatile compounds obtained from the whole plants, aerial parts, and roots of *E. densa*.

Constituents	RI	MS%		
		Whole plants	Aerial Parts	Roots
Toluene	668.6	36.25	34.34	55.01
Oxirane-2-carboxylic acid, ethyl ester	684.6	tr		2.44
Acetic acid, butyl ester	692.9	7.16	8.77	6.06
1-Butanol, 3-methyl-, acetate	731.1	5.62	5.71	5.02
Octane, 3,3-dimethyl-	828.9	tr		3.88
Decane, 4-methyl-	830.0	4.38	6.3	
Nonane, 4,5-dimethyl-	852.5	tr		5.45
Undecane, 5,7-dimethyl-	853.2	6.2	6.72	
Allyl n-octyl ether	881.4	9.31	10.1	
Decane, 2,6,7-trimethyl-	952.8	4.22	4.81	
Undecane, 2,4-dimethyl-	982.3	5.68		3.45
Dodecane, 2,6,10-trimethyl-	991.7	6.97	5.29	
Heptadecane	1102.1	4.18		7.74
Hexadecane	1121.1	11.42	13.48	8.02
Eicosane	1195.8	4.27	4.49	

Table S3 Chemical composition of extracts from *E. densa* obtained via hydrodistillation, solvent extraction, and ultrasound-assisted extraction.

Constituents	RI	MS%		
		HD	SE	UAE
Toluene	668.6			7.61
Acetic acid, butyl ester	691.8		2.83	2.52
1-Octen-3-ol	803.9	2.90		
5-Hepten-2-one, 6-methyl-	807.9	6.50		
Allyl n-octyl ether	832.9	-	1.08	0.48
Eucalyptol	839.8	1.38		1.02
1,3,6-Octatriene, 3,7-dimethyl-, (Z)-	848.2	5.06		
Acetophenone	867.5	6.22		
Octen-1-ol, acetate	889.2	4.99		3.53
2,6-Octadienal, 3,7-dimethyl-, (Z)-	975.5	7.62		
2,6-Octadienal, 3,7-dimethyl-, (E)-	992.8	7.78		
Caryophyllene	1075.2	7.85		
Cedrene	1079.4	2.44	2.24	4.86
α-Caryophyllene	1093.4	14.34	9.03	10.01
Benzene, 1-(1,5-dimethyl-4-hexenyl)-4-methyl-	1104.9	3.68	2.41	
α -Farnesene	1109.1	2.92		
Naphthalene,1,2,3,5,6,8a-hexahydro-4,7-dimethyl-1-(1-	1122.1	2.68		

methylethyl)-, (1S-cis)- 1,5,5,8-Tetramethyl-12-oxabicyclo[9.1.0]dodeca-3,7-diene	1166.7	2.43	
3,7,11,15-Tetramethyl-2-hexadecen-1-ol	1249.5	4.26	3.65
N-Benzyl-2-aminocinnamate, methyl ester	1487.7	4.36	-
2,4-Di-tert-butylphenyl benzoate	1509.4	9.65	5.85
3-Benzoyl-2-t-butyl-4-isopropyl-4-methyl-oxazolidin-5-one	1530.6		9.12
3-Isopropyl-6a,10b-dimethyl-8-(2-oxo-2-phenyl-ethyl)-dodecahydro-benzo[f]chromen-7-one	1546.9	2.02	2.11
Octadecane, 3-ethyl-5-(2-ethylbutyl)-	1590.0	8.19	1.8
Nonacosane	1601.8	9.92	2.47
Stearic acid, 3-(octadecyloxy)propyl ester	1649.6	3.63	0.97
6,10-Dodecadien-3-ol, 3,7,11-trimethyl-	1663.5	3.19	2.63
Octadecane, 1,1'-[1,3-propanediylbis(oxy)]bis-	1684.0	1.5	0.97
Tetrapentacontane	1695.4	31.16	10.69
Hexatriacontane	1924.5		12.91
Pentatriacontane	2256.7		8.63

HD: hydrodistillation; SE: solvent extraction; UAE: ultrasound-assistant extraction

Table S4 Chemical composition of oils extracted from *E. densa* and *M. chinensis*.

Compounds	RI	MS%	
		<i>E.densa</i>	<i>M.chinensis</i>
Hexanal	684.3	0.43	0.13
Bicyclo[3.1.0]hex-2-ene, 2-methyl-5-(1-methylethyl)-(1R)-2,6,6-Trimethylbicyclo[3.1.1]hept-2-ene	765.4	0.25	0.96
Camphene	771.1	0.18	1.31
Benzaldehyde	782.1	0.18	0.17
Bicyclo[3.1.0]hexane, 4-methylene-1-(1-methylethyl)-	793.6		3.26
1-Octen-3-ol	798.9		15.51
5-Hepten-2-one, 6-methyl-	803.9	2.90	
Benzene, 1-methyl-2-(1-methylethyl)-	807.9	6.50	
Eucalyptol	835.0		2.86
1,3,6-Octatriene, 3,7-dimethyl-, (Z)-	839.3	1.38	1.45
1,4-Cyclohexadiene, 1-methyl-4-(1-methylethyl)-	848.2	5.06	
Acetophenone	857.1	0.77	2.69
Octen-1-ol, acetate	867.5	6.22	1.54
Thujone	889.3	4.99	
3-Cyclohexen-1-ol, 4-methyl-1-(1-methylethyl)-	898.2		2.78
Benzaldehyde, 4-(1-methylethyl)-	938.5	0.94	4.17
2,6-Octadienal, 3,7-dimethyl-, (Z)-	947.5	0.03	0.31
2,6-Octadienal, 3,7-dimethyl-, (E)-	975.5	7.62	
	992.8	7.78	

Thymol	1010.5		7.72
Phenol, 2-methyl-5-(1-methylethyl)-	1016.4		7.69
Caryophyllene	1075.2	7.85	5.29
1,6-Cyclodecadiene	1079.0	2.44	
1,6,10-Dodecatriene, 7,11-dimethyl-3-methylene-, (E)-	1086.7		7.33
α-Caryophyllene	1093.3	14.34	2.32
Asarone	1096.9		2.54
Benzene, 1-(1,5-dimethyl-4-hexenyl)-4-methyl-	1104.9	3.68	
α -Farnesene	1109.2	2.92	
Naphthalene, 1,2,3,5,6,8a-hexahydro-4,7-dimethyl-1-(1-methylethyl)-, (1S-cis)-	1122.1	2.68	
1,3-Benzodioxole, 4-methoxy-6-(2-propenyl)-	1124.9		6.43
Caryophyllene oxide	1155.4		3.15
1,5,5,8-Tetramethyl-12-oxabicyclo[9.1.0]dodeca-3,7-diene	1166.7	2.43	
Apiol	1169.8		8.32
1H-Indene, 2,3-dihydro-1,1,3-trimethyl-3-phenyl-	1208.1	0.21	1.02

Table S5 Nonvolatile compounds in *E. densa* and *M. chinensis*.

Compounds	RI	MS%	
		<i>E.densa</i>	<i>M.chinensis</i>
Toluene	668.6	6.25	32.24
Acetic acid, butyl ester	692.9	7.16	
Heptane, 2,4-dimethyl-	695.0		3.10
1-Butanol, 3-methyl-, acetate	730.0	3.92	
Decane, 4-methyl-	828.6	4.38	
Undecane, 5,7-dimethyl-	852.1	10.2	
Nonane, 4,5-dimethyl-	855.7		2.37
Allyl n-octyl ether	881.4	13.31	
Bicyclo[3.1.0]hexan-3-one, 4-methyl-1-(1-methylethyl)-	893.9		5.54
Decane, 2,6,7-trimethyl-	952.1	4.22	
Undecane, 2,4-dimethyl-	982.3	5.68	
Dodecane, 2,6,10-trimethyl-	991.7	6.97	
Benzene, 1,2-dimethoxy-4-(2-propenyl)-	1070.3		13.38
1,6,10-Dodecatriene, 7,11-dimethyl-3-methylene-	1090.2		3.97
Heptadecane	1102.1	4.18	
Hexadecane	1121.1	11.42	3.27
Eicosane	1195.8	10.27	
1,4-Benzenediol, 2,5-bis(1,1-dimethylethyl)-	1203.2	6.1	
Benzene, (1-ethyloctadecyl)-	1243.5	3.96	
9-Octadecenamide, (Z)-	1450.7		30.43
8-Methyl-6-nonenamide	1657.8		5.68

Figure S1 Free radical scavenging properties of the methanolic sub-fractions of *E.densa*

Figure S2 α -glucosidase inhibitory effects of the methanolic sub-fractions of *E.densa*