SUPPLEMENTARY MATERIAL

Chemical composition and cytotoxicity of the essential oil from different parts of *Datura metel* L.

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Abstract

The essential oil from different parts of *Datura metel* L. were extracted using hydro-distillation and GC-MS was used to analyse the essential oil. The main components of flowers were ketone (23.61%), ethyl palmitate (15.84%). The main components of leaves were ketone (18.84%), phytol (18.71%). Ketone (39.45%) and phytol (31.32%) were the major components of petioles. Palmitic acid (30.60%) and ethyl linoleate (21.56%) were the major components of seeds. The major ingredient of roots was palmitic acid (52.61%). The main ingredients of the stems were palmitic acid (38.38%), ethyl linoleate (17.38%). All the different parts of essential oil were screened for cytotoxicity. The roots and stems showed the inhibitory effects against HepG-2 with IC₅₀ levels of

34 613.88 mg/L and 341.12 mg/L. The leaves and roots showed the inhibitory
35 effects against HeLa with IC₅₀ levels of 267.76 mg/L and 348.35 mg/L. All the
36 six parts have inhibitory effects against SGC-7901 cell lines.

37 **Keywords:** *Datura metel L.*, *essential oil*, *GC-MS*, *cytotoxicity*.

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1. Experimental

1.1. Plant material and essential oil isolation

The flowers, leaves, petioles, seeds, roots and stems of *D. metel* were all collected at Lingao County, Hainan Province, People's Republic of China, in September 2012, and was identified by Prof. Zhenyue Wang (Department of Chinese Medicine Resources, Heilongjiang University of Chinese Medicine). A voucher specimen (2012184) has been deposited at the Herbarium of Heilongjiang University of Chinese Medicine, P. R. China

1.2 Essential oil isolation

The fresh flowers, leaves, petioles, seeds, roots and stems (250g respectively) of *D. metel* were hydrodistilled for 4 h using a Clevenger apparatus. The six samples all yielded yellowish oil with a pleasant smell, and the content of essential oil is 0.15%, 0.10%, 0.05%, 0.15%, 0.10% and 0.12% (on a dry mass), respectively. The obtained essential oil was dried over anhydrous sodium sulfate and stored at 4 °C before analysis and bioassay.

1.3 Essential oil analysis

Analysis of the volatile extracts was carried out by gas chromatography (GC) and by gas chromatography-mass spectrometry (GC-MS). Analytical GC was carried out in a gas chromatograph (Agilent, Model 7890A), equipped with a flame ionization detector (FID), an autosampler (Agilent, Model 7693), Agilent DB-17 fused silica column (5 % phenyl-methylpolysiloxane), 60 m × 0.25 mm i.d., film thickness 0.25 µm, and a Agilent ChemStation software system(E.02.01). Oven temperature was settled at 120 °C, raising at 3 °C min⁻¹ to 280 °C and then held 2min at 280 °C; injector temperature: 250 °C; carrier gas: helium at 1.2 mL/min; splitting ratio 1:10; detectors temperature: 300 °C. GC-MS was carried out in a Agilent 6890 gas chromatograph equipped with a mass spectrometer(Agilent, Model 5975C), GC parameters as described above; interface temperature: 250 °C; MS source temperature: 230 °C; MS quadrupole temperature: 150°C; ionization energy: 70 eV; scan range: 20–550 units. Identification of individual compound was made by comparison of their mass spectra with those of the internal reference mass spectra library (NIST08), or with authentic

compounds and confirmed by comparison of their retention index with authentic compounds or those reported in the literature (Adams 2007).

1.4 Cytotoxicity activity

1.4.1 Cell lines

The cytotoxicity activities of the *Datura metel* L. essential oil were tested against three cell lines. HepG-2 (ATCC HB-8065), HeLa (ATCC HTB-22) and SGC-7901 (ATCC CRL-1740).

1.4.2 Cytotoxicity assay

The cytotoxicity activities of the *Datura metel* L. essential oil were tested against t The cytotoxic activity of essential oil of six parts against human cancer cell lines HepG-2, HeLa, SGC-7901. 5-Fluorouracil (Sigma Chemical Co; purity $\geq$ 98%) was used as reference compound to assess the cytotoxicity of tested essential oils. Cell numbers were counted with a hemocytometer by trypan blue dye exclusion technique to make sure that cell viability exceeded 95 %. Briefly, cells were plated in 96-well plates 24 h before treatment and continuously exposed to different concentrations of six parts of essential oil. Then cells were cultured in triplicate for 48 h with the test compounds. Cells cultured with medium alone were used as controls. MTT (5 mg/mL) reagent was added 4 h before the end of culture, the plates were centrifuged (1400 rpm, 5 min), and the untransformed MTT was removed carefully by pipetting. DMSO working solution (150 μ L) was added to each well, and the absorbance was evaluated using an ELISA reader at 490 nm. The percentage of cell death was calculated using the following formula: Cytotoxicity % = [Compounds (OD490)-background (OD490)] / [control (OD490)-background (OD490)] $\times$ 100%

1.5 Statistical analysis

The cytotoxicity assays were carried out in triplicate. The data of inhibition rates are expressed as means $\pm$ SD. One-way analysis of variance and Duncan's multiple range tests were carried out to determine significant differences ($p<0.05$) between the means.

References

Adams RP. 2007. Identification of essential oil components by gas chromatography/mass spectroscopy. Carol Stream, IL: Allured Publishing Corporation.

Table S1 Chemical composition and relative contents of the essential oil from six parts of *Datura metel* L.

RI	compounds	% Area					
		flowers	leaves	petioles	seeds	roots	stems
1013	(E)-2-Octenal					0.33	
1101	Nonanal					0.36	
1112	Trans-2-nonenal					1.01	
1204	n-Decanal					0.28	
1285	2,5-Dimethyl-dodecane		0.27			0.18	
1293	2-Methyldecan-1-al	1.96	4.63			0.82	
1435	Thujopsene					2.80	
1436	β -Selinene	7.64					
1455	6,10-Dimethylundeca-5,9-dien-2-one		0.72			0.46	
1457	β -Ionone		0.48			0.36	
1460	α -Curcumene	1.32					
1484	β -Ionol		1.85			0.41	
1494	Isocaryophyllene		10.89			3.45	
1494	β -Caryophyllene		7.71				
1507	Caryophyllene oxide	1.27	5.57			1.53	
1520	Elemol		0.49			0.39	
1523	Guaiene	1.28					
1536	Spathulenol		0.59				
1544	Isolongifolene		1.49				
1564	Nerolidol	1.47					
1563	3,7,11-Trimethyl-1-dodecanol		0.96				
1593	β -Eudesmol					0.55	
1601	Myristic aldehyde	1.99		2.86			
1635	Isolongifolol		4.56			1.19	
1668	Santalol		9.88			2.45	
1673	α -Cyperone	1.92				8.26	
1754	Phytone	23.61	18.84	39.45		1.77	2.92
1878	Methyl palmitate	6.91	0.60	2.15	0.87	0.44	2.65
1899	Isovegetable alcohol		0.90	3.25			
1902	Farnesylacetone		2.06	2.97			
1939	Cembrene				4.02		4.77
1978	Ethyl palmitate	15.84		5.64	6.93		7.40
1979	Palmitic acid	6.42			30.60	52.61	38.83
2085	Methyl elaidate				0.62		1.42
2093	Methyl linoleate	2.13		2.49	2.22		3.34
2077	Methyl stearate	1.45					
2097	Phytol	5.30	18.71	31.32		0.61	1.22
2185	Ethyl Oleate	1.58			6.23		5.51
2193	Ethyl linoleate	5.49		3.84	21.56		17.38
2197	Ethyl Stearate	2.98					1.08
2197	Ethyl linolenate	1.73		1.70			
2109	n-Heneicosane				0.88	0.91	
2123	Linoleic acid				17.07	1.95	

2175	Oleate	1.77	1.14
2407	Tetracosane	1.08	
2506	n-Pentacosane	1.26	0.48
2606	Hexacosane	1.22	
2705	Heptacosane	1.07	
2804	n-Octacosane	0.87	
2904	n-Nonacosane	0.73	
		92.29	91.20
		95.67	99.00
		85.72	87.66

Fig S1. Total ion chromatogram of flowers

Fig S2. Total ion chromatogram of leaves

Fig S3. Total ion chromatogram of petioles

Fig S4. Total ion chromatogram of seeds

Fig S5. Total ion chromatogram of roots

124 **Fig S6.** Total ion chromatogram of stems

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