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Composition, cytotoxicity and antimicrobial activity of essential oil from *Dictamnus dasycarpus*

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Abstract

The composition of the essential oil from *Dictamnus dasycarpus* was analysed by GC–MS; 47 constituents (88.9% of the total oil) were identified. The main compounds were *syn*-7-hydroxy-7-anisylnorbornene (29.4%), pregeijerene (15.5%) and geijerene (11.4%). The antimicrobial activity of the essential oil was evaluated against nine microorganisms using disc diffusion and broth microdilution methods. The oil showed the strongest bactericidal activity against *Staphylococcus aureus* ATCC 25923 and methicillin-resistant *S. aureus*. The *in vitro* cytotoxicity of the oil on six human cancer cell lines was also examined. The cytotoxicity of the oil on three human breast cancer cell lines was significantly stronger than on other cell lines.
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Keywords: *Dictamnus dasycarpus*; Essential oil; Cytotoxicity; Antimicrobial

1. Introduction

The genus *Dictamnus* has about five species and is widespread in Europe and Asia. Two species grow in China, *Dictamnus dasycarpus* Turcz has a wide distribution in China, while *Dictamnus angustifolius* G. Don mainly occurs in Xinjiang (Fan, Zhang, & Wang, 2003). The root bark of *D. dasycarpus* Turcz (Rutaceae) is a traditional Chinese medicine used for treatment of jaundice, cough and rheumatism. It has also been widely used to treat some skin diseases. The water extract of the root bark was reported to inhibit the growth of many kinds of human pathogenic fungi *in vitro* (Jiangsu New Medical College, 1977). The root bark is known to contain alkaloids, lactones and sesquiterpene glycosides, known as dictamnosides, such as eudesmane-type sesquiterpene glycosides and trinorguaiane-type sesquiterpene glycosides (Chang, Xuan, Xu, & Zhang, 2001; Du, Yang, & Tu, 2005; Zhao et al., 1998a; Zhao, Wang, Hostettmann, Qin, & Xu, 1999; Zhao, Wang, Qui, Xu, & Hostettmann, 2001). Six compounds isolated from the dichloromethane extract of the root bark were found to be active against the plant pathogenic fungus *Cladosporium cucumerinum* (Zhao, Wolfender, Hostettmann, Xu, & Qin, 1998b). In the water-soluble constituents of the root bark, one terpene glycoside, dasycarpuside A, was found to have weak cytotoxic activity against A-549 (human lung adenocarcinoma) cell line, and two phenolic glycosides inhibited the proliferation of T-cells *in vitro* (Chang, Xuan, Xu, & Zhang, 2002). To our knowledge, there are no published reports on the chemical composition and bioactivities of the essential oil from *D. dasycarpus*. Therefore we focused our study on the chemical composition, cytotoxicity and antimicrobial activity of this essential oil.

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2. Materials and methods

2.1. Plant material

The root bark of *D. dasycarpus* was collected in Hubei (China) in September 2003. The voucher specimen was deposited at the herbarium of the College of Pharmacy, Wuhan University, China.

2.2. Essential oil extraction and analysis

The root bark of *D. dasycarpus* was air dried and then distilled for 3 h, using a Clevenger-type apparatus. Anhydrous sodium sulphate was used to absorb the little water that the essential oils contained. The oil was then stored at −10 °C until tested.

GC–MS analyses were performed with a Thermo-Finnigan Trace GC coupled with a Trace MS Plus (EI, 70 eV) from the same company. The analyses were carried out using two different fused-silica capillary columns: DB-5 (30 m × 0.25 mm i.d.; film thickness 0.25 μm) and HP-Innowax (30 m × 0.25 mm i.d.; film thickness 0.25 μm). The oven temperature was programmed from 50 to 250 °C at 3 °C/min and held at this temperature for 10 min. Injector and interface temperatures were 220 and 250 °C, respectively. Carrier gas was helium at a flow rate of 1 ml/min. Diluted sample (1/10 in ether) of 1.0 μl was injected manually with a split ratio of 40:1. Components were identified by comparison of their mass spectra with those in the NIST98 GC–MS library and those in the literature (Adams, 2001), as well as by comparison of their retention indices with literature data (Adams, 2001; Agnihotri et al., 2004; Tabanca et al., 2006). Retention indices of the components were determined relative to the retention times of a series of *n*-alkanes.

2.3. Cell lines and culture

Human breast cancer cell lines (MCF-7, ZR-75-30 and MDA-MB-435S), human liver carcinoma cell lines (Bel-7402 and Hep G2) and human renal adenocarcinoma cell line (ACHN) were purchased from China Centre for Type Culture Collection. The human cell lines were grown and maintained in a humidified incubator at 37 °C and in 5% CO_2 atmosphere. Minimum Essential Medium (MEM) supplemented with 10% foetal bovine serum (FBS), 100 units/ml penicillin and 100 μg/ml streptomycin, was used for cell cultures of MCF-7, ZR-75-30, MDA-MB-435S, ACHN and Hep G2. RPMI 1640 medium supplemented with 10% FBS, 100 units/ml penicillin and 100 μg/ml streptomycin was used as the culture medium of Bel-7402.

2.4. MTT assay

This assay detects the reduction of MTT [3-(4,5-dimethylthiazolyl)-2,5-diphenyltetrazolium bromide] by mitochondrial dehydrogenase, to blue formazan product, which reflects the normal functioning of mitochondrial and cell viability (Lau et al., 2004). Briefly, after being harvested from culture flasks, 1×10^4 cells were incubated in 96-well plates, containing 100 μl of the growth medium per well for MCF-7 and HepG2, while for Bel-7402, ZR-75-30, MDA-MB-435S and ACHN, 5×10^3 cells were seeded per well. Cells were permitted to adhere for 24 h, and then treated with the essential oil dissolved in medium for 48 h; 20 μl of 5 mg/ml MTT in phosphate buffered saline (PBS) was added to each well and the plate was incubated at 37 °C for 4 h. The medium was removed, and 100 μl DMSO were then added to each well. After incubation at 37 °C for 10 min, absorbance in the control (treated with 0.1% DMSO) and in the essential oil-treated cells was measured at 570 nm, using a microplate ELISA reader.

2.5. Microbial strains

The essential oil was tested against nine microorganisms. Reference strains were: *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853 *and Candida albicans* ATCC 14053; clinically isolated strains were as follows: methicillin-resistant *S. aureus*, *Staphylococcus simulans, Enterococcus faecalis* (Group D), *Salmonella typhi* and *Proteus mirabilis*. These microorganism strains were conserved at the College of Life Sciences, Wuhan University, China.

2.6. Antimicrobial screening

The agar disc diffusion method was employed for the determination of antimicrobial activity of the essential oil (NCCLS, 1997). Briefly, a suspension of the test microorganism (2×10^8 cfu/ml) was spread on the solid media plates. Filter paper discs (6 mm in diameter) were individually impregnated with 15μl of the oil, then placed on the inoculated plates, for 2 h at 4 °C, following which they were incubated at 37 °C for 24 h for the bacteria and at 30 °C for 48 h for the yeasts. The diameters of the inhibition zones were measured in millimetres.

2.7. Determinations of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC)

A broth microdilution method was used to determine the MIC and MBC (NCCLS, 1999; Bassole et al., 2003). All tests were performed in Mueller Hinton broth supplemented with Tween 80 (final concentration of 0.5% (v/v)), with the exception of the yeasts (Sabouraud dextrose broth + Tween 80). A serial doubling dilution of the oil was prepared in a 96-well microtitre plate, over the range 0.78–100.00 mg/ml.

Overnight broth cultures of each strain were prepared and the final concentration in each well was adjusted to 2×10^4 cfu/ml. Plates were incubated at 37 °C for 24 h for bacteria and at 30 °C for 48 h for the yeasts. The MIC is defined as the lowest concentration of the essential

oil at which the microorganism does not demonstrate visible growth. Microorganism growth was indicated by turbidity.

To determine MBC, broth was taken from each well and inoculated in Mueller Hinton Agar for 24 h at 37 °C for bacteria or in Sabouraud dextrose agar for 48 h at 30 °C for the yeasts. The MBC is defined as the lowest concentration of the essential oil at which inoculated microorganism was completely killed. Each test was performed in duplicate and repeated twice. Levofloxacin was used as a positive control.

3. Results and discussion

3.1. Chemical composition of the essential oil

The essential oil was obtained by hydrodistillation from the root bark of *D. dasycarpus* with a yield of 0.2% (v/w) on a dry weight basis. Forty-seven compounds, representing 88.9% of the oil, were identified using two chromatographic stationary phases. Qualitative and quantitative analytical results are shown in Table 1.

Table 1
Main components (%) of the essential oil from the root bark of *Dictamnus dasycarpus*

Components	RI[a]	Percentage	Identification
Hexyl alcohol	868	0.2	MS, RI
α-Pinene	937	0.1	MS, RI
β-Pinene	976	0.3	MS, RI
α-Myrcene	989	0.2	MS, RI
α-Phellandrene	1006	0.1	MS, RI
p-Cymene	1023	0.1	MS, RI
β-Phellandrene	1029	3.3	MS, RI
Linalool	1100	0.1	MS, RI
3,4-Diethenyl-3-methyl-cyclohexene	1132	1.0	MS
Geijerene	1141	11.4	MS, RI
Terpene-4-ol	1179	0.1	MS, RI
L-Cryptone	1185	0.1	MS, RI
α-Terpineol	1192	0.1	MS, RI
6,7-Dimethyl-1,2,3,5,8,8a-hexahydronaphthalene	1211	0.4	MS
Thymol methyl ether	1232	1.2	MS, RI
1-Methoxy-4-methyl-2-(1-methylethyl)-benzene	1239	0.3	MS
Isogeijerene C	1253	0.5	MS, RI
α-Methyl-4-isopropylstyrene	1275	0.2	MS
1,2,3,6-Tetramethyl-bicyclo[2.2.2]octa-2,5-diene	1287	1.6	MS
Pregeijerene	1291	15.5	MS, RI
(*E*,*E*)-2,4-Decadienal	1322	0.3	MS, RI
3-*tert*-Butyl-4-methoxyphenol	1349	0.1	MS
8-Epi-Dictamnol	1377	2.1	MS, RI
(*E*)-β-Damascenone	1382	0.2	MS, RI
β-Elemene	1388	0.2	MS, RI
Thymohydroquinone dimethyl ether	1411	0.1	MS
(*E*)-Caryophyllene	1416	0.1	MS, RI
cis-8-Methyl-1α-acetyl-hydrindane	1420	1.1	MS
Dictamnol	1431	3.5	MS, RI
2-Acetyl-2-carene	1437	0.5	MS
9-Epi-(*E*)-caryophyllene	1463	0.2	MS, RI
trans-Cadinal-1(6),4-diene	1477	0.4	MS, RI
Elemol	1547	2.4	MS, RI
Dodecanoic acid	1565	0.5	MS, RI
4-(2-Norbornyl)-2,6-xylenol	1576	1.3	MS
Mansonone C	1578	1.5	MS
syn-7-Hydroxy-7-anisylnorbornene	1608	29.4	MS
10-Epi-γ- eudesmol	1627	0.3	MS, RI
α-Eudesmol	1650	0.8	MS, RI
Patchoulol	1661	0.9	MS, RI
(*Z*,*Z*)-Farnesol	1716	0.4	MS, RI
Tetradecanoic acid	1762	0.4	MS
Guaiazulene	1779	0.2	MS, RI
4,4,5,7,8-Pentamethyl-3,4-2H-isocoumarin-3-one	1799	0.3	MS
Pentadecanoic acid	1834	0.2	MS
8S,13-Cedranediol	1898	2.7	MS, RI
Palmitic acid	1967	2.0	MS

[a] Retention indices obtained on a DB-5 column.

Syn-7-hydroxy-7-anisylnorbornene (29.4%) was the main compound of the essential oil, followed by pregeijerene (15.5%) and geijerene (11.4%). Among other compounds, considerable amounts of dictamnol (3.5%), β-phellandrene (3.3%), 8-epi-dictamnol (2.1%), elemol (2.4%), 8S,13-cedranediol (2.7%) and palmitic acid (2.0%) were detected.

3.2. Cytotoxicity

The growth inhibitory effects of the essential oil on six human cancer cell lines were examined. The oil exhibited a marked growth inhibitory effects on all cell lines tested in a dose-dependent manner. The IC_{50} values are shown in Table 2. The growth inhibition effects of the essential oil on three human breast cancer cell lines were significantly stronger than those on other cell lines, with IC_{50} values of 28–45 μg/ml. Among three human breast cancer cell lines, ZR-75-30 cell line was the most sensitive to the essential oil, with an IC_{50} value of 28.8 ± 2.7 μg/ml. Among other cancer cell lines, Hep-G2 cell line was of similar sensitivity to the oil to ACHN cell line, with IC_{50} values of 55.7 ± 3.8 and 57.8 ± 3.6 μg/ml, respectively. Bel-7402 cell line exhibited the lowest sensitivity to the oil with an IC_{50} value of 81.1 ± 5.7 μg/ml.

Palmitic acid and β-elemene have been reported to have strong cytotoxicity (Harada et al., 2002; Wang et al., 2005). The cytotoxicity of the essential oil could be due to the synergistic effects of these active chemicals with the other main constituents of the essential oil.

Table 2
Cytotoxicity of the essential oil from the root bark of *Dictamnus dasycarpus* on six human cancer cell lines

Cell lines	IC_{50} (μg/ml)[a]
ACHN	57.8 ± 3.6
MCF-7	45.1 ± 2.3
ZR-75-30	28.8 ± 2.7
MDA-MB-435S	37.5 ± 3.1
Hep-G2	55.7 ± 3.8
Bel-7402	81.1 ± 5.7

[a] IC_{50} values were expressed as the mean ± SD, determined from the results of MTT assay in triplicate experiments.

3.3. Antimicrobial activity

The antimicrobial activity of the essential oil was evaluated against nine microorganisms, using disc diffusion and broth microdilution methods. The disc diameters of zone of inhibition (DD), minimum inhibitory concentrations (MIC) and minimum bactericidal concentrations (MBC) of the essential oil for the microorganisms tested are shown in Table 3.

The data obtained from the disc diffusion method indicated that *S. aureus* ATCC 25923 was the most sensitive microorganism tested, with the strongest inhibition zone (16 mm), followed by methicillin-resistant *S. aureus*, *S. simulans*, *C. albicans* ATCC 14053 and *Proteus mirabilis,* all with strong inhibition zones (13–14 mm). *P. aeruginosa* ATCC 27853 exhibited a weak inhibition zone (8 mm).

The results of the MIC determination indicated the oil inhibited all microorganisms tested. *S. aureus* ATCC 25923 and methicillin-resistant *S. aureus* had the lowest MIC (3.13 mg/ml). *P. aeruginosa* ATCC 27853, *E. faecalis* (Group D) and *Salmonella typhi* had the highest MIC (25.0 mg/ml).

As can be seen in Table 3, the MBC and MIC results varied for the three microorganisms tested. The lowest MBC was 3.13 mg/ml for *S. aureus* ATCC 25923 and methicillin-resistant *S. aureus*. *C. albicans* ATCC 14053, *S. simulans* and *Proteus mirabilis* had a low MBC of

Table 3
Antimicrobial activity of the essential oil from the root bark of *Dictamnus dasycarpus*

Microorganisms	Essential oil			Levofloxacin		
	DD[a]	MIC[b]	MBC[b]	DD[c]	MIC[d]	MBC[d]
Reference strains						
Staphylococcus aureus ATCC 25923	16	3.13	3.13	34	0.30	0.30
Escherichia coli ATCC 25922	10	6.25	12.5	34	0.30	0.61
Pseudomonas aeruginosa ATCC 27853	8	25.0	50.0	20	0.15	0.30
Candida albicans ATCC 14053	13	6.25	6.25	NT	NT	NT
Clinically isolated strains						
Methicillin-resistant *Staphylococcus aureus*	14	3.13	3.13	10	9.75	19.5
Staphylococcus simulans	13	6.25	6.25	30	0.61	0.61
Enterococcus faecalis (Group D)	11	25.0	25.0	22	9.75	19.5
Salmonella typhi	10	25.0	50.0	30	1.22	1.22
Proteus mirabilis	13	6.25	6.25	30	1.22	1.22

DD, diameter of zone of inhibition (mm) including disc diameter of 6 mm. NT, not tested.
[a] Tested at a concentration of 10.0 mg/disc.
[b] Values given as mg/ml.
[c] Tested at a concentration of 5 μg/disc.
[d] Values given as μg/ml.

6.25 mg/ml. *P. aeruginosa* ATCC 27853 and *Salmonella typhi* had the highest MBC values of 50.0 mg/ml.

Although some compounds such as palmitic acid, α-pinene and β-pinene have been reported to have significant antimicrobial activity (Benkendorff, Davis, Rogers, & Bremner, 2005; Dorman & Deans, 2000; Yff, Lindsey, Taylor, Erasmus, & Jäger, 2002), these compounds comprise a small percentage of the oil. Therefore they could not be the main compounds responsible for the observed antimicrobial activity of the oil. The main antimicrobial compounds may be abundant compounds in the oil.

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