Chemical composition and anti-MRSA activity of the essential oil from Chinese eaglewood

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Abstract: To analyze the constituents of essential oil from Chinese eaglewood [resinous wood of *Aquilaria sinensis* (Lour.) Gilg] and its anti-methicillin-resistant *Staphylococcus aureus* (MRSA) activity. The essential oil was extracted by water-steam distillation and analyzed by GC/MS method. The relative contents of the compounds were determined by normalization. The compounds were characterized by NIST05 and WILEY275L database matching and comparison of their MS spectra with those of literature data. Antibacterial activity of the oil was assayed by the filter paper disc agar diffusion method. The oil showed significant antibacterial activity against MRSA. Sixty-six chromatographic peaks were detected, among them thirty compounds comprising 59.80% of the total essential oil were characterized. Twenty-six compounds comprising 54.26% of the oil were identified as sesquiterpenes. β-Agarofuran (8.96%), kusunol (7.82%), (-)-jinkoh-eremol (5.04%), agarospirol (4.53%), baimuxifuranic acid (4.09%) were the major sesquiterpenes. Four nor-sesquiterpenes and some other sesquiterpenes, such as 10-epi-γ-eudesmol, α-agarofuran, epi-ligulyl oxide, *etc.* were detected in Chinese eaglewood oil for the first time. This is the first report about anti-MRSA activity of Chinese eaglewood oil from *A. sinensis*.

Keywords: Chinese eaglewood; *Aquilaria sinensis* Lour. Gilg; Essential oil; GC/MS; Antibacterial activity; MRSA

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1. Introduction

Chinese eaglewood (Chinese name: Chenxiang) is a resinous wood from the tree of *Aquilaria sinensis* (Lour.) Gilg, and has been widely used in traditional Chinese medicine for the treatment of stomachache, asthma, vomiting, *etc*. The essential oil of Chinese eaglewood is rich in sesquiterpenes with special skeletons. Some sesquiterpenes, such as agarospirol, baimuxinic acid, α-agarofuran, have been reported to possess sedative or hypnotic effect[1]. In 1998, Yang reviewed all the thirteen sesquiterpenes and three aromatic compounds identified up to 1993 from Chinese eaglewood. The constituents are similar to those of agarwood from *A. agallocha* Roxb., which is mainly distributed in India or Malaysia[2]. In the same year, Lawrence reported in a review covering the literatures from 1983 to 1995 that about twenty new sesquiterpenes had been identified in agarwood oil, mainly from *A. agallocha*, and a few from *A. malaccensis*, which is found in Indonesia, Malaysia, Thailand and the mountainous areas of Vietnam and Cambodia[3]. However, there are few studies on the Chinese eaglewood oil from *A. sinensis*. In our recent research on the oils of five different kinds of Chinese eaglewood, several sesquiterpenes, such as hinesol, nootkatone, valerenic acid, velleral, viridiflorol, *etc.*, were found to be present in the oils by GC/MS analysis for the first time[4]. The present paper describes the GC/MS analysis and anti-methicillin-resistant *Staphylococcus aureus* (MRSA) results of a new sample of Chinese eaglewood oil, in which some sesquiterpenes, especially four nor-sesquiterpenes were characterized for the first time.

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2. Experimental

2.1. Sample preparation

Naturally produced Chinese eaglewood from the tree of *A. sinensis* was collected in Tunchang county of Hainan Province of China in February 2007. The plant was identified by associate Professor Zheng-Fu Dai and a voucher specimen (No. 070286) is deposited at the Institute of Tropical Bioscience and Biotechnology. Hydrodistillation of 260 g of the milled Chinese eaglewood was carried out using a Clevenger-type apparatus for 4 h. After drying with anhydrous sodium sulfate, 0.55 mL pale yellow oil with pleasant odour was obtained and the yield was 0.21% (v/w).

2.2. GC/MS analysis

A Hewlett Packard gas chromatography instrument (GC 6890) coupled with a mass selective detector (5973) (Hewlett Packard Company, California, Palo Alto, USA) was used for the analysis. Separation of the analytes by gas chromatography was carried out by using a HP-Innowax polyethylene glycol capillary column (30 m × 0.32 mm, 0.25 μm) (Hewlett Packard Company). Separation of the compounds involved injecting 1.0 μL of essential oil into the front inlet of the gas chromatograph operating at 250 °C in the splitless mode. The flow of helium (carrier gas) was 2.0 mL/min with 50:1 of split ratio. The oven program commenced at 50 °C (2 min) and increased at a rate of 4 °C/min to 230 °C where it was held for 10 min. The interface temperature was 280 °C. Ionization of the analytes by electron impact (EI) was obtained using an emission current of 70 eV. The ion source temperature was set at 230 °C and scan scope was from 10 to 550 amu.

Figure 1 shows the total ion chromatogram of essential oil of Chinese eaglewood. The relative contents of the compounds were determined by normalization. The compounds were characterized by NIST05 and WILEY275L database matching and comparison of their MS spectra with those of literature data.

2.3. Antibacterial assay

Antibacterial activity of the oil was assayed by the filter paper disc agar diffusion method[5] using strain MRSA 9551 conserved in the Institute of Tropical Bioscience and Biotechnology, Chinese Academy of Tropical Agriculture Sciences, Haikou, China. The media nutrient agar (NA) was used to culture the bacteria. The sterile agar media was poured into Petri-plates to a uniform depth of 5 mm and was allowed to solidify. The microbial suspensions were streaked over the surface of media using a sterile cotton swab. Thirty and fifty μL oil dissolved in acetone at a concentration of 50 mg/mL were impregnated on sterile Whatman filter paper discs of 6 mm size, respectively. These discs were then aseptically applied to the surface of the agar plates at well-spaced intervals. Control discs impregnated with 50 μL of acetone and 50 μL of kanamycin sulfate (0.08 mg/mL) were also used alongside the test discs in the experiment. The plates were incubated at 36 °C for 24 h. Then the diameters of the observed zones of inhibition surrounding each disc including the 6 mm disc diameter were measured. The activities are expressed in mm diameter of the inhibition zone. Experiments were carried out in triplicate and the results are expressed as the mean value.

3. Results

Sixty-six chromatographic peaks were detected and thirty compounds comprising 59.80% of the total essential oil were characterized (Table 1). Twenty-six compounds comprising 54.26% of the oil were sesquiterpenes. β-Agarofuran (8.96%), kusunol (7.82%), (-)-jinkoh-eremol (5.04%), agarospirol (4.53%), and baimuxifuranic acid (4.09%) were the major sesquiterpenes. Most of the sesquiterpenes reported before have been found in our present work. In addition, four nor-sesquiterpenes and some other sesquiterpenes, such as 10-epi-γ-eudesmol, α-agarofuran, epi-ligulyl oxide *etc.* were detected in Chinese eaglewood oil for the first time. The four nor-sesquiterpenes, compounds **1**, **3** – **5**, had been characterized in agarwood oil from *A. agallocha* as new compounds[6]. They are different from nor-ketoagarofuran, the only nor-sesquiterpene reported from Chinese eaglewood oil before. 10-Epi-γ-eudesmol and α-agarofuran had also been identified from *A. malaccensis*[7], whereas epi-ligulyl oxide, elemol, aristolenepoxide have never been reported from Chinese eaglewood or agarwood oil before.

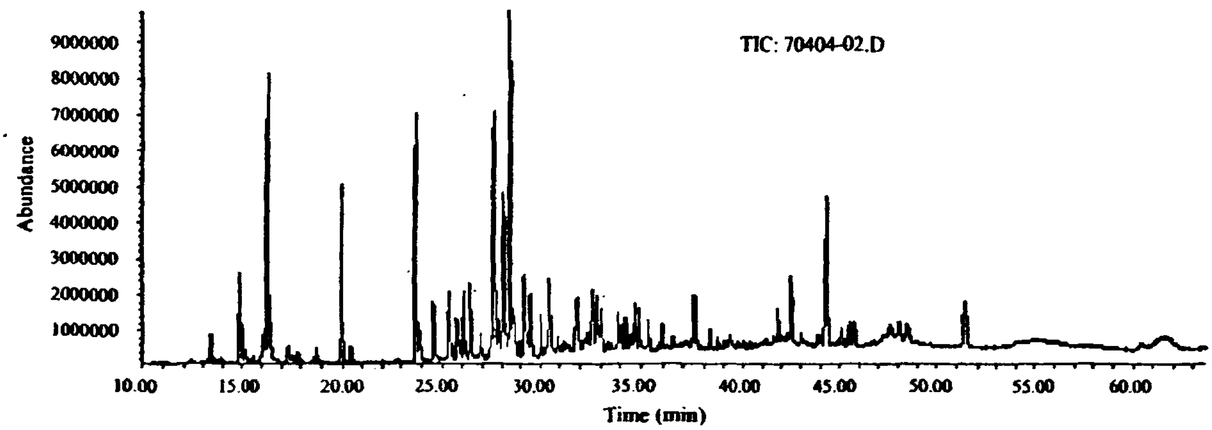

Figure 1. GC/MS chromatogram of essential oil of Chinese eaglewood.

Table 1. Chemical composition of the Chinese eaglewood oil

No.	Retention time (min)	Compound	Molecular formula	Molecular weight	Relative content (%)	Reference
1	13.34	(1): (1*R*,6*S*,9*R*)-6,10,10-Trimethyl-11-oxatricyclo[7.2.1.0^{1,6}]dodecane	$C_{14}H_{24}O$	208	0.85	[6]
2	16.06	Dihydro-β-agarofuran	$C_{15}H_{26}O$	222	1.07	[3], [8]
3	16.28	β-Agarofuran	$C_{15}H_{24}O$	220	8.96	[9]
4	17.81	Epi-ligulyl oxide	$C_{15}H_{26}O$	222	0.48	[10]
5	19.94	Benzyl acetone	$C_{10}H_{12}O$	148	2.97	[9]
6	20.39	α-Agarofuran	$C_{15}H_{24}O$	220	0.60	[7]
7	23.65	(2):Unidentified			5.97	
8	23.87	(3): (*S*)-4a-Methyl-2-(1-methylethyl)-3,4,4a,5,6,7-hexahydronaphthalene	$C_{14}H_{22}$	190	0.78	[6]
9	24.55	(4): (1*R*,2*R*,6*S*,9*R*)-6,10,10-Trimethyl-11-oxatricyclo[7.2.1.0^{1,6}]dodecan-2-ol	$C_{14}H_{24}O_2$	224	1.47	[6]
10	25.32	(5): (*S*)-4a-Methyl-2-(1-methylethylidene)-1,2,3,4,4a,5,6,7-octahydronaphthalene	$C_{14}H_{22}$	190	1.74	[6]
11	25.49	Elemol	$C_{15}H_{26}O$	222	0.18	[11]
12	25.70	10-Epi-γ-eudesmol	$C_{15}H_{26}O$	222	0.94	[7]
13	26.40	α-Santalol	$C_{15}H_{24}O$	220	1.79	[4]
14	27.60	Agarospirol	$C_{15}H_{26}O$	222	4.53	[9], [12]
15	27.83	Hinesol	$C_{15}H_{26}O$	222	0.99	[4]
16	28.08	(-)-Jinkoh-eremol	$C_{15}H_{26}O$	222	5.04	[13], [14]
17	28.39	(6): Kusunol (Valerianol) or Guaiol	$C_{15}H_{26}O$	222	7.82	[14], [15]
18	28.55	4-Hydroxydihydroagarofuran	$C_{15}H_{26}O_2$	238	0.95	[16]
19	28.63	β-Eudesmol	$C_{15}H_{26}O$	222	0.91	[4]
20	29.14	Viridiflorol	$C_{15}H_{26}O$	222	2.32	[4]
21	30.01	Anisylacetone	$C_{11}H_{14}O_2$	178	0.77	[9]
22	30.44	Sinenofuranol	$C_{15}H_{26}O_2$	238	2.26	[17]
23	31.86	Neopetasane	$C_{15}H_{22}O$	218	1.60	[13]
24	32.83	Dihydrokaranone	$C_{15}H_{22}O$	218	1.46	[13], [14]
25	33.87	Baimuxinol	$C_{15}H_{26}O_2$	238	0.74	[12], [18]
26	34.68	Aristolenepoxide	$C_{15}H_{24}O$	220	0.91	[19]
27	36.03	*Iso*baimuxinol	$C_{15}H_{26}O_2$	238	0.61	[9]
28	37.54	Baimuxinal (Oxo-agarofuran)	$C_{15}H_{24}O_2$	236	1.17	[7], [20]
29	38.66	Myristic acid	$C_{14}H_{28}O_2$	228	0.20	[4]
30	42.51	Palmitic acid	$C_{16}H_{32}O_2$	256	1.60	[4]
31	44.34	Baimuxifuranic acid	$C_{15}H_{24}O_3$	252	4.09	[21]

The oil showed significant antibacterial activity against MRSA. The inhibition zone diameters were 9 mm and 12 mm at the ration of 1.5 mg and 2.5 mg, respectively. The inhibition zone diameter of 4 μg kanamycin sulfate was 15 mm.

4. Discussion

MRSA is a major pathogen of iatrogenic infection, which has been a serious problem with more and more detectable rate and aggravation tolerance[22,23]. This is the first report on the anti-MRSA activity of Chinese eaglewood oil from *A. sinensis*. It is known that sesquiterpenes usually possess anti-microbial activity. The essential oil of Chinese eaglewood was found to be rich in sesquiterpenes. That maybe the reason why it showed significant antibacterial activity against MRSA. The main structural types of the sesquiterpenes in Chinese eaglewood oil were listed in Table 2. It should be mentioned that Ishihara *et al.* reported the chemical composition of four different sources of agarwood, and two of them were tentatively identified as *A. sinensis*[24]. Five sesquiterpenes of eudesmane skeleton, selina-3,11-dien-9-one, selina-3,11-dien-9-ol, selina-3,11-dien-14-al, selina-3,11-dien-14-ol, and selina-4,11-dien-14-al, together with two sesquiterpenes of guaiane skeleton, guaia-1(10),11-dien-15-al, and guaia-1(10),11-dien-15,2-olide were identified from the two samples. However, these samples were collected in Vietnam and imported via Hong Kong, and their species identification need further confirmation. Thus, the chemical compositions reported in this paper are not listed in Table 2.

Table 2. Main structural types of the sesquiterpenes in Chinese eaglewood oil

Structure type	Compounds
Agarofuran skeleton	β-Agarofuran, baimuxinol, *iso*baimuxinol, dehydrobaimuxinol, baimuxifuranic acid, dihydro-β-agarofuran, α-agarofuran, 4-hydroxydihydroagarofuran
Agarospirane skeleton	Agarospirol, baimuxinal, baimuxinic acid, hinesol
Elemophilane skeleton	(-)-Jinkoh-eremol, kusunol, neopetasane, dihydrokaranone, dehydrojinkoh-eremol
Guaiane skeleton	Sinenofuranal, sinenofuranol, epi-ligulyl oxide
Eudesmane skeleton	β-Eudesmol, 10-epi-γ-eudesmol
Nor-sesquiterpene	Nor-ketoagarofuran, (1*R*,6*S*,9*R*)-6,10,10-trimethyl-11-oxatricyclo[7.2.1.$0^{1,6}$]dodecane, (*S*)-4a-methyl-2-(1-ethylethyl)-3,4,4a,5,6,7-hexahydronaphthalene, (1*R*,2*R*,6*S*,9*R*)-6,10,10-trimethyl-11-oxatricyclo[7.2.1.$0^{1,6}$]dodecan-2-ol, (*S*)-4a-methyl-2-(1-methylethylidene)-1,2,3,4,4a,5,6,7-octahydronaphthalene

Although more and more sesquiterpenes have been identified in Chinese eaglewood, a lot of compounds have yet to be identified. The relative content of compound **2** is high (5.97%), it should be a compound reported before. After consulting the references, we consider it could be nor-ketoagarofuran. Unfortunately, the MS data could not been found in our database, so the compound was not characterized. Compound **6** was identified by database matching as guaiol, which has not been reported in Chinese eaglewood or agarwood before. Since the relative content is high (7.82%), it should be a compound reported before. After consulting the references, it was tentatively identified as kusunol (valerianol). And it's hard to tell whether the newly detected sesquiterpenes in agarwood oil from *A. agallocha* or *A. malaccensis*[3] exist in Chinese eaglewood oil from *A. sinensis* or not. But to the best of our knowledge, sinenofuranal and sinenofuranol, two guaiane sesquiterpenes with tetrahydrofuran ring, which found in Chinese eaglewood as new compounds in 1988, have not been found in agarwood oil from *A. agallocha* or *A. malaccensis*. Thus, these two compounds could be the characteristic sesquiterpenes of *A. sinensis*. It should be mentioned that epi-ligulyl oxide, which has been detected in Chinese eaglewood oil in our study, is also a guaiane sesquiterpene with tetrahydrofuran ring.

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中国沉香挥发油的化学成分与抗耐甲氧西林金葡菌活性

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摘要: 分析中国沉香的挥发油成分, 并测试其抗耐甲氧西林金葡菌活性。采用水蒸气蒸馏法提取中国沉香挥发油进行GC/MS分析, 峰面积归一化法计算各成分相对含量, 采用NIST05和 WILEY275L 数据库匹配, 以及将质谱图与文献数据进行对照的方法进行鉴定。采用滤纸片琼脂扩散法测试挥发油抗菌活性。挥发油对耐甲氧西林金葡菌显示强活性。共检测到66个色谱峰, 其中30个化合物得到鉴定, 占挥发油总量的59.80%。26个化合物被鉴定为倍半萜类化合物, 占挥发油总量的54.26%。β-沉香呋喃 (8.96%), 枯苏醇 (7.82%), (-)-jinkoh-eremol (5.04%), 沉香螺旋醇 (4.53%), 白木香呋喃酸 (4.09%) 为主要的倍半萜。4个降倍半萜和其他一些倍半萜, 如10-表-γ-桉叶油醇, α-沉香呋喃, epi-ligulyl oxide等为首次从中国沉香中检出。本文首次报道了中国沉香挥发油对耐甲氧西林金葡菌具有抗菌活性。

关键词: 中国沉香; 白木香; 挥发油; 气相色谱-质谱; 抗菌活性; 耐甲氧西林金葡菌