

# Chemical composition of the volatile oil from *Zanthoxylum avicennae* and antimicrobial activities and cytotoxicity

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## ABSTRACT

**Background:** Through literature retrieval, there has been no report on the research of the chemical components in *Zanthoxylum avicennae* (Lam.) DC. This paper extracted and determined the chemical components of the volatile oil in *Z. avicennae*, and at the same time, measured and evaluated the bioactivity of the volatile oil in *Z. avicennae*. **Materials and Methods:** We extract the volatile oil in *Z. avicennae* by steam distillation method, determined the chemical composition of the volatile oil by GC-MS coupling technique, and adopt the peak area normalization method to measured the relative percentage of each chemical composition in the volatile oil. Meanwhile, we use the Lethal-to-prawn larva bioactivity experiment to screen the cytotoxicity activities of the volatile oil in *Z. avicennae*, and using the slanting test-tube experiment to determine and evaluate its antibacterial activities *in vitro* for the eight kinds of plant pathogenic fungi in the volatile oil of the *Z. avicennae*. **Results:** The results show that 68 kinds of compounds are determined from the volatile oil of *Z. avicennae*. The determined part takes up 97.89% of the total peak area. The main ingredients in the volatile oil of *Z. avicennae* are sesquiterpenoids and monoterpene. The test results show that the volatile oil in *Z. avicennae* has strong antibacterial activities and cytotoxicity, with the strongest antibacterial activity against the *Rhizoctonia solani* AG1-1A. **Conclusion:** This research results will provide reference data for understanding the chemical composition of the volatile oil in the aromatic plant of *Z. avicennae* and its bioactivity, and for its further development and application.

**Key words:** Antimicrobial, cytotoxicity, GC-MS, volatile oil, *Zanthoxylum avicennae*

## INTRODUCTION

The *Zanthoxylum avicennae* (Lam.) DC belongs to the plant of *Zanthoxylum* genus of the *Rutaceae* family. Its other names are pepper, local pepper, small-leaf pepper, etc.<sup>[1]</sup> Its roots, leaves, and fruit can all be used as medicine. Its roots can be picked all the year round, while its leaves can be picked at any time and its fruit can only be picked in winter. Dry them up after picking. Its fresh leaves, root, and fruit skins all have pepper smell. It has viscosity when chewing them, with bitter taste and numbing tongue. The fruit skin and the root skin have the strongest smell, which are used as herbal medicine among the common people. According to the literature reports<sup>[2,3]</sup> the fruit skin and the leaves of

*Z. avicennae* contain much volatile oil composition. The root bark, stalk bark, and the leaf contain the components of alkaloids and coumarins, thus having the medical effects of dispelling wind, removing dampness, reducing phlegm, promoting blood circulation, and relieving pain, etc., clinically it can be used in curing many different kinds of pain-related diseases, such as traumatic injuries, lumbar muscle degeneration, and mastitis, etc., It can also be used in curing jaundice-type hepatitis, nephritis, dropsy, rheumatic arthritis. Furthermore, it can be used as roundworm repellents. Even though *Z. avicennae* is used widely among the common people and has some practical value in its developing and application, there has been no report on its foundation research in any literature.

Through literature retrieval, there has been no report on the research of the volatile oil chemical components and its bioactivity in *Z. avicennae*. There is much volatile oil in this aromatic plant, and the uncultivated *Z. avicennae* is

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abundant in the wild. This paper extracted and determined the chemical components of the volatile oil in *Z. avicennae*, used the coupling technique of Gas Chromatograph-Mass Spectrometer to determine the chemical composition of the volatile oil, adopted the peak area normalization method to measure the relative percentage of the chemical composition in volatile oil screened preliminary the cytotoxicity of the volatile oil in *Z. avicennae* by doing the prawn-lethal bioactivity-experiment using slanting test-tube method, through measuring the MIC value to evaluate the *in vitro* antibacterial activities of the eight kinds of plant pathogenic fungi in *Z. avicennae*, and thus providing the reference data for further developing this plant.

## MATERIALS AND METHODS

### Instruments and materials

Trace MS Gas Chromatograph-Mass Spectrometer (American Phinnigan Corp.); Chromatographic column is DB-WAX (30 m × 0.25 mm, 0.25 mm) elastic Quartz Capillary Column; UV-2102 PCS Ultraviolet and Visible Spectrophotometer (Shanghai Unico Instrument Corp.); 96-hole plate (Zhejiang Gongdong Medical Plastic Factory); Volatile oil Extractor; N-1001-type super-clean workbench; PRX-350-typr biochemical incubator.

The leaves of the *Z. avicennae* were picked in Sanya city, Hainan Island, in August 2011 and were appraised by Professor Huang Shi-man, who majored in medicinal plant taxonomy, Hainan University.

The selected experiment strains: *Fusarium graminearum*, *Fusarium graminearum* Schwabe, *Fusarium oxysporum*, *Exserobolium turcicum*, *Rhizoctonia solani*, *Botrytis cinerea*, *Rhizoctonia solani* AG1-IA, *Sclerotinia sclerotiorum* eight kinds of fungi were provided by the Forest Protection Lab of Zhejiang Agriculture and Forestry University; the prawn-egg in salina produced by Tianjin Fengnian Aquiculture Corp, and the DMSO is reagent of analytical pure.

### Extracting of the volatile oil

Take 200 g of the dried leaf-powder of the *Z. avicennae* after sifting through the 20-hole sieve; then put it in the round-bottomed flask and add suitable amount of distilled water into it, and then extract it by using the volatile oil extractor according to the XD extracting standards<sup>[4]</sup> stipulated in the appendix, Part One, 2010 version of *The Pharmacopoeia of The People's Republic of China*. Collect the distillate and extract it by using diethyl ether. We can get the volatile oil after drying the extract liquid by the anhydrous sodium sulphate. The volatile oil is the oily liquid with faint yellow color and rich fragrance. The oil-obtaining rate of the *Z. avicennae* is 2.10%.

### Analytical conditions of the GC-MS

The GC conditions: The Chromatographic column is DB-WAX (30 m × 0.25 mm, 0.25 mm) elastic quartz capillary column; temperature programming: Keep the initial temperature at 45°C for 3 min, then raise the temperature to 100°C at the speed of 10°C·min<sup>-1</sup>, and again raise the temperature to 170°C at the speed of 5°C·min<sup>-1</sup>, then again to 240°C for 7 min at the speed of 10°C·min<sup>-1</sup>. The temperature at the sample-feeding gate is 250°C, the temperature in the carburetor room is 250°C, the carrier gas is helium; its flow velocity is 0.8 mL·min<sup>-1</sup>, and the split sampling speed is 20 mL·min<sup>-1</sup>.

The mass spectrum conditions: Let the electrons bombard the EI ionization source; the ionizing energy is 70 eV; the temperature of the ionization source is 200°C; the voltage of the detector is 350 V; the scanning quality range is between 40 and 300 m/z; the retrieved atlas databank is the standard mass spectrum depot of Willey and NIST; the scanning speed is 0.5 s; the temperature of the quadruple rod is 130°C. Each mass spectrogram corresponding to each chromatographic peak is qualitatively determined by computer chart-base; the relative content of each component is calculated by the Peak Area Normalization method according to its total ion current chart.

### Calculation of the RI value

This experiment adopts *n*-alkane-mixed reference sample to analyze according to the gas chromatographic and mass spectrum conditions, and use the Peak Area Normalization method to determine the relative percentage of each content of the chemical component in the volatile oil. Then we calculate the RI value of each component by the linear warming equation according to the retention time of each *n*-alkane.  $RI = 100n + 100 \frac{(t_x - t_n)}{t_{n+1} - t_n}$ . here we analyze and group  $t_x$ ,  $t_n$  and  $t_{n+1}$ , respectively, with the carbon number as the retention time (min)<sup>[5]</sup> of the outflow peak of *n* and *n* + 1 *n*-alkane ( $t_n < t_x < t_{n+1}$ ).

### Bioactivity determination of the lethal-to-prawn

The preparation of the sample solution: Weigh precisely 0.02 g of the volatile oil sample; and use DMSO to dissolve it to constant volume 10 mL, we will get the sample solution with the concentration of 2 mg mL<sup>-1</sup>. Then use DMSO to prepare it to the following sample solutions with five different concentration gradient: 10, 50, 100, 500, 1000 µg mL<sup>-1</sup>.

The incubation of prawns: Weigh respectively sodium bicarbonate 2 g, iodine-free sea salt 2 g, prawns eggs 2-3 g, and add them to distilled water 1000 mL. Hatch these eggs for 24 h in dark place with good ventilation. Then close the ventilation, precipitate it, we will get the hatched prawn larva.

Take the sample solutions with different concentration and 25-30 prawn larva and experiment it in the 96-hole porous plate. Only add DMSO into the control group. Then cultivate it for 24 h in the dark at the room temperature and calculate the number of the dead prawn larva under the microscope in each trough. And finally calculate the mortality rate of the prawn larva according to the equation below:  $M = (A-B-N)/(G-N) \times 100\%$ . Here M is the mortality rate after 24 h, A is the total deaths after 24 h, B is the total deaths in the control group after 24 h, N is the total deaths before adding the samples, G is the total number of the prawns chosen for the testing. According to the average death rate under different concentrations we calculate its half-number-death concentration  $LC_{50}$  by the SPSS method.

### Antibacterial activities determination

We determined the *in vitro* antibacterial activities of the volatile oil in the *Z. avicennae* for the eight kinds of plant pathogenic fungi by the slanting test-tube experiment. Choose potatoes, glucose, and prepare them to PDA with water. Disinfect the prepared PDA by the wet method. And then melt the curdled PDA by heating on the super-clean workbench. Put the 700  $\mu\text{L}$  PDA into the 2 mL centrifugal tube when it is still warm. Then place it slantingly, and cool and curdle it till it becomes slanting culture medium. Use the DMSO as the solvent to prepare the volatile oil sample with 12 different concentration gradient: 200, 100, 50, 25, 12.5, 6.25, 3.125, 1.563, 0.781, 0.391, 0.195, 0.098  $\mu\text{L mL}^{-1}$ , with the highest concentration 200  $\mu\text{L mL}^{-1}$ , the lowest 0.098  $\mu\text{L mL}^{-1}$ . First use the highest and lowest concentrations to make preliminary screenings and then make screenings, respectively.

Spray the prepared volatile oil sample solution onto the PDA surface of the centrifugal test-tube with each sample-feeding amount 40  $\mu\text{L}$ . Then shake it instantly and make it touch the PDA surface evenly. Put only DMSO to the PDA surface in the negative contrast group, but add nothing to the PDA surface in the blank group.

Use the puncher to perforate after train the eight plant pathogenic fungi in the culture dish, and then put the punched fungi in the centrifugal test-tube containing the prepared sample solution. Train them in the incubator for 48 h and observe their growing processes. Regulate the concentration of the volatile oil according to their fungi growth. From the watching, we found that the fungi grow very well in each concentration gradient of the blank group and the negative control group. Use the lowest solution concentration of volatile oil of the fungi free-survival as the lowest antibacterial concentration MIC.<sup>[6-11]</sup> The experiment in each group is repeated three times, and we take the average value in the experiment results.

## RESULTS AND DISCUSSION

### Analytical results of the GC-MS

Take the obtained volatile oil by the vapor distillation method, with the sample-feeding amount 5.0  $\mu\text{L}$ . According to the above GC-MS condition, we analyzed and determined it by the Gas chromatography-Mass spectrometer, and got the total ion flow chart of the volatile oil in the *Z. avicennae*, which can be seen in Figure 1. After mass-spectrum scanning for each chromatographic peak, we get the mass spectrogram. Through the mass spectrum retrieval by NIST2008 standard and the relevant mass spectrum data, we quantified the compounds by Hewlett-Packard software processing system and calculated each peak area according to the peak area normalization method. Moreover, we calculated the relative percentage of the volatile oil in each component and chose the probable matter with high matching degree to calculate the RI value, and combined the manual analysis to determine the chemical components of the volatile oil in *Z. avicennae*, which can also be seen in Figure 1.

From Table 1, we can see that 68 kinds of compounds are determined in the volatile oil of *Z. avicennae*, which account for 97.89% of the total peak area in the appraised components. We also know that the highest contents in the volatile oil of *Z. avicennae* are sesquiterpenoids and monoterpene in addition to the small-molecule aromatic components. The high contents of the components in the volatile oil of *Z. avicennae* are mainly Compound 1: 2-methoxy-3-(2-propenyl)-phenol (42.94%); Compound 2: Caryophyllene (23.33%); Compound 3: 1,1,4,8-tetramethyl-cis, cis-4,7,10- cycloundecatriene (8.98%); Compound 4: 1-ethenyl-1-methyl -2,4-bis (1-methylethenyl)-[1S-(1 $\alpha$ , 2 $\alpha$ , 4 $\alpha$ )]-cyclohexane (6.20%); Compound 5: 3,7-dimethyl-1,6-octadien -3-ol (1.73%); Compound 6: Caryophyllene oxide (1.58%); Compound 7: 1,2-dimethoxy-4- (2-propenyl)-benzene (1.40%);

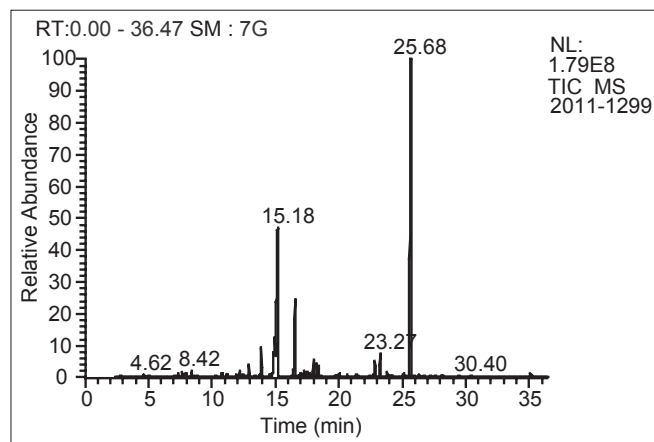


Figure 1: GC-MS total ion current chromatogram of the essential oil from the *Z. avicennae*

**Table 1: Analytical results of chemical constituents of the essential oil from the *Z. avicennae* by GC/MS**

Name of components	RT (min)	M.F	RI	Relative concent(%)
Ethyl acetate	2.74	C <sub>4</sub> H <sub>8</sub> O <sub>2</sub>	884.211	0.09
Benzene	3.39	C <sub>6</sub> H <sub>6</sub>	937.589	0.06
1R-a-Pinene	4.62	C <sub>10</sub> H <sub>16</sub>	1020.231	0.23
2-methyl-3-buten-2-ol	5.02	C <sub>5</sub> H <sub>10</sub> O	1043.353	0.06
6,6-dimethyl-2-methylene-(1S)-bicyclo[3.1.1]heptane	7.07	C <sub>10</sub> H <sub>16</sub>	1162.573	0.09
1-methyl-4-(1-methylethyl)-1,3-cyclohexadiene	7.33	C <sub>10</sub> H <sub>16</sub>	1177.778	0.17
D-Limonene	7.65	C <sub>10</sub> H <sub>16</sub>	1196.491	0.23
a-Phellandrene	7.80	C <sub>10</sub> H <sub>16</sub>	1205.488	0.21
2-Hexenal	7.96	C <sub>6</sub> H <sub>10</sub> O	1215.244	0.20
1-methyl-4-(1-methylethyl)-1,4-cyclohexadiene	8.42	C <sub>10</sub> H <sub>16</sub>	1243.293	0.28
1-methyl-2-(1-methylethyl)-benzene	8.80	C <sub>10</sub> H <sub>14</sub>	1266.463	0.06
1-methyl-4-(1-methylethylidene)-cyclohexene	9.03	C <sub>10</sub> H <sub>16</sub>	1280.488	0.06
2-methyl-2-buten-1-ol	9.67	C <sub>5</sub> H <sub>10</sub> O	1317.877	0.06
6-methyl-5-hepten-2-one	9.93	C <sub>8</sub> H <sub>14</sub> O	1332.402	0.05
1-Hexanol	10.23	C <sub>6</sub> H <sub>14</sub> O	1349.162	0.08
3-Hexen-1-ol	10.79	C <sub>6</sub> H <sub>12</sub> O	1380.447	0.25
(E)-2-Hexen-1-ol	11.18	C <sub>6</sub> H <sub>12</sub> O	1402.062	0.18
5-ethenyltetrahydro-a, a, 5-trimethyl-trans-2-furanmethanol	11.89	C <sub>10</sub> H <sub>18</sub> O <sub>2</sub>	1438.660	0.16
Acetic acid	11.99	C <sub>2</sub> H <sub>4</sub> O <sub>2</sub>	1443.814	0.11
Furfural	12.21	C <sub>5</sub> H <sub>4</sub> O <sub>2</sub>	1455.155	0.47
5-ethenyltetrahydro-a, a, 5-trimethyl-cis-2-furanmethanol	12.43	C <sub>10</sub> H <sub>18</sub> O <sub>2</sub>	1466.495	0.15
Copaene	12.88	C <sub>15</sub> H <sub>24</sub>	1489.691	1.04
Benzaldehyde	13.40	C <sub>7</sub> H <sub>6</sub> O	1515.764	0.14
Octahydro-7-methyl-3-methylene-4-(1-methylethyl)-[3αS-(3αa, 3αb, 4a, 7a, 7αS*)]-1H-cyclopenta[1,3]cyclopropa[1,2]benzene	13.77	C <sub>15</sub> H <sub>24</sub>	1533.990	0.22
3,7-dimethyl-1,6-octadien-3-ol	13.89	C <sub>10</sub> H <sub>18</sub> O	1539.901	1.73
Octyl ester formic acid	14.14	C <sub>9</sub> H <sub>18</sub> O <sub>2</sub>	1552.217	0.05
5-methyl-2-furancarboxaldehyde	14.39	C <sub>6</sub> H <sub>6</sub> O <sub>2</sub>	1564.532	0.04
1-ethenyl-1-methyl-2,4-bis (1-methylethenyl)-[1S-(1a, 2a, 4a)]-cyclohexane	14.96	C <sub>15</sub> H <sub>24</sub>	1592.611	6.20
Caryophyllene	15.18	C <sub>15</sub> H <sub>24</sub>	1603.349	23.33
2-methylene-(3a, 5a)-cholestan-3-ol	16.20	C <sub>28</sub> H <sub>48</sub> O	1652.153	0.05
1,2,4a, 5,8,8a-hexahydro-4,7-dimethyl-1-(1-methylethyl)-[1S-(1a, 4αa, 8αa)]-naphthalene	16.31	C <sub>15</sub> H <sub>24</sub>	1657.416	0.09
1,1,4,8-tetramethyl- cis, cis, cis-4,7,10-cycloundecatriene	16.57	C <sub>15</sub> H <sub>24</sub>	1669.856	8.98
1,2,3,4,4α,5,6,8a-octahydro-7-methyl-4-methylene-1-(1-methylethyl)- (1a, 4αa, 8αa)-naphthalene	16.84	C <sub>15</sub> H <sub>24</sub>	1682.775	0.15
a, a4-trimethyl-3-cyclohexene-1-methanol	17.02	C <sub>10</sub> H <sub>18</sub> O	1691.388	0.20
1,2,3,4,5,6,7,8-octahydro-1,4-dimethyl-7-(1-methylethenyl)-[1S-(1a, 4a, 7a)]-azulene	17.13	C <sub>15</sub> H <sub>24</sub>	1696.651	0.09
Germacrene D	17.27	C <sub>15</sub> H <sub>24</sub>	1703.365	0.43
1,2,3,5,6,7,8,8a-octahydro-1,8a-dimethyl-7-(1-methylethenyl)-[1S-(1a, 7a, 8αa)]-naphthalene	17.39	C <sub>15</sub> H <sub>24</sub>	1709.135	0.19
Eudesma-4 (14),11-diene	17.48	C <sub>15</sub> H <sub>24</sub>	1713.462	0.33
1,2,3,4,4a, 5,6,8a-octahydro-4a, 8-dimethyl-2-(1-methylethenyl)-[2R-(2a, 4αa, 8αa)]-naphthalene	17.58	C <sub>15</sub> H <sub>24</sub>	1718.269	0.36
1-ethenyl-1-methyl-2-(1-methylethenyl)-4-(1-methylethylidene)-cyclohexane	17.77	C <sub>15</sub> H <sub>24</sub>	1727.404	0.25
a-Farnesene	18.03	C <sub>15</sub> H <sub>24</sub>	1739.904	1.02
1,2,3,5,6,8a-hexahydro-4,7-dimethyl-1-(1-methylethyl)-(1S-cis)-naphthalene	18.28	C <sub>15</sub> H <sub>24</sub>	1751.923	0.88
Methyl salicylate	18.55	C <sub>8</sub> H <sub>8</sub> O <sub>3</sub>	1764.904	0.13
1,2,3,4,4a, 7-hexahydro-1,6-dimethyl-4-(1-methylethyl)-	18.74	C <sub>15</sub> H <sub>24</sub>	1774.038	0.07
2,6-Octadien-1-ol, 3,7-dimethyl-(Z)- naphthalene	19.14	C <sub>10</sub> H <sub>18</sub> O	1793.269	0.08
1,2,3,4-tetrahydro-1,6-dimethyl-4-(1-methylethyl)-(1S-cis)-naphthalene	19.74	C <sub>15</sub> H <sub>22</sub>	1822.549	0.12
Hexanoic acid	20.02	C <sub>6</sub> H <sub>12</sub> O <sub>2</sub>	1836.275	0.17
3,7-dimethyl-(ñ)-1,6-octadien-3-ol	20.08	C <sub>10</sub> H <sub>18</sub> O	1839.216	0.25
6,10-dimethyl-(E)-5,9-undecadien-2-one	20.18	C <sub>13</sub> H <sub>22</sub> O	1844.118	0.07
Benzyl alcohol	20.65	C <sub>7</sub> H <sub>8</sub> O	1867.167	0.19

Contd...

**Table 1: Contd**

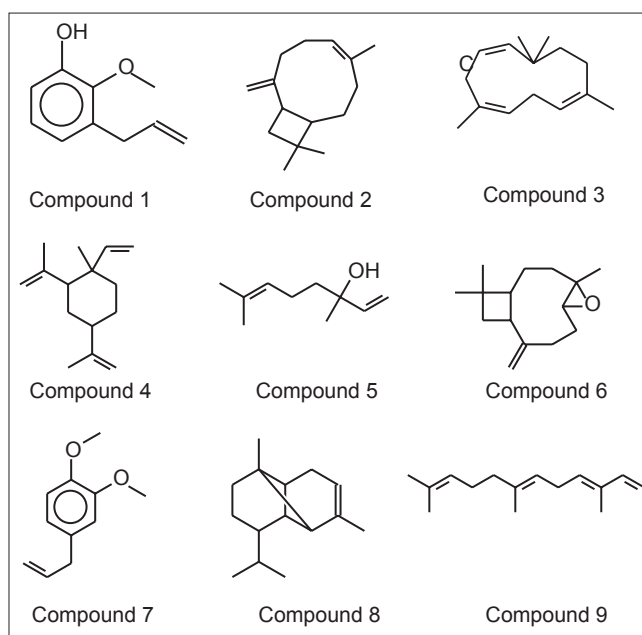
Name of components	RT (min)	M.F	RI	Relative content(%)
Phenylethyl alcohol	21.37	C <sub>8</sub> H <sub>10</sub> O	1902.591	0.22
(E)-2-Hexenoic acid	22.44	C <sub>6</sub> H <sub>10</sub> O <sub>2</sub>	1958.031	0.09
Caryophyllene oxide	22.84	C <sub>15</sub> H <sub>24</sub> O	1978.756	1.58
1,2-dimethoxy-4-(2-propenyl)-benzene	23.27	C <sub>11</sub> H <sub>14</sub> O <sub>2</sub>	2001.274	1.40
1,5,5,8-tetramethyl-[1R*(1R*,3E,7E,11R*)]-12-oxabicyclo[9.1.0]dodeca-3,7-diene	23.80	C <sub>15</sub> H <sub>24</sub> O	2035.032	0.39
(Z, Z, Z)-8,11,14-eicosatrienoic acid	24.01	C <sub>20</sub> H <sub>34</sub> O <sub>2</sub>	2048.408	0.04
Cubanol	24.06	C <sub>15</sub> H <sub>26</sub> O	2051.592	0.12
8aH-Cedran-8-ol	25.00	C <sub>15</sub> H <sub>26</sub> O	2116.981	0.06
(-)-Spathulenol	25.10	C <sub>15</sub> H <sub>24</sub> O	2126.415	0.25
6,10,14-trimethyl-2-pentadecanone	25.21	C <sub>18</sub> H <sub>36</sub> O	2136.792	0.12
2-methoxy-3-(2-propenyl)-phenol	25.68	C <sub>10</sub> H <sub>12</sub> O <sub>2</sub>	2181.132	42.94
1,2,4-trimethoxy-5-(1-propenyl)-(Z)-benzene	26.32	C <sub>12</sub> H <sub>16</sub> O <sub>3</sub>	2215.789	0.11
Globulol	26.69	C <sub>15</sub> H <sub>26</sub> O	2248.246	0.10
10,12-Octadecadiynoic acid	27.61	C <sub>18</sub> H <sub>28</sub> O <sub>2</sub>	2332.353	0.06
3-(2-hydroxyphenyl)-(E)-2-propenoic acid	28.17	C <sub>9</sub> H <sub>8</sub> O <sub>3</sub>	2387.255	0.08
2',3',4'-Trimethoxyacetophenone	29.44	C <sub>11</sub> H <sub>14</sub> O <sub>4</sub>	2509.756	0.06
Phytol	30.40	C <sub>20</sub> H <sub>40</sub> O	2587.805	0.17
Total				97.89

Compound 8: Copaene (1.04%); Compound 9:  $\alpha$ -Farnesene (1.02%). and their structures can be seen in Figure 2.

### Cytotoxicity analysis

At present in China, the commonly used screening methods of the anti-cancer medicine are external method and internal method of body. These two methods both use human or animal tumor cells as their screening systems or use the animal transplanted tumors as the pathological models which call for sterile operation during the experiment. These methods are strict, high-cost and their observing time is long, thus causing much inconvenience for the preliminary screening of the large-amount anti-cancer medicine. However, the advantage of the prawn - larva - lethality bioassay method is that the prawn larva eggs can live for several years in dry condition and that these eggs can be hatched if they are placed in the sea under the room temperature. Therefore, the source of the animal materials for the medicine screening becomes very quick and simple; there is no need for sterile operation, no need for the animal blood serum; and it has the advantages of very low-cost, small dose of medicine. This method is convenient in biological statistics in large amount. This is really a new and economical way of preliminary screening for the anti-cancer medicine.

According to the method in the literature,<sup>[12]</sup> and after hatching the prawn eggs to the larva, we put the larva (25-30 larva in each group) into the prepared solutions with different concentrations (diluting again the samples with 1% DMSO, with added DMSO man-made seawater



**Figure 2:** Representative volatile compounds of essential oil from the *Z. avicennae*

solutions as the control group). Then we observe the survival conditions of the larva under the room temperature for 24 h, and calculate the percentage death rate and the lethal concentration of 50% LC<sub>50</sub>, which can be seen in Table 2.

It is shown through the death-causing experiment for the prawn larva that there is no death in the control group, that under the volatile oil concentration of 10  $\mu\text{g mL}^{-1}$ , the death rate is 38%; under the volatile oil

**Table 2: The determining results of the lethal bioactivity of prawn larva**

Samples concentration of essential oil	10 $\mu\text{g mL}^{-1}$	50 $\mu\text{g mL}^{-1}$	100 $\mu\text{g mL}^{-1}$	500 $\mu\text{g mL}^{-1}$	1000 $\mu\text{g mL}^{-1}$
Percent deaths at 24 h	38%	58%	96%	100%	100%
LC50 ( $\mu\text{g mL}^{-1}$ )	34				

concentration of 50  $\mu\text{g mL}^{-1}$ , the death rate is 58%; and when the concentration of the volatile oil is greater than 100  $\mu\text{g mL}^{-1}$ , the death rate is nearly 100%. After careful calculation, when the  $\text{LC}_{50}$  value is 34  $\mu\text{g mL}^{-1}$ , the volatile oil has stronger cytotoxicity for the prawn larva. And the preliminary screening for the prawn lethal bioactivity shows that the volatile oil in the *Z. avicennae* has relatively strong anti-tumor activity.

### Antibacterial activities analysis

The minimum antibacterial concentration MIC of the volatile oil in *Z. avicennae* on the eight kinds plant pathogenic fungi can be seen in Table 3. The experiment results show that the volatile oil in *Z. avicennae* has an obvious inhibitory action on the experiment bacterial strain of the eight kinds of plant pathogenic fungi. Among them, the antibacterial activity for *Rhizoctonia solani* AGI-IA is the strongest, with MIC value 0.195  $\mu\text{L mL}^{-1}$ ; the antibacterial activity for *Botrytis cinerea* is relatively weak, with MIC value 100  $\mu\text{L mL}^{-1}$ .

## CONCLUSIONS

We totally determined 68 kinds of components from the volatile oil in *Z. avicennae*. The components determined account for 97.89% of the total peak area. The highest contents in the volatile oil of *Z. avicennae* are sesquiterpenoids and monoterpene in addition to the small-molecule aromatic components.

The biological determination of the prawn larva fatality is often used as a way to detect the pesticide residues and to analyze the mycete toxin in the United States. The research results show that,<sup>[13]</sup> the *Z. avicennae* is the natural plant with bioactivity. Nearly all the anticancer medicine has some toxicity when taken in large doses. It has a correlation between the fatality of the simple animal prawn larva and the inhibitory rate of the cancer cells. Therefore, a satisfactory result has been achieved by using the Prawn Larva Fatality Determination method to make the preliminary screening for the anticancer medicine.

**Table 3: The antifungal activity of the essential oil from the *Z. avicennae* as MIC**

Microorganisms	Concentration of samples ( $\mu\text{L mL}^{-1}$ )	The growth of the fungi	MIC ( $\mu\text{L mL}^{-1}$ )
<i>Botrytis cinerea</i>	200	-	100
	100	-	
	50	+	
<i>Exerohilum turcicum</i>	50	-	25
	25	-	
<i>Rhizoctonia solani</i> AG1-IA	12.5	+	0.195
	0.391	-	
	0.195	-	
<i>Fusarium graminearum</i>	0.098	+	50
	100	-	
	50	-	
<i>Sclerotinia sclerotiorum</i>	25	+	0.781
	1.563	-	
	0.781	-	
<i>Fusarium oxysporum</i>	0.391	+	25
	50	-	
	25	-	
<i>Rhizoctonia solani</i> Kühn	12.5	+	3.125
	6.25	-	
	3.125	-	
<i>Fusarium graminearum</i> Schwabe	1.563	+	12.5
	25	-	
	12.5	-	
	6.25	+	

"+" stands for fungi grow, "-" stands for fungi don't grow

It is reported that,<sup>[14]</sup> when the  $\text{LC}_{50}$  value of the crude plant extracts is less than 1000  $\mu\text{g mL}^{-1}$ , and the  $\text{LC}_{50}$  value of the monomeric compound is less than 50  $\mu\text{g mL}^{-1}$ , it shows that the tested sample has a relatively strong cytotoxicity, that is, a stronger anticancer activity. This experiment uses the method of the prawn larva fatality biological experiment to test the cytotoxicity of the volatile oil in *Z. avicennae*, the results show that the  $\text{LC}_{50}$  value of the volatile oil in *Z. avicennae* is 34  $\mu\text{g mL}^{-1}$ , and thus it has strong cytotoxicity.

The antibacterial activities experiment shows that the volatile oil in *Z. avicennae* has certain antibacterial activity on eight kinds different plant pathogenic fungi, The volatile oil has the strongest antibacterial activity on *R. solani* AG1-IA and relatively weak antibacterial activity on *B. cinerea*. This plant of *Z. avicennae* is abundant in the wild nature. It also has a very high oil content and strong biological activity,

so this natural plant will have a very bright future in its development and application.

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