

# Phytochemical compositions of volatile oil from *Blumea balsamifera* and their biological activities

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## ABSTRACT

**Background:** This research, extract compositions of volatile oil in *Blumea balsamifera* (Linn.) DC by steam distillation method. The gas chromatograph-mass spectrometer technology (GC-MS) was used to separate and determine chemical components of volatile oil. Meanwhile, the percentage of the chemical components was determined in the volatile oil by peak area normalization method. At the same time, we determine and evaluate antitumor, antibacterial, and antioxidation activities of chemical components of volatile oil in *Blumea balsamifera* of the aromatic plant. **Materials and Methods:** We screened the cytotoxicity of volatile oil in *Blumea balsamifera* by using prawn larva and by prawn-lethal bioactivity experiment. Use the slanting test tube method to evaluate the antibacterial activity of volatile oil in *Blumea balsamifera* for eight kinds of plant pathogenic fungi. Taking Trolox as the contrast, the research uses DPPH method to study the radical-scavenging function (IC<sub>50</sub>) of the volatile oil in *Blumea balsamifera*. **Results:** The results show that 42 kinds of compounds are separated from volatile oil of *Blumea balsamifera*. The appraised components take up 97.65% of total peak area. The volatile oil in *Blumea balsamifera* mainly contains sesquiterpenoids. The results also show that it has relatively strong activity of antitumor and anti-plant pathogenic fungi and some antioxidation activity. **Conclusion:** This research provided the reference data for further development of this natural resource, and at the same time, we understood more of the chemical components of volatile oil and bioactivity of this aromatic plant.

**Key words:** Antimicrobial, antioxidant, antitumor, *Blumea balsamifera*, GC-MS, volatile oil

## INTRODUCTION

*Blumea balsamifera* (Linn.) DC belongs to the composite family. Its branches, twigs, and leaves can all be used as medicine and can be picked all the year round.<sup>[1,2]</sup> *Blumea balsamifera* is nicknamed as Big Wind mugwort, Borneol mugwort, etc., It is mainly distributed in south China, Fujian, Taiwan, Guizhou, Hainan and Yunnan, etc., *Blumea balsamifera* has medical effects of insect-killing, dispelling wind and eliminating dampness, checking diarrhea, blood activating and detoxification, detumescence and pain-relieving, etc., It is used mainly to cure wind-cold, head-wind and headache, rheumatism and cold-damp diarrhea, spleen worm disease, snake-biting, arthralgia, traumatic injuries, ringworm,<sup>[3,4]</sup> etc., The pharmacologic research shows that injection of extracts of *Blumea balsamifera* into animals can cause

reduced blood pressure, inhibition of sympathetic nervous system, which can be used in curing over-excitement, insomnia, or hyperpietic. Also, there are reports of it having functions of diuresis and invigorating blood circulation, dispelling wind and eliminating dampness, worm-killing,<sup>[5,6]</sup> etc.

The prawn-larva-fatality biological determining method was first put forward by R. F. Brown, which is very simple, convenient, and low cost determining method of internal biological activity. This method is used to determine toxic components like aflatoxin in food<sup>[7]</sup> and also widely used in detecting and determining toxic matters in fungi.<sup>[8]</sup> The prawn larva are also used in all kinds of biological determining system. In these applications, it also includes detecting insecticides, fungus cytotoxicity, river pollution, anesthetics, morphine components, and toxic matters in the sea. In 1982, scientists like B. N. Meyer used this method to make a biological determination for known advanced plant extracts with different bioactivity. The results show that these components have clear fatality function on the prawn larva. Melaughlin, an American

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professor at the Medicine College of Purdue found that there was an obvious correlation between natural compounds with bioactivity on prawn larva mortality and inhibitory rate on cancer cells. These materials show that prawn-larva-fatality biological determining method; which is a very simple, convenient, and low cost; can also be used to guide separation and determination of the plant active components. It also shows that the prawn larva mortality is over 70%, indicating that the test sample has a strong cytotoxicity, that is, a relatively strong antitumor activity. Through literature retrieval, there have been no reports on research about antitumor, antifungi, and antioxidant activity of volatile oil of this aromatic plant of *Blumea balsamifera*. This plant belongs to the fragrant plants containing much volatile oil and it is abundant in the natural world. It is very important for us to research on chemical components in volatile oil and its bioactivity. This research will also have a very important value in theory and practice.

## MATERIALS AND METHODS

### Instruments and materials

The instruments used in this study were: Trace 2000 instrument (Finnigan MS Co, San Jose, CA), the chromatographic column is an elastic quartz capillary column (DB-WAX 30 m × 0.25 mm, 0.25 μm), Infinite M 200 Microplate Reader (Swiss Tecan), UV-2102 PCS Ultraviolet Visible Spectrophotometer (Shanghai Unico Instrument Corporation), 96-hole ELISA plate (Zhejiang Gongdong Medical Plastics Plant), volatile oil extractor, N-1001 clean bench; PRX-350 Biochemical Incubator, MOV-212 Dry-heat Sterilization Pot, DGG-924A Electric Heat Constant Temperature Drying Oven (Shanghai Senxin Experimental Instrument Corporation, Ltd), KQ-250 B Ultrasonic Cleaner (Kunshan City Ultrasonic Instrument Corporation), and R201B Rotatory Evaporating Instrument (Shanghai Shensheng Biotechnical Limited Company). *Blumea balsamifera* was picked up from Sanya, Hainan, China, in August 2011, and was identified by Huang Shiman, medicinal plant taxonomic professor of Hainan University. The eight selected experiment strains are *Fusarium graminearum*, *Botrytis cinerea*, *Exserohilum turcicum*, *Mucor*, *Sclerotinia sclerotiorum*, *Rhizoctonia solani* AG1-IA, *Rhizoctonia solani* Kühn, and *Fusarium graminearum* Schwabe, all provided by Forest-Protection Lab, Zhejiang Agriculture and Forestry University. The culture medium used in the fungi is potato and glucose agar culture medium. 1,1-diphenyl-2-bitter diazanyl free radicals (1,1-Diphenyl-2-picryl-hydrazyl, DPPH), 6-Hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic, Trolox are all purchased from Sigma company; Artemia salina prawn egg are purchased from Tianjin Fengnian Aquaculture Corp, Ltd; and dimethyl sulfoxide (DMSO) is analytically pure.

### Extraction of volatile oil

Take 200 g of dried leaf powder of *Blumea balsamifera* after sifting through a 20-hole sieve, put it in a round-bottomed flask and add 600 mL of distilled water to it, and then extract it by using the volatile oil extractor according to the XD extracting standards<sup>[9]</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 extracted liquid by anhydrous sodium sulfate. The volatile oil is the oily liquid with faint yellow color and rich fragrance. The oil-obtaining rate of the *Blumea balsamifera* is 3.25%.

### The analytical conditions of GC-MS

The GC conditions: The chromatographic column is DB-WAX (30 m × 0.25 mm, 0.25 μm) elastic quartz capillary column; temperature programming: Keep the initial temperature at 45°C for 3 min, then raise the temperature to 100°C at a speed of 10°C per min, and again raise the temperature to 170°C at a speed of 5°C per min, then again to 240°C for 7 min at a speed of 10°C per min. 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, and the split sampling speed is 20 mL/min.

The mass spectrum conditions: Let the electrons bombard the electron impact (EI) ionization source; ionizing energy is 70 eV; temperature of the ionization source is 200°C; voltage of the detector is 350 V; scanning quality range is between 40-300 m/z; the retrieved atlas databank is the standard mass spectrum depot of Willey and NIST; scanning speed is 0.5 s; and 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.

### Retention indices value

This experiment adopts n-alkane mixed reference sample to analyze data by the gas chromatographic and mass spectrum conditions, and uses the peak area normalization method to determine relative percentage of each chemical component in volatile oil. Then we calculated the RI value of each component by linear warming equation according to the retention time of each n-alkane.  $RI = 100n + 100(t_x - t_n)/t_{n+1} - t_n$ ; here we analyze and group  $t_x$ ,  $t_n$ , and  $t_{n+1}$  with the carbon number as the retention time (min) of the outflow peak of n and n + 1 n-alkane ( $t_n < t_x < t_{n+1}$ ).<sup>[10]</sup>

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

Preparation of the sample solution: Weigh precisely 0.02 g of volatile oil sample and use DMSO to dissolve it to a

constant volume of 10 mL, we will get the sample solution with a concentration of 2 mg/mL. Then use DMSO to prepare the following sample solutions with five different concentration gradients: 10, 50, 100, 500, and 1000 µg/mL. Take the sample solutions with different concentrations and 25-30 prawn larva and experiment it in the 96-hole porous plate. Only add DMSO into the control group and cultivate it for 24 h in the dark at room temperature and calculate the number of dead prawn larva under the microscope in each trough. Finally, calculate the mortality rate of prawn larva according to the equation, According to the average death rate under different concentrations, we calculate its half-number-death concentration  $LC_{50}$  by the SPSS method.

#### Determination of antibacterial activity

We determined the *in vitro* antibacterial activity of volatile oil in *Blumea balsamifera* for eight kinds of plant pathogenic fungi by the slanting test tube method.<sup>[11,12]</sup> Choose potatoes, glucose, and add water to prepare potato dextrose agar (PDA). Disinfect the prepared PDA by wet method and then melt the curdled PDA by heating on super-clean workbench. Put 700 µL PDA into 2 mL centrifugal tube when it is still warm. Then place it slantingly and cool and curdle till it becomes a slanting culture medium. Use DMSO as a solvent to prepare volatile oil samples with 12 different concentration gradients of 200, 100, 50, 25, 12.5, 6.25, 3.125, 1.563, 0.781, 0.391, 0.195, and 0.098 µL/mL.

Spray the prepared volatile oil sample solution onto the PDA surface of centrifugal test tube with each sample feeding amount of 40 µL. Then shake it instantly and make it touch the PDA surface evenly. Put only DMSO to PDA surface in the negative contrast group, but add nothing to the PDA surface in the blank group. Use the puncher to perforate after training the eight plant pathogenic fungi in the culture dish, and then put the punched fungi in 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 volatile oil according to their fungi growth. Use the lowest solution concentration of fungi-free volatile oil as the lowest antibacterial concentration, that is, minimum inhibitory concentration (MIC). The experiment in each group is repeated three times, and we take the average value<sup>[13]</sup> in the experiment results.

#### Determination of antioxidant activity

DPPH is a stable, free radical in the organic solvent. When its paired electrons are at 517 nm, it will have a strong absorption (displaying purple color). When the organic scavenging agents exist, lone pair electrons will be mated, absorbed, dispelled, or weakened. Through determining its absorption degree, we can evaluate the activity of free radical scavenging agents.<sup>[14-16]</sup>

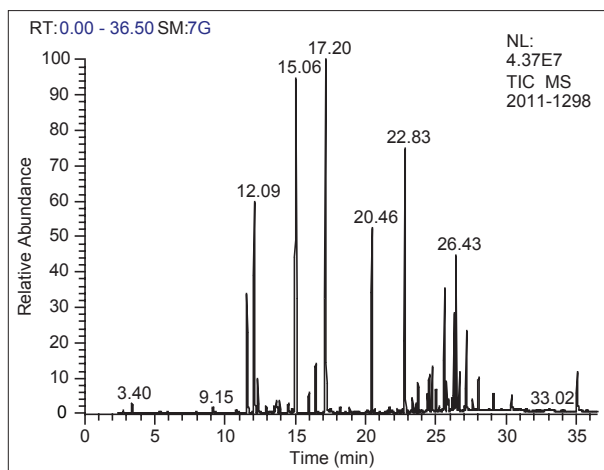
Use Trolox as the contrast, adopt DPPH method to measure the antioxidant activity of volatile oil; weigh exactly 50 µL volatile oil and put it into the 10 mL flask, mixing the absolute ethyl alcohol till it reaches the given volume and shake it evenly for later use. Put the 100 µL sample and 200 µL DPPH free radical solution (the concentration is 31.28 mg/mL) into the enzyme microplate reader and shake for 30 s. Keep it for 30 min under the temperature of 37°C and measure its ultraviolet absorption value. Meanwhile, we should measure the ultraviolet absorption value of free radical sample solution without adding DPPH and absolute ethanol solution with DPPH. According to the following formula, we calculate the free radical scavenging ability<sup>[17]</sup>: Scavenging % =  $1 - (A_p - A_c) / A_{max} \times 100\%$ . In this formula,  $A_p$  is the absorbance of 200 µL DPPH solution and 100 µL solution ready to be tested.  $A_c$  is the absorbance of 200 µL solution to be tested and 100 µL absolute ethanol.  $A_{max}$  is the absorbance of 100 µL DPPH solution and 200 µL absolute ethyl alcohol.

Use ethanol to dilute Trolox reserve solution (mass concentration is 0.2800 mg/mL<sup>-1</sup>) into the different concentration gradients and according to the above method and formula, calculate Trolox's free radical scavenging ability for DPPH free radicals with different concentrations. Then we use fresh working solution to correct it, and regard Trolox concentration as the X-axis and scavenging rate for the DPPH free radicals as the Y-axis to draw a standard curve. At the same time, use ethanol to dilute volatile oil into different concentration gradient and use sample concentration as the X-axis and scavenging rate for the DPPH free radicals as the Y-axis to draw a standard curve.

## RESULTS AND DISCUSSION

#### The analytical results of GC-MS

Take the obtained volatile oil by steam distillation method with sample-feeding amount of 2.0 µL. According to the above GC-MS condition, we analyzed and determined it by GC-MS, and got the total ion flow chart of volatile oil in *Blumea balsamifera*, which can be seen in Figure 1. After mass spectrum scanning for each chromatographic peak, we got the mass spectrogram. Through the mass spectrum retrieval by NIST2008 standard and relevant mass spectrum data, we quantified the compounds by Hewlett-Packard software processing system and calculated each peak area according to peak area normalization method. Moreover, we calculated the relative percentage of volatile oil and chose the probable matter with high matching degree to calculate the RI value and combined manual analysis to determine chemical components of volatile oil in *Blumea balsamifera*, which can also be seen in Figure 1 [Table 1].



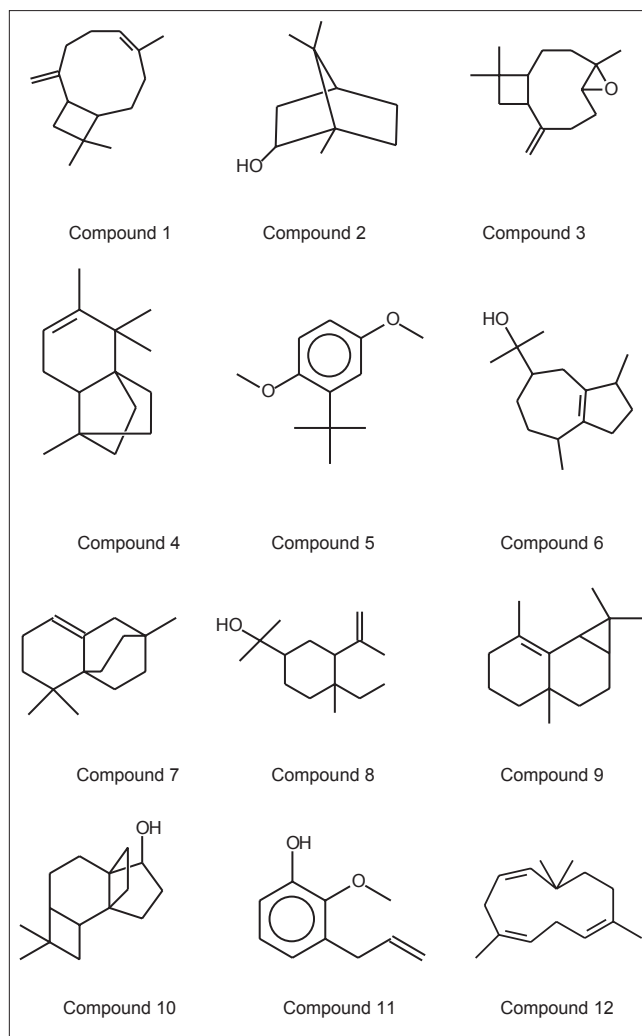
**Figure 1:** GC-MS total ion current chromatogram of the volatile oil of *Blumea balsamifera*

From Figure 1, we can see that 42 kinds of compounds are determined from volatile oil in *Blumea balsamifera*. The appraised components take up 97.65% of the total peak area. The highest content compounds in volatile oil of *Blumea balsamifera* are all sesquiterpene. In addition, there are also small molecule aromatic components. It has been known that the above sesquiterpenes have very strong antibacterial and certain antioxidant activity as well as the cytotoxicity activity.<sup>[18-20]</sup>

The high contents of the components in volatile oil of *Blumea balsamifera* are mainly compound 1: Caryophyllene (19.28%); compound 2: 1,7,7-trimethyl-(1S-endo)-bicyclo[2.2.1]heptan-2-ol (15.54%); compound 3: Caryophyllene oxide (11.20%); compound 4: Thujopsene (10.36%); compound 5: 3-*t*-butyl-4-methoxyphenol methyl derivative (6.04%); compound 6: Guaiol (5.03%); compound 7: 1,3,4,5,6,7-hexahydro-2,5,5-trimethyl-2H-2,4 $\alpha$ -ethanonaphthalene (4.89%); compound 8: Decahydro- $\alpha$ ,  $\alpha$ , 4 $\alpha$ -trimethyl-8-methylene-[2R-(2 $\alpha$ , 4 $\alpha$ , 8 $\alpha$ )]-2-naphthalenemethanol (3.83%); compound 9: 1 $\alpha$ , 2,3,3 $\alpha$ , 4,5,6,7 $\beta$ -octahydro-1,1,3 $\alpha$ , 7-tetramethyl-[1 $\alpha$ R-(1 $\alpha$ , 3 $\alpha$ , 7 $\alpha$ )]-1H-cyclopropa[a]naphthalene (2.97%); compound 10: 4,4-dimethyl-tetracyclo[6.3.2.0(2,5).0(1,8)]tridecan-9-ol (2.54%); compound 11: 2-methoxy-3-(2-propenyl)-phenol (1.93); compound 12: 1,1,4,8-tetramethyl-*cis*, *cis*, *cis*-4,7,10-cycloundecatriene (1.67); and their structures can be seen in Figure 2.

#### Antitumor activity analysis

According to the method recorded in literature,<sup>[21]</sup> we put the larva (25-30 larva each group) into prepared solutions with different concentration group after the prawn eggs are hatched (use 1% DMSO to solubilize the samples, artificial seawater solution by DMSO to dissolve seawater as blank control). Observe survival condition of larva for 24 h



**Figure 2:** Representative volatile compounds of *Blumea balsamifera*

under room temperature and calculate percentage death rate and lethal concentration of 50% ( $LC_{50}$ ). Through the prawn-larva-lethal experiment, we know that the larva in control group have no deaths, but when the concentration of volatile oil is at 10  $\mu\text{g}/\text{mL}$ , the death rate is 28%; at 100  $\mu\text{g}/\text{mL}$ , the death rate is 64%; at 1000  $\mu\text{g}/\text{mL}$ , the death rate is 100%, and the  $LC_{50}$  value is 65  $\mu\text{g}/\text{mL}$  which shows that its volatile oil has relatively strong cytotoxicity on prawn larva, and that the volatile oil in *Blumea balsamifera* has a certain antitumor activity.

#### Antibacterial activity analysis

Use the slanting test tube method to determine antibacterial activity of volatile oil. The minimum antibacterial concentrations of volatile oil in *Blumea balsamifera* for eight kinds of plant pathogenic fungi can be seen in Figure 2. The experiment results show that the volatile oil has obvious inhibitory activity on the experiment fungal strains of eight kinds of plant pathogenic fungi, and among them, the antibacterial activity on *Rhizoctonia solani* Kühn, *Rhizoctonia*



**Table 1: Analytical results of chemical constituents of the volatile oil from the *Blumea balsamifera* by GC/MS**

Name of components	RT (min)	MF	RI	Relative concentration (%)
Ethanol	3.40	C <sub>2</sub> H <sub>6</sub> O	938.298	0.28
3-hydroxy-2-butanone	9.15	C <sub>4</sub> H <sub>8</sub> O <sub>2</sub>	1287.805	0.18
3-hexen-1-ol	10.80	C <sub>6</sub> H <sub>12</sub> O	1381.006	0.09
1,3,4,5,6,7-hexahydro-2,5,5-trimethyl-2H-2,4a-ethanonaphthalene	11.57	C <sub>15</sub> H <sub>24</sub>	1422.165	4.89
Thujopsene	12.09	C <sub>14</sub> H <sub>22</sub>	1448.969	10.36
4,4-dimethyl-3-(3-methylbut-3-enylidene)-2-methylenebicyclo[4.1.0]heptane	13.50	C <sub>15</sub> H <sub>22</sub>	1520.690	0.27
2,3,6,7,8,8a-hexahydro-1,4,9,9-tetramethyl-(1a, 3aa, 7a, 8aa)-1H-3a, 7-methanoazulene	13.65	C <sub>15</sub> H <sub>24</sub>	1528.079	0.72
3,7-dimethyl-1,6-octadien-3-ol	13.87	C <sub>10</sub> H <sub>18</sub> O	1538.916	0.34
4,11,11-trimethyl-8-methylene-[1R-(1R*,4Z,9S*)]-bicyclo[7.2.0]undec-4-ene	14.53	C <sub>15</sub> H <sub>24</sub>	1571.429	0.55
1,2,3,4,5,6,7,8-octahydro-1,4-dimethyl-7-(1-methylethenyl)-[1S-(1a, 4a, 7a)]-azulene	14.77	C <sub>15</sub> H <sub>24</sub>	1583.251	0.19
Caryophyllene	15.06	C <sub>15</sub> H <sub>24</sub>	1597.537	19.28
Decahydro-1,1,7-trimethyl-4-methylene-[1αR-(1αa, 4αa, 7a, 7αa, 7ab)]-1H-cycloprop[e] azulene	15.97	C <sub>15</sub> H <sub>24</sub>	1641.148	0.66
1,1,4,8-tetramethyl- cis, cis, cis-4,7,10- cycloundecatriene	16.45	C <sub>15</sub> H <sub>24</sub>	1664.115	1.67
1,7,7-trimethyl-(1S-endo)-bicyclo[2.2.1]heptan-2-ol	17.20	C <sub>10</sub> H <sub>18</sub> O	1700.000	15.54
4-methylene-1-methyl-2- (2-methyl-1-propen-1-yl)-1- vinyl-cycloheptane	17.43	C <sub>15</sub> H <sub>24</sub>	1711.058	0.10
Longifolene	17.52	C <sub>15</sub> H <sub>24</sub>	1715.385	0.21
Aromadendrene oxide	17.55	C <sub>15</sub> H <sub>24</sub>	1716.827	0.11
1,2,4a, 5,8,8a-hexahydro-4,7-dimethyl-1-(1-methylethyl)-[1S-(1a, 4aa, 8aa)]-naphthalene	18.20	C <sub>15</sub> H <sub>24</sub>	1748.077	0.22
7-methyl-3-methylene-6-octen-1-ol	18.83	C <sub>10</sub> H <sub>18</sub> O	1778.365	0.16
3,7-dimethyl-2,6-octadien-1-ol	20.08	C <sub>10</sub> H <sub>18</sub> O	1839.216	0.14
3-t-butyl-4-methoxyphenol methyl derivative	20.46	C <sub>12</sub> H <sub>18</sub> O <sub>2</sub>	1857.843	6.04
2,2-dimethyl-3-methylene-(1R)-bicyclo[2.2.1]heptane	20.67	C <sub>10</sub> H <sub>16</sub>	1868.137	0.24
Decahydro-1,1,4,7-tetramethyl-[1αr-(14Ah-cycloprop[e] azulen-4a-ol	21.70	C <sub>15</sub> H <sub>26</sub> O	1919.689	0.17
Diepicedrene-1-oxide	22.26	C <sub>15</sub> H <sub>24</sub> O	1948.705	0.15
Caryophyllene oxide	22.83	C <sub>15</sub> H <sub>24</sub> O	1978.238	11.20
1,2-dimethoxy-4-(2-propenyl)-benzene	23.24	C <sub>11</sub> H <sub>14</sub> O <sub>2</sub>	1999.482	0.08
Ledol	23.59	C <sub>15</sub> H <sub>26</sub> O	2021.656	0.28
1,5,5,8-tetramethyl-[1R-(1R*,3E,7E,11R*)]-12-oxabicyclo[9.1.0]dodeca-3,7-diene	23.74	C <sub>15</sub> H <sub>24</sub> O	2031.210	1.08
4-ethenyl- a, a, 4-trimethyl-3-(1-methylethenyl)-[1R-(1a, 3a, 4a)]-cyclohexanemethanol	24.37	C <sub>15</sub> H <sub>26</sub> O	2071.338	0.47
Epiglobulol	24.45	C <sub>15</sub> H <sub>26</sub> O	2076.433	0.12
1,2,3,4,5,6,7,8-octahydro-a, a, 3,8-tetramethyl-5-azulenemethanol	24.53	C <sub>15</sub> H <sub>26</sub> O	2081.529	1.34
Decahydro-a, a, 4a-trimethyl-8-methylene-[2R-(2a, 4aa, 8aa)]-2-naphthalenemethanol	25.01	C <sub>15</sub> H <sub>28</sub> O	2117.925	0.59
2-methoxy-3-(2-propenyl)-phenol	25.56	C <sub>10</sub> H <sub>12</sub> O <sub>2</sub>	2169.811	1.36
1a, 2,3,3a, 4,5,6,7b-octahydro-1,1,3a, 7-tetramethyl-, [1αR-(1αa, 3aa, 7ab)]-1H-cyclopropa[a] naphthalene	25.65	C <sub>15</sub> H <sub>26</sub>	2178.302	2.97
Thymol	25.82	C <sub>10</sub> H <sub>14</sub> O	2194.340	0.26
Guaiol	26.31		2214.912	5.03
Decahydro-a, a, 4a-trimethyl-8-methylene- [2R-(2a, 4aa, 8aa)]-2-naphthalenemethanol	26.43	C <sub>15</sub> H <sub>26</sub> O	2225.439	3.83
decahydro-1,4a-dimethyl-7-(1-methylethylidene)-[1R-(1a, 4aa, 8aa)]-1-naphthalenol	26.69	C <sub>15</sub> H <sub>26</sub> O	2248.246	0.93
4,4-dimethyl-tetracyclo[6.3.2.0 (2,5).0 (1,8)]tridecan-9-ol	27.21	C <sub>15</sub> H <sub>24</sub> O	2293.860	2.54
3-(1,1-dimethylethyl)-4-methoxy-phenol	29.10	C <sub>11</sub> H <sub>16</sub> O <sub>2</sub>	2423.156	0.42
Phytol	30.39	C <sub>20</sub> H <sub>40</sub> O	2586.992	0.66
Tetradecanoic acid	35.08	C <sub>14</sub> H <sub>28</sub> O <sub>2</sub>	2991.345	1.93
Total				97.65

RT: Real time, MF: Molecular formula, RI: Refractive index

*solani* AG1-IA, and *Sclerotinia sclerotiorum* is relatively strong; MIC value for all is 0.195 μL/mL. The antibacterial activity

on *Exerobillum turcicum* is the weakest and its MIC value is 100 μL/mL [Table 2].

**Table 2: The antifungi activity of the volatile oil from the *Blumea balsamifera* as MIC**

Microorganisms	MIC (μL/mL)	Microorganisms	MIC (μL/mL)
<i>Botrytis cinerea</i>	25	<i>Sclerotinia sclerotiorum</i>	0.195
<i>Exerohilum turcicum</i>	100	<i>Rhizoctonia solani</i> AG1-IA	0.195
<i>Mucor</i>	1.563	<i>Rhizoctonia solani</i> Kühn	0.195
<i>Fusarium graminearum</i>	25	<i>Fusarium graminearum</i> Schwabe	1.563

MIC: Minimum inhibitory concentration

### Antioxidant activity analysis

DPPH method is widely used in determining scavenging activity for the free radicals in plant and volatile oil. Put the volatile oil of *Blumea balsamifera* and the artificial antioxidant agent Trolox into five concentrations with equal difference, and then determine them with the DPPH method, and make a regression analysis for experiment results. Use the concentration of test samples as the X-axis and obtained free radical scavenging rate as the Y-axis to draw a standard curve, we will get the regression equation, and calculate 50% inhibitory concentration (IC<sub>50</sub>) value according to the regression equation. Among them, the regression equation of Trolox is  $Y = 0.0792X + 0.1948$ ,  $R^2 = 0.9974$ , and IC<sub>50</sub> is 0.1873 μg/mL; the regression equation of volatile oil from *Blumea balsamifera* is  $Y = 0.6234X + 12.4541$ ,  $R^2 = 0.9734$ , and IC<sub>50</sub> is 0.6342 μL/mL. This experiment results show that the volatile oil of *Blumea balsamifera* has a certain free radical scavenging activity for DPPH, and scavenging activity for DPPH free radicals will increase with the concentration of volatile oil, which shows that there is a positive correlation between scavenging rate and concentration of the volatile oil.

### CONCLUSION

Forty-two kinds of compounds are determined from volatile oil in *Blumea balsamifera*. The appraised components take up 97.65% of the total peak area. The highest content compounds in volatile oil of *Blumea balsamifera* are all sesquiterpene. In addition, there are also small molecule aromatic components.

The antibacterial experiment results show that the volatile oil in *Blumea balsamifera* has a certain antibacterial activity on eight kinds of plant pathogenic fungi and among them, the antibacterial activity on *Rhizoctonia solani* Kühn, *Rhizoctonia solani* AG1-IA, and *Sclerotinia sclerotiorum* is relatively strong; MIC value for all of them is 0.195 μL/mL.

IC<sub>50</sub> value is used as the indicator of free-radical scavenging ability and IC<sub>50</sub> value is a parameter often used to evaluate the

antioxidant ability, and it refers to the needed concentration for scavenging 50% of the DPPH free radicals. The smaller its value, the smaller the concentration dose of the free-radical scavenging agents when it reaches the 50% scavenging rate and stronger the scavenging rate, and at the same time, it has stronger antioxidant activity of the corresponding test samples. From this antioxidant experiment, it shows that the IC<sub>50</sub> value of volatile oil for DPPH free radicals is 0.6342 μL/mL, which shows that the volatile oil in *Blumea balsamifera* has a certain antioxidant activity.

This experiment uses the prawn-larva-fatality biological determining method to measure cytotoxicity of volatile oil in *Blumea balsamifera*. The experiment result shows the LC<sub>50</sub> value of volatile oil in *Blumea balsamifera* is 65 μg/mL, so it has a certain antitumor activity. The method of prawn-larva-fatality is a new kind of speedy, concise, and practical screening method for sifting antitumor activity part of the plant and microbes. It is reported that when the LC<sub>50</sub> value of the plant crude extracts is less than 1000 μg/mL, and the LC<sub>50</sub> value of monomeric compound is less than 50 μg/mL, it will have a strong antitumor activity.

This wild plant is rich in the natural world, and has very rich oil content as well as a strong bioactivity, so it will have a very bright prospect in its development and application.

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