Research Article

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GC-MS analysis of Vespa velutina auraria Smith and its anti-inflammatory and antioxidant activities in vitro

Abstract: Vespa velutina auraria Smith is an edible and medicinal insect in China. This study demonstrated the in vitro antioxidant and anti-inflammatory bioactivities and the volatile composition identification determined by Gas chromatography mass spectrometry (GC-MS). The antioxidant activity screening results showed that the ethanol extracts of both the fresh and dried samples exhibited an efficient antioxidant activity for three models, 2,2’-azino-bis (3-ethylbenzo-thiazoline-6-sulfonic acid diamonium salt) free radicals scavenging capacity, 1,1-diphenyl-2-picrylhydrazyl scavenging capacity, and ferric reducing antioxidant power. The anti-inflammatory activity screening in vitro indicated that ethanol extracts had considerable inhibitory effect on Tumor Necrosis Factor-α and Interleukin-1β (IL-1β) in macrophages, but had no influence on IL-6 expression. GC-MS analyses of volatile composition of V. auraria identified 46 components, representing 75.76% of the total peak areas from fresh sample, and 34 components, 84.70% of the total peak areas from dried ones. The volatile constituents were very different in the petroleum ether part of fresh and dried ones. The three major components are hentriacon (7.76%), n-hexadecanoic acid (6.54%), and palmitoleic acid (4.50%) in the fresh sample, while they are benzeneacetaldehyde (13.11%), dodecanoic acid (7.08%), and oleic acid (6.72%) in the dried sample.

Keywords: Vespa velutina auraria Smith, GC-MS, volatile composition, antioxidant, anti-inflammatory

1 Introduction

Insects have been widely used in traditional medicine for centuries in East Asia, Africa, and South America, and this has gradually attracted attention as sources of modern drugs. In China, more than 100 medicinal insects, including the wasp, were recorded in the “Compendium of Materia Medica,” which was also known as Ancient Chinese Encyclopedia. The natural products and extracts of insects have various pharmacological activities [1–3]. The wasp, vespae nidus and wasp venom have been used in the treatment of arthritis, headache, hemorrhage, analgesic, and sexual vigor [4–6]. We were committed to insect biomedical R&D and had developed a wasp venom plastic for treating pain, thrombus, and Ischemia reperfusion injury [7–9]. Although the wasp
stings threaten health and wasp is a natural enemy of bees, it can catch agricultural pests such as Heliothis armigera Habner, Ascotis selenaria, and Cnaphalocrocis medinalis Guenee.

Vespa velutina auraria Smith is a species of wasp. Most of them are widely distributed in north-eastern India, southern and central China (including Yunnan, Sichuan, Tibet, and Taiwan), and Indonesia [10]. They mainly spread in farmland, river ditch, mountain, forest, orchard, and other places with suitable climate and sufficient food source [11]. V. auraria is used as a folk medicine in China for the treatment of arthritis and rheumatism since ancient times. Simultaneously, it is used to treat these diseases by making wasp wine [12,13], which was widely used in Jingpo, a Chinese national minority, and has been recorded in the Pharmacopoeia of the People's Republic of China. In addition, the wasp pupae are fried and used as a culinary delicacy in China due to its abundant nutrient contents [14].

The research on V. auraria mainly focuses on elemental and protein analysis [14,15], melanization [16], invasion, and distribution [17]. However, the active ingredients and pheromone are not clear, and the mechanism of pharmacological action is a terra incognita. Therefore, the antioxidant, the anti-inflammatory, and the antibacterial activity attached to ethanolic extracts of fresh and dried V. auraria were tested and compared. The volatile compounds of the fresh and dried material activity attached to ethanolic extracts of fresh and dried wasps consist of about 65.57 and 2.99% water, respectively.

2 Materials and methods

2.1 Wasp samples

The wasp used for this study were collected in a fresh condition from Yunnan province, China, and identified to be V. auraria by Professor Zi-Zhong Yang at Yunnan Provincial Key Laboratory of Entomological Biopharmaceutical R&D, Dali University. Voucher specimens (number: 201506) were deposited at the same laboratory. Prior to the experiments, the live insects were frozen at −20°C and divided into two parts after they expired. One part is dried in a drying cabinet at 45°C and the other part is not processed. Both of them are crushed into a certain size of particles. The fresh and dried wasps consist of about 65.57 and 2.99% water, respectively.

2.2 Preparation of extracts

50 g samples of fresh and dried V. auraria were ultrasonecally extracted 3 times with 95% ethanol, at a solid to liquid ratio of 1:10, 30 min for each time. The extracted solution was filtrated, concentrated, and lyophilized to obtain the ethanol extracts, with a yield of 286.48 mg/g for fresh and 320.72 mg/g for dried sample. Taking 50 mg of above ethanol extracts and is extracted again with petroleum ether to get petroleum ether part, which was used for volatile composition analysis by GC-MS.

2.3 Antioxidant activity determination of ethanol extracts

2.3.1 2,2’-Azino-bis (3-ethylbenzo-thiazoline-6-sulfonic acid diammonium salt) (ABTS) scavenging capacity assay

ABTS free radicals scavenging capacity was measured according to the reported method [18]. Ascorbic acid was used as positive control. ABTS radical cation (ABTS⁺) was produced by reacting ABTS stock solution (7 mM) with potassium persulfate (2.45 mM) and the mixture was kept in the dark at room temperature for 12 h before use. The concentrated ABTS was diluted with ethanol to a final absorbance of 0.72 ± 0.02 at 734 nm on the UV-6000PC UV-Vis spectrophotometer (Metash, Shanghai, China). Then, 100 µL of the examined ethanol extract solution was added to 2 mL of ABTS solution and equilibrated at 30°C for 10 min, and the absorbance of the solution was measured at 734 nm. The inhibition ratio (%) was calculated as \( I = \left( \frac{A_0 - A_S}{A_0} \right) \times 100\% \), where \( A_0 \) is the absorbance of ABTS solution without sample, and \( A_S \) is the absorbance after adding sample. A series of extract solutions with concentrations of 0.125, 0.25, 0.50, 1.00, and 2.00 mg/mL was tested to estimate the inhibition activity and calculate the IC50.

2.3.2 1,1-Diphenyl-2-picrylhydrazyl (DPPH) scavenging capacity assay

The free radical scavenging activity of extracts on 1,1-diphenyl-2-picrylhydrazyl (DPPH) was assessed using the reported method [19]. Ascorbic acid was used as the
positive control. 3 mL of 50% ethanol and 1 mL of DPPH ethanol solution (1 mM) were added to 1 mL of the examined ethanol extract solutions (with the final concentration of 0.125, 0.25, 0.50, 1.00, and 2.00 mg/mL) at room temperature for 40 min, and then the absorbance of the solution was tested at 517 nm. The inhibition ratio (%) calculated was consistent with the above.

### 2.3.3 Ferric reducing antioxidant power (FRAP) assay

The FRAP of ethanol extracts was measured according to the method [20]. Ascorbic acid was used as positive control. The FRAP reagent was prepared before the test by mixing 10 mL of acetate buffer (300 mM and pH 3.6) with 1 mL of TPTZ solution (10 mM in 40 mM HCl) and 1 mL of FeCl$_3$ (20 mM). Then, the mixture was incubated at 37°C. 200 µL of the examined ethanol extract solution or different concentrations of FeSO$_4$ solution were added to 3 mL of FRAP and equilibrated at 37°C for 10 min. The absorbance of the solution was measured at 593 nm. The results were expressed in mmol FeSO$_4$ equivalents per gram of ethanol extracts and ascorbic acid.

### 2.4 Anti-inflammatory activity determination of ethanol extracts

RAW 264.7 cells were maintained in RPMI 1640 medium supplemented with 10% fetal bovine serum, penicillin (100 U/mL), and streptomycin (100 U/mL). They were incubated at 37°C in a humidified atmosphere containing 5% CO$_2$. The cells were passaged by trypsinization during logarithmic phase. They ($1 \times 10^6$ cells per mL) were treated with Lipopolysaccharide (LPS, 10 µg/mL) for 4 h and then cultivated with or without ethanol extract solutions (75, 150, and 300 µg/mL) for 24 h. 50 µL of the culture supernatant was taken out to determine the level of Tumor Necrosis Factor-α (TNF-α), Interleukin-1β (IL-1β), and Interleukin-6 (IL-6) using respective enzyme-linked immunosorbent assay (ELISA) kits according to the manufacturer’s instructions.

### 2.5 Analysis of the volatile constituents by gas chromatography mass spectrometry (GC-MS)

GC-MS was performed with a gas chromatography instrument (Agilent Technologies 7890A, Agilent Technologies, Inc. Santa Clara, CA, USA) coupled to a mass spectrometer (Agilent Technologies 5975C, Agilent Technologies, Inc. Santa Clara, CA, USA).

GC/MS (electron ionization; EI) conditions: Helium was used as the carrier gas (0.9 mL/min). All analyses were performed using the following temperature ramp: the program was initiated by a column temperature set at 80°C and maintained for 1 min, increased from 80 to 240°C at the rate of 5°C/min and then kept at 240°C for 5 min. The splitless injection was conducted. The mass spectrometer was operated with EI ion source at 70 eV, and the mass range was from $m/z$ 40 to $m/z$ 400. The scan rate is 0.2 s. The transfer line temperature and ionization source temperature were 280 and 230°C, respectively. The components were identified by matching their recorded mass spectra with those of the reference compounds in the National Institute of Standards and Technology (NIST) mass spectral library, and the similarity was greater than or equal to 80%. The relative percentage of the constituents was expressed as percentages by peak area normalization.

### 2.6 Statistical analysis

The experimental data are represented as $\bar{x} + s$. A one-way analysis of variance was performed with SPSS 26.0 software. After the homogeneity of the variance test, the experimental data with uniform variance were statistically analyzed by the pairwise comparison LSD method. Besides, the data with uneven variance were analyzed by the rank sum test. Values of $P < 0.05$ were considered statistically significant. According to the results, GraphPad Prism 6.01 software was used for mapping.

### 3 Results

#### 3.1 Antioxidant activity of $V. auraria$ ethanol extracts

The methods of ABTS, DPPH, and FRAP are based on single-electron transfer reaction. In the present study, the ABTS assay values of the two ethanol extracts showed that the clearance rate of ABTS free radicals increases as the concentration increases in the range of 0.125–2.00 mg/mL (Figure 1a), and there was a positive correlation between the increase in concentration and the inhibition in the
DPPH scavenging capacity assay in the concentration range of 0.125–1.00 mg/mL (Figure 1b). The free radical scavenging activities of DPPH and ABTS were evaluated by IC₅₀ values, and the amount of Fe²⁺ equivalent per gram of ethanol extracts is also shown in Table 1. The extract of dried wasp had a better activity for clearance of ABTS free radicals and DPPH scavenging with IC₅₀ values of 748.50 ± 22.90 and 365.33 ± 19.55 μg/mL, while the values were 957.51 ± 51.46 and 478.19 ± 28.22 μg/mL for extract of fresh wasp, respectively. Fresh and dried wasp ethanol extracts showed similar total reducing capacity for FRAP, 1 g ethanol extracts of fresh and dried wasp were equivalent to the antioxidant capacity in 5.12 ± 0.07 and 5.49 ± 0.07 mmol/g Fe²⁺, respectively. The results obtained in this work showed that *V. auraria* ethanol extracts possessed antioxidant activity. Comparing the IC₅₀ values of the free radical scavenging activities of DPPH and ABTS for dried and fresh wasps, it was determined that the ethanol extract of dried wasp had a little stronger antioxidant activity than that of the fresh one. Phenolics have favorable antioxidant activity naturally. We hypothesized that the stronger antioxidant activity of dried wasp extract must be due to the phenolic contents. Further study of the phenolic content testing by using Folin–Ciocalteu method [21] supported the above speculation, the content was 27.15 mg/g for fresh wasp extract and 38.54 mg/g for dried one.

### 3.2 Effect of V. auraria extract on the expression of inflammatory mediators

In the research, the RAW 264.7 cells were treated with LPS to produce large amounts of inflammatory factors and they were tested after adding the ethanol extracts. Results found that LPS increased the amount of TNF-α, IL-1β, and IL-6 produced by macrophages and the extract from dried wasp reduced the production of TNF-α and IL-1β (Figure 2). TNF-α was considerably suppressed in a dose-dependent manner. Ethanol extracts had no influence on IL-6 expression. *V. auraria* may inhibit inflammatory responses primarily by regulating TNF-α.

### 3.3 The volatile compositions in V. auraria

As shown in Table 2, GC-MS analyses of the petroleum ether part of fresh wasp were extracted for the identification of 46 different components, representing 75.76% of the total peak areas, and 34 components were identified from the corresponding part of dried wasp that accounted for 84.70% of the total peak areas. Carboxylic acids and carboxylic esters, representing 28.37% (15) and 55.23% (21) of the total peak areas, were identified in the fresh and dried samples, respectively. The three major components are hentriacontane (7.76%), n-hexadecanoic acid (6.54%), and palmitoleic acid (4.50%) in the fresh sample, while they are benzeneacetaldehyde

### Table 1: Antioxidant activities of the V. auraria extracts (x ± s, n = 3)

| Samples       | ABTS IC₅₀ (μg/mL) | DPPH IC₅₀ (μg/mL) | FRAP Fe²⁺ (mmol/g) |
|---------------|------------------|------------------|-------------------|
| Fresh wasp    | 957.51 ± 51.46   | 478.19 ± 28.22   | 5.12 ± 0.07       |
| Dried wasp    | 748.50 ± 22.90   | 365.33 ± 19.55   | 5.49 ± 0.07       |
| Ascorbic acid | 30.00 ± 0.07     | 17.57 ± 0.02     | 20.58 ± 0.03      |
(13.11%), dodecanoic acid (7.08%), and oleic acid (6.72%) in the dried sample. It can also be seen from Figure 3 that the samples are different.

The antibacterial research was carried out on the petroleum ether and the ethanol extracts of fresh and dried V. auraria (data not shown). Inhibition effect of Staphylococcus aureus, Candida albicans, and Escherichia coli were not seen in the ethanol extracts.

4 Discussion

In general, the body’s antioxidant defense system is dynamically balanced, and the generation and removal of free radicals in the body are also in equilibrium. They may lead to chain reactions in the body and are able to cause DNA fragmentation, cell damage, and lipid peroxidation when the generation of free radicals exceeds the system’s ability to eliminate them. Modern medical research shows that the generation and development of aging, cancer, cardiovascular disease, and inflammation can be induced by free radicals and their metabolites [19]. Determination of the antioxidant properties of samples is important for the investigation of their use in various fields such as food, medicine, and cosmetics. Insects possess antioxidant compounds which aid in curbing various pathologies [22]. There are few studies of the Vespa extracts. In previous studies, only the aqueous extract of V. affinis have been researched about the antioxidant activities. It is reported that the aqueous extract of V. affinis had significant antioxidant enzyme activities and could prevent the H₂O₂-induced intracellular reactive oxygen species production in monocytes [23]. This study demonstrated the bioactivities about the antioxidant and anti-inflammatory characteristics of the V. auraria ethanol extracts for the first time. Results showed that the ethanol extract of V. auraria demonstrated a dose-dependent increase in the inhibition of ABTS.

TNF-α is an important inflammatory factor in the inflammatory response and is widely found in the blood, synovial tissue, and synovial fluid of patients with rheumatoid arthritis (RA) [24]. It is involved in a variety of pathophysiological processes in RA (including the onset of anemia) and is an important driving and maintenance factor for the development of RA. In this research, the TNF-α was considerably suppressed in a dose-dependent manner.

Pheromone usually consists of two types of compounds, one is straight-chain aliphatic alcohols, aldehydes or esters, and the other is acyclic mono-, sesqui-, and diterpenes (alcohols or acetates) [25]. The major
Table 2: Volatile composition in the petroleum ether parts of the fresh and dried *V. auraria*

| No. | Retention time (min) | Name | Formula | Molecular weight | Fresh Content (%) | Similarity (%) | Dried Content (%) | Similarity (%) |
|-----|----------------------|------|---------|------------------|------------------|----------------|------------------|----------------|
| 1   | 4.987                | 1-Tridecene | C₁₃H₂₆ | 182              | 1.68             | 95             | —                | —              |
| 2   | 6.831                | Pyrazine, tetramethyl- | C₄H₂N₂ | 136              | —                | 5.60           | 91               | —              |
| 3   | 7.564                | Benaldehyde    | C₇H₄O   | 106              | —                | 2.88           | 94               | —              |
| 4   | 9.031                | Hexadecane     | C₁₆H₃₆  | 226              | 0.58             | 95             | 0.46             | 94             |
| 5   | 9.964                | Benzeneacetaldehyde | C₆H₆O | 120              | 0.39             | 92             | 13.11            | 91             |
| 6   | 13.597               | Hexadecane, 2,6,10,14-tetramethyl- | C₂₀H₄₂  | 282              | 0.19             | 93             | —                | —              |
| 7   | 14.352               | Octadecane     | C₁₈H₃₈  | 254              | 0.47             | 98             | —                | —              |
| 8   | 15.719               | Heptadecanoic acid, ethyl ester | C₁₉H₃₈O₂ | 298              | 0.09             | 91             | —                | —              |
| 9   | 16.052               | Heptadecane, 2-methyl- | C₁₈H₃₈  | 254              | 0.14             | 91             | —                | —              |
| 10  | 16.63                | Benzyl alcohol  | C₇H₈O   | 108              | —                | 2.11           | 96               | —              |
| 11  | 17.541               | 2-Bromo dodecane | C₁₂H₂₅Br | 248              | 0.68             | 90             | —                | —              |
| 12  | 17.729               | Phenylethyl alcohol | C₈H₁₆O | 152              | —                | 2.24           | 93               | —              |
| 13  | 17.796               | Butylated hydroxytoluene | C₁₈H₃₂O | 220              | 0.33             | 98             | —                | —              |
| 14  | 18.174               | Benzeneacetaldehyde, alpha.-ethyldiene- | C₁₀H₁₀O | 146              | 0.09             | 83             | 2.00             | 94             |
| 15  | 19.262               | Octanoic acid   | C₈H₁₈O₂ | 130              | —                | 0.72           | 91               | —              |
| 16  | 20.918               | Heptadecane     | C₁₇H₃₈  | 240              | 1.05             | 91             | —                | —              |
| 17  | 22.529               | Tetradecanoic acid, ethyl ester | C₁₆H₃₂O₂ | 256              | 0.26             | 92             | 2.90             | 96             |
| 18  | 22.895               | Heptadecanoic acid, methyl ester | C₁₇H₃₄O₂ | 270              | —                | 0.25           | 99               | —              |
| 19  | 23.751               | Ethyl 9-tetradecenoate | C₁₆H₃₂O₂ | 254              | —                | 0.06           | 99               | —              |
| 20  | 24.362               | Nonadecane      | C₁₉H₃₈   | 268              | 1.86             | 95             | 0.18             | 95             |
| 21  | 26.562               | Nonanoic acid   | C₁₀H₁₈O₂ | 158              | —                | 1.49           | 90               | —              |
| 22  | 27.075               | Megastigmatrienone | C₁₃H₂₀O | 190              | —                | 0.12           | 98               | —              |
| 23  | 27.795               | Eicosane        | C₂₀H₄₂  | 282              | 1.38             | 91             | —                | —              |
| 24  | 28.228               | Hexadecanoic acid, methyl ester | C₁₇H₃₄O₂ | 270              | —                | 0.25           | 99               | —              |
| 25  | 28.884               | Cyclohexane, (1-octynyl)- | C₂₃H₄₆ | 322              | 0.10             | 91             | —                | —              |
| 26  | 29.55                | Hexadecanoic acid, ethyl ester | C₁₈H₃₆O₂ | 284              | 0.94             | 93             | 4.01             | 97             |
| 27  | 30.172               | n-Decanoic acid | C₁₀H₂₀O₂ | 172              | —                | 1.02           | 98               | —              |
| 28  | 30.339               | E-11-Hexadecenoic acid, ethyl ester | C₁₈H₃₆O₂ | 282              | 0.83             | 98             | 2.49             | 99             |
| 29  | 31.094               | Heneicosane, 11-decyl- | C₂₀H₄₂ | 437              | —                | 0.16           | 94               | —              |
| 30  | 31.205               | Heneicosane     | C₂₁H₄₄  | 296              | 1.90             | 91             | —                | —              |
| 31  | 31.572               | 1-Docosene      | C₂₂H₄₄  | 308              | 0.37             | 98             | —                | —              |
| 32  | 32.383               | Octadecanal     | C₁₈H₃₆O | 268              | —                | 0.17           | 95               | —              |
| 33  | 32.416               | Triacylacetate  | C₁₂H₂₄O₂ | 480              | 0.25             | 95             | —                | —              |
| 34  | 32.872               | Diethylphthalate | C₁₂H₁₄O₂ | 222              | —                | 0.37           | 98               | —              |
| 35  | 34.516               | Nonadecane, 9-methyl-indole | C₁₀H₃₈N | 117              | 0.34             | 76             | 0.22             | 95             |
| 36  | 34.916               | Triacetylacetate | C₁₂H₂₄O₂ | 480              | 0.24             | 95             | —                | —              |
| 37  | 35.838               | Triacetylacetate | C₁₂H₂₄O₂ | 480              | 0.24             | 95             | —                | —              |
| 38  | 36.127               | Octadecane, 2,6,10,14-tetramethyl- | C₂₂H₄₆ | 310              | 0.36             | 94             | —                | —              |
| 39  | 36.305               | Octadecanoic acid, ethyl ester | C₂₀H₃₆O₂ | 312              | —                | 0.24           | 99               | —              |
| 40  | 36.86                | Ethyl oleate    | C₂₀H₃₆O₂ | 310              | 2.40             | 99             | 5.75             | 99             |
| 41  | 37.127               | Dodecanoic acid | C₁₂H₂₄O₂ | 200              | 0.98             | 91             | 7.08             | 99             |
| 42  | 37.749               | Pentacosane      | C₁₅H₃₂  | 352              | 3.51             | 98             | 0.21             | 96             |
| 43  | 39.804               | Tetracosane      | C₁₄H₃₀  | 338              | 1.15             | 99             | —                | —              |
| 44  | 40.36                | 9,12,15-Octadecatrienoic acid, ethyl ester, (Z,Z,Z)- | C₂₀H₃₆O₂ | 306              | 1.52             | 94             | 1.44             | 99             |
| 45  | 40.86                | Heptadecane, 9-octyl- | C₁₉H₃₂  | 352              | 2.76             | 90             | —                | —              |
constituents of petroleum ether part were dominated by aliphatic hydrocarbons, carboxylic acids, and esters in fresh wasp, and they were the possible components of pheromones, while for dried ones, aliphatic hydrocarbons were less both in quantity and content. 23 aliphatic hydrocarbons, that is, 40.30% of the total peak areas were identified in fresh wasp. These hydrocarbons were mainly from fresh wasp, while for dried ones, aliphatic hydrocarbons were less both in quantity and content. The content of aliphatic hydrocarbons in fresh wasp was 40.30% of the total peak areas, while for dried ones, it was 23. The content of aliphatic hydrocarbons in fresh wasp was 75.76%, while for dried ones, it was 84.70%.

Table 2: Continued

| No. | Retention time (min) | Name                                      | Formula               | Molecular weight | Fresh Content (%) | Similarity (%) | Dried Content (%) | Similarity (%) |
|-----|----------------------|-------------------------------------------|-----------------------|------------------|-------------------|----------------|------------------|----------------|
| 46  | 41.471               | Triacontane, 1-bromo-                      | C_{30}H_{61}Br        | 500              | 0.79              | 92             | —                | —              |
| 47  | 43.104               | Dibutyl phthalate                          | C_{16}H_{22}O_{4}     | 278              | 0.42              | 93             | 0.79             | 93             |
| 48  | 43.659               | Tetradecanoic acid                         | C_{14}H_{28}O_{2}     | 228              | 3.66              | 99             | 5.51             | 99             |
| 49  | 43.904               | Hexacosane                                 | C_{26}H_{54}          | 366              | 2.41              | 91             | —                | —              |
| 50  | 44.426               | 1-Hexacosene                               | C_{26}H_{52}          | 364              | 0.92              | 94             | —                | —              |
| 51  | 44.981               | Z-7-Tetradecenoic acid                     | C_{14}H_{26}O_{2}     | 226              | —                 | —              | 0.80             | 98             |
| 52  | 46.815               | Octacosane                                 | C_{28}H_{58}          | 394              | 4.37              | 99             | —                | —              |
| 53  | 47.359               | Nonacosane                                 | C_{29}H_{60}          | 408              | 1.32              | 96             | —                | —              |
| 54  | 48.781               | 2,6,10,14-Tetramethyl-7-(3-methylpent-4-enylidene) pentadecane | C_{25}H_{48}          | 348              | 0.71              | 90             | —                | —              |
| 55  | 49.748               | n-Hexadecanoic acid                        | C_{16}H_{32}O_{2}     | 256              | 6.54              | 98             | 5.51             | 99             |
| 56  | 50.703               | Palmitoleic acid                           | C_{16}H_{30}O_{2}     | 254              | 4.50              | 99             | 3.74             | 99             |
| 57  | 52.358               | Triacontane                                | C_{30}H_{62}          | 422              | 3.41              | 92             | —                | —              |
| 58  | 55.002               | Hentriacontane                             | C_{31}H_{64}          | 437              | 7.76              | 94             | —                | —              |
| 59  | 56.191               | Oleic acid                                 | C_{18}H_{32}O_{2}     | 282              | 2.66              | 99             | 6.72             | 99             |
| 60  | 57.558               | 9,12-Octodecadienoic acid (Z,Z)-           | C_{18}H_{32}O_{2}     | 280              | 3.59              | 99             | —                | —              |
| 61  | 59.346               | 9,12,15-Octadecatrienoic acid, (Z,Z,Z)-    | C_{18}H_{30}O_{2}     | 278              | 1.39              | 99             | 1.89             | 99             |
| 62  | 60.057               | Octadecane, 1-iodo-                        | C_{18}H_{37}I         | 380              | 2.57              | 93             | —                | —              |
|     | Total content        |                                           |                       |                  | 75.76             | 84.70          |                  |                |

Figure 3: Total ion current of volatile composition analyzed on *V. auraria*. (a) The petroleum ether part of the fresh *V. auraria*; (b) The petroleum ether part of the dried *V. auraria*. 
were identified in the fresh sample, but just 4 alkanes were identified in the dried one. Phenylethyl alcohol is a very common semiochemical that is found in a broad range of insect species, and it usually as the aggregation, sex, alarm pheromone to use. [26] Phenylethyl alcohol was 2.24% in the dried sample. Fewer components have been identified in dried wasps than fresh samples, and fresh samples contain large amounts of aliphatic hydrocarbons, while the main components in dried samples are carboxylic acids and esters. This indicates that *V. auraria* is rich in aliphatic hydrocarbons, and most of the volatile hydrocarbons were lost in the drying process.

5 Conclusion

Insects are used for the treatment of different types of diseases all over the world. This study reported the bioactivities about antioxidant and anti-inflammatory characteristics of *V. auraria* ethanol extracts for the first time. The ethanol extract from dried wasp had higher antioxidant activity than the fresh ones. Meanwhile, the results of anti-inflammatory activity demonstrated that the ethanol extract of dried wasp had considerable inhibitory effect on TNF-α in a dose-dependent manner in the macrophages and it also had a significant inhibitory effect on IL-1β. The volatile constituents were very different in fresh and dried ones. The straight-chain aliphatic alcohols, aldehydes, or esters may be the possible components of wasp pheromones. In order to have a clear picture, further investigations are required to identify the active principles present and more possible pheromones in *V. auraria* and it is currently in progress.

Funding information: This work was supported by The National Natural Science Foundation of China (No. 82160822 and 82060765); The Special Program of Science and Technology of Yunnan Province (202002AA100007); Medicine Pieces Industry Development Special Fund of Yunnan Province [Grant number 2019-YG-067]; The Natural Science Foundation of Yunnan Province [Grant number 2017FA050]; Key Science and Technology Support Project of Dali [Grant number D2019NA01].

Author contributions: Huai Xiao and Xiu-Mei Wu conceived and designed the experiments; Si-Tong Zhou, Lian-Li Ni, Shi-Meng Yuan, Zhi-Bin Yang, and Yue-Hua Li performed the experiments; Si-Tong Zhou and Xiu-Mei Wu analyzed the data; Qi Wang, Si-Tong Zhou, Xiu-Qin Pang, and Huai Xiao wrote the manuscript; and Huai Xiao polished it. Huai Xiao and Zhi-Bin Yang acquired funding for the research. All authors reviewed and approved the final version.

Conflict of interest: The authors report no conflicts of interest.

Ethical approval: The conducted research is not related to either human or animal use.

Data availability statement: All the data of this manuscript are available from the authors.

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