

## Short Communication

# GC-MS analysis of volatile oils from *Bupleurum chinense* DC. f. *vanheurckii* (Muell.-Arg.) Shan et Y.Li

LIU Ze-kun<sup>1</sup> and Chen-Haixia<sup>2\*</sup>

<sup>1</sup>Marine College, Shandong University at Weihai, Weihai, Shandong 264209, China.

<sup>2</sup>Department of Pharmacy, Qilu Hospital of Shandong University, Jinan, Shandong 250012, China.

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The aim of this study was to analyze the volatile oil from *Bupleurum chinense* DC. f. *vanheurckii* (Muell.-Arg.) Shan et Y.Li. The volatile oil was extracted from *B. chinense* DC. f. *vanheurckii* (Muell.-Arg.) Shan et Y.Li by steam distillation, its components was analyzed and identified by gas chromatography-mass spectrometry (GC-MS) method. 27 compounds, which represent 93.3% of total oil, were identified. Predominant constituents over 5% were 4,7,10,13,16,19-Docosahexaenoic acid, methylester, (all-Z) - (19.1%); Germacrene D (14.8%); Retinyl acetate(7.3%); Cycloisolongifolene,8,9-dehydro-9-formyl-(6.9%);  $\alpha$ -Cadinol(5.6%); and  $\tau$ -Muurolo (5.3%).

**Key words:** *Bupleurum chinense* DC. f. *vanheurckii* (Muell.-Arg.) Shan et Y.Li, volatile oil, gas chromatography-mass spectrometry (GC-MS) analysis.

## INTRODUCTION

*Bupleurum chinense* DC. f. *vanheurckii* (Muell.-Arg.) Shan et Y.Li is an herb of 20 to 30 cm height and belongs to the Apiaceae family. It is a variety of *B. chinense* DC. but rich in volatile oil. There are 40 species and 17 varieties of *Bupleurum* in China and the majority of them are produced in Liaoning, Gansu, Hebei, Henan, Shandong, Hubei, Jiangsu, Sichuan, Anhui and other provinces (Sun and Zhu, 1994). *B. chinense* DC. f. *vanheurckii* (Muell.-Arg.) Shan et Y.Li differs from *B. chinense* DC. Its original species by its 4 to 5 ovate-lanceolated green small bracts with white edge, slightly longer than umbel and the half of the fruit umbrella (Li and Shan 1974). In Chinese medicine, the plant is used as traditional drugs. The main active ingredients of *Bupleurum* are saponins and volatile oil. Up to now, there are no studies of its volatile oil. Therefore, the relationship between effective ingredients and the efficacy of *B. chinense* DC. f. *vanheurckii* (Muell.-Arg.) Shan et Y.Li should be discussed, so as to find out its clinical range and using standard and to apply it into clinical practice. Based on this, the volatile oils were extracted from the plant by steam distillation. Gas

chromatography-mass spectrometry (GC-MS) method was used to analyze and identify the components, in order to lay foundations for the further research and development of their medicinal value and for the expansion of clinical range.

## MATERIALS AND METHODS

### Research material

*Bupleurum chinense* DC. f. *vanheurckii* (Muell.-Arg.) Shan et Y.Li were collected in Kunyu Mountain, Yantai City on August 25, 2010, and were identified by professor Zhang Qinde in Shandong College of Traditional Chinese Medicine. The plants were cut into decoction pieces of 1 cm and were dried under the sun.

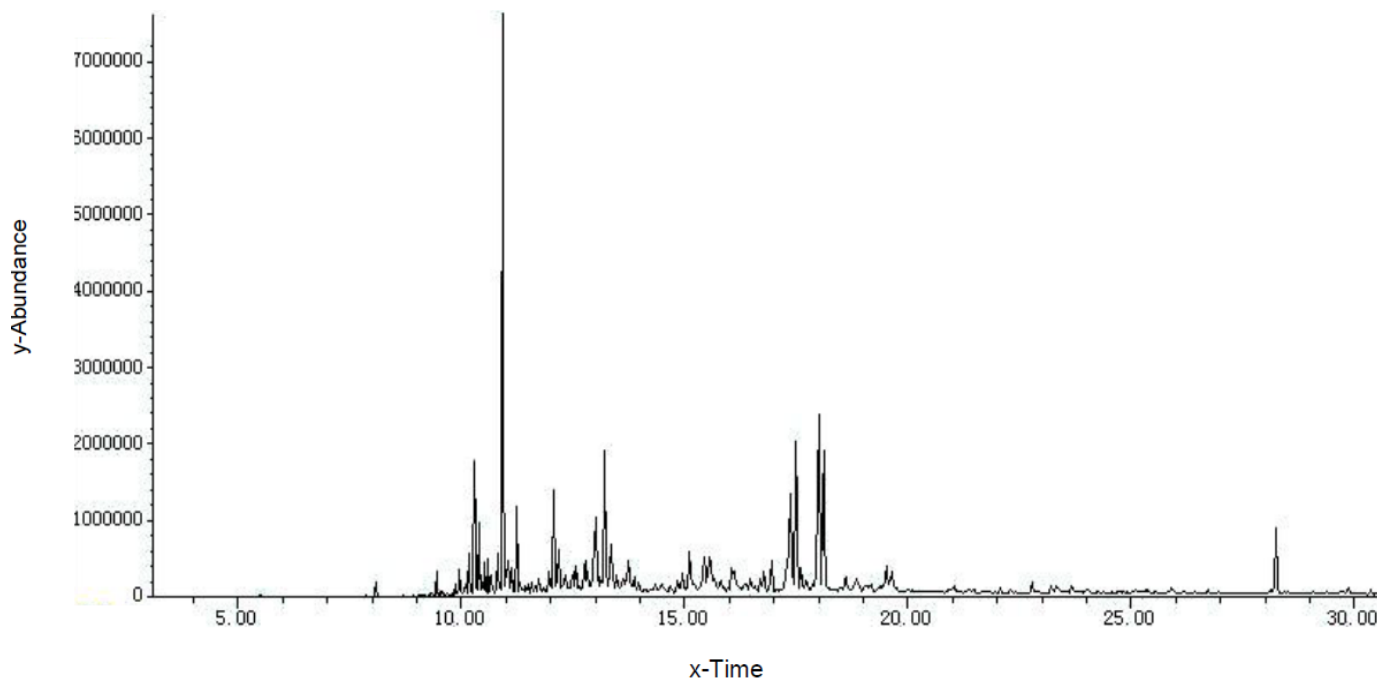
### Main reagents

Both anhydrous ether and anhydrous sodium sulfate were analytically pure sold in the market.

### Main instruments

7890 to 5975 gas chromatography-mass spectrometry (GC-MS) is from Agilent Technologies and other instruments were conical flask, volatile oil extractor, electric heating jacket, injector, and 0.45 m

\*Corresponding author. E-mail: 423603242@qq.com.



**Figure 1.** GC-MS total ion chromatogram of volatile oil from *Bupleurum chinense* DC. f. *vanheurckii* (Muell.-Arg.) Shan et Y.Li.

micro porous membrane.

## Procedure

### Extraction of volatile oil

The method of steam distillation in appendix U of *Chinese Pharmacopoeia* was adopted for the extraction of volatile oil (*Chinese Pharmacopoeia*, 2000). 100 g decoction pieces of *B. chinense* DC. f. *vanheurckii* (Muell.-Arg.) Shan et Y.Li were grinded, sieved through 40 meshes, and then put into a steam distillation vessel. After heating reflux for 8 h and collecting the distilled fluid, NaCl was added until it reached saturation. Then ethyl ether was used for the extraction for three times. The extracts were dried by anhydrous sodium sulfate. And volatile oil was obtained by rotary evaporator after removing the ethyl ether. The volatile oil was sealed and stored in the refrigerator. Samples were sent to the Shandong Institute for Drug Control to carry out GC-MS analysis.

### Chromatographic conditions for GC-MS analysis

GC conditions were as follows: HP-5MS 5% Phenyl Methyl Siloxane fused silica capillary column (30 m × 0.25 mm × 0.25 μm), injector temperature at 250°C, helium as the carrier gas, 72 kPa column pressure, 1 μl injection volume (n-hexane solution), and 1.2 ml/min flow rate; the temperature programming started at 60°C for 2 min, then increased to 180°C for 5 min at a rate of 15°C/min, and finally reached 250°C for 3 min at a rate of 5°C/min. The MS conditions were as follows: EI, ion source temperature at 230°C, 70 eV electron energy, interface temperature at 270°C, solvent delay for 3 min, and mass scan range within 60 to 600 amu. NIST mass spectral database was used and compared with the standard spectrum, in order to identify the components peaks. According to the peak area normalization method, relative mass fraction of each component in volatile oil was calculated.

## RESULTS

Total ion chromatogram of volatile oil components was obtained (Figure 1) based on the GC-MS analysis of volatile oil from *B. chinense* DC. f. *vanheurckii* (Muell.-Arg.) (Li and Shan, 1974). A total of 27 compounds, which represent 93.3% of total oil, were identified by computer retrieval of NIST05 mass spectral database and manual spectrogram analysis. Table 1 reported that the main components of volatile oils from the *B. chinense* DC. f. *vanheurckii* (Muell.-Arg.) (Li and Shan, 1974) were terpenoids, low-molecular aliphatic compounds, and a minority of alcohol compounds. According to the method of area normalization, predominant constituents over 5% were 4,7,10,13,16,19-Docosahexaenoic acid, methyl ester, (all-Z) - (19.1%); Germacrene D (14.8%); Retinyl acetate (7.3%); Cycloisolongifolene, 8,9-dehydro-9-formyl - (6.9%); α-Cadinol (5.6%); and τ-Muurolo (5.3%).

## DISCUSSION

A total of 27 compounds were identified, which were mainly terpenoids, low-molecular aliphatic compounds and alcohol compounds. It is reported that eight kinds of chemical compounds including τ-Muurolo, β-Caryophyllene, Aromadendrene, citronellyl acetate, Elemene, Guaiene and elemol are significantly related to the inhibition of *Staphylococcus aureus* and some other microbe, in which elemol is negatively correlated with the inhibition (Chen et al., 2010) and in this work,

**Table 1.** GC-MS analysis results of volatile oil from *Bupleurum chinense* DC. f. *vanheurckii* (Muell.-Arg.) Shan et Y.Li.

S/N	Compound	Retention time	Content (%)
1	Elixene	9.439	0.598
2	$\alpha$ -Elemene	9.964	0.607
3	Bicyclo[4.1.0]heptane,7-bicyclo[4.1.0]hept-7-ylidene-	10.166	1.088
4	$\alpha$ -Cubebene	10.281	4.881
5	$\beta$ -Caryophyllene	10.314	1.262
6	Cyclosativene	10.535	0.802
7	$\tau$ -Cadinene	10.613	0.824
8	$\tau$ -Muurolene	10.810	1.844
9	Germacrene D	10.927	14.843
10	$\alpha$ -Farnesene	10.983	0.910
11	$\zeta$ -Elemene	11.065	1.937
12	$\epsilon$ -Cadinene	11.252	2.622
13	Isoaromadendrene epoxide	11.937	0.757
14	Ent-Spathulenol	12.072	3.451
15	Caryophyllene oxide	12.580	2.681
16	$\beta$ -Farnesol	12.777	1.305
17	$\tau$ -Muurolo	13.009	5.264
18	$\alpha$ -Cadinol	13.217	5.609
19	4-(2,2-Dimethyl-6-methylenecyclohexylidene)-3-methylbutan-2-one	13.351	2.734
20	Calarene epoxide	13.753	1.519
21	Aromadendrene oxide-(1)	15.115	2.093
22	Murolan-3,9(11)-diene-10-peroxy	15.576	3.618
23	Spiro[tricyclo[4.4.0.0(5,9)]decane-10,2'-oxirane],1-methyl-4-isopropyl-7,8-dihydroxy-	16.953	1.566
24	Retinyl acetate	17.375	7.330
25	4,7,10,13,16,19- Docosahexaenoic acid,methyl ester, (all-Z)-	17.508	19.1
26	Cycloisolongifolene, 8,9-dehydro-9-formyl-	18.118	6.913
27	Falcarinol	19.520	0.856

Germacrene D,  $\tau$ -Muurolo, Elemene and  $\beta$ -Caryophyllene are discovered in the volatile oil and they are totally more than 20%. Also, some of the other compounds have antibiotic effect. This fact maybe a good explanation of Chinese folk's using *B. chinense* DC. f. *vanheurckii* (Muell.-Arg.) Shan et Y.Li as traditional medicine. However, the specific effect is not thoroughly cleared and further discussion is still needed on whether the volatile oil is the main active components on some efficacies such as dizziness and headache alleviation, blood activation, fever bring down, dampness and wind elimination. This research provided experimental basis for the further research on the development and utilization of physiological activity and medicinal resources of *B. chinense* DC. f. *vanheurckii* (Muell.-Arg.) Shan et Y.Li.

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