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Analysis of Volatile Components and Antibacterial Activity of Silver Wormwood Essential Oils from Different Habitats by E-Nose Combined with GC-MS

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Abstract: Electronic nose (E-nose) combined with gas chromatography–mass spectrometry (GC-MS) was used to analyze the volatile components of silver wormwood from different habitats, and the antibacterial activity of essential oils was also studied, to provide a scientific basis for quality control of silver wormwood and rational utilization of their essential oils. In this study, the total content of essential oils in silver wormwood was determined by steam distillation; the volatile components were conducted in an overall analysis by E-nose combined with chemometrics; the volatile components were analyzed and identified by GC-MS; and two G-negative bacteria and one Gram-positive bacteria were used as test bacteria to determine the antibacterial activity of the essential oils from silver wormwood. The results showed that principal component analysis (PCA) and linear discriminant analysis (LDA) of E-nose could distinguish the essential oils of silver wormwood from different habitats, and the odor difference of essential oils was obvious. A total of 87 volatile components were identified by GC-MS, and there were significant differences in components and contents in silver wormwood from different habitats; PCA and hierarchical cluster analysis (HCA) could effectively distinguish silver wormwood from different habitats. The essential oils from silver wormwood from different habitats all had a certain inhibitory effect on *Bacillus subtilis*, *Staphylococcus aureus*, and *Escherichia coli*. Therefore, the combination of E-nose and GC-MS could quickly distinguish silver wormwood from different habitats and provide a reference for quality control, drug selection, and comprehensive utilization of silver wormwood.

Keywords: silver wormwood; essential oil; E-nose; GC-MS; antibacterial activity; habitat identification

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1. Introduction

Silver wormwood is the dry leaf of *Artemisia argyi Lévl*. et Vant and contains flavonoids, essential oils, polysaccharides, and other active ingredients [1,2]. Essential oil is the main active component of silver wormwood and mainly contains monoterpene and its derivatives, sesquiterpene and its derivatives, ketones (aldehydes), alcohols (phenols), acids (esters), alkanes (alkenes), and other chemical components [3]. Silver wormwood is bitter and pungent in flavor and warm in nature and has the functions of warming meridians, stopping bleeding, dispelling cold, and relieving pain [4]. Silver wormwood has pharmacological effects such as antioxidant, antibacterial, antivirus, antitumor, and immune regulation [5–7]. Silver wormwood has a high medicinal value and abundant resources and is mainly produced in Henan province and Hubei province. Silver wormwood is widely used. In addition to being used as medicinal materials, it can also be made into practical articles such as moxa wool, moxa-wool moxibustion, and inkpad. In addition, it is also closely related to food and can be used to make wormwood leaves bread, fresh

wormwood leaves, wormwood oxalic acid, wheat fermented by wormwood leaves, and other foods [8]. Therefore, the development and utilization of silver wormwood have attracted the attention of researchers, and considering how to control the quality of silver wormwood has become an important prerequisite for medicinal safety and food safety.

As the main active component of silver wormwood, the essential oil has a strong special odor and is regarded as the standard to evaluate the medicinal quality of silver wormwood, and its content can directly reflect the quality of silver wormwood [9,10]. Due to different planting conditions and methods and harvesting methods in different habitats, the volatile components of silver wormwood from different habitats are quite different. Most researchers only focus on the analysis of the content differences of the essential oils in silver wormwood from different habitats, leading to the inability to scientifically and comprehensively reflect the quality differences of silver wormwood from different habitats.

In this study, electronic nose (E-nose) combined with gas chromatography–mass spectrometry (GC-MS) was used to analyze the essential oils in silver wormwood from different habitats. Odor, an important sensory indicator of essential oil in traditional Chinese medicine, is directly related to the quality and efficacy of traditional Chinese medicine [11,12]. For traditional Chinese medicine that contains essential oils, the differences in the types and contents of volatile components lead to differences in the odors of different traditional Chinese medicine. At present, the evaluation of the quality of traditional Chinese medicine based on odor and other traits mainly uses sensory evaluation, which has various limitations, such as strong subjectivity [13]. E-nose is a simulated biological olfactory system that can digitize, model, and visualize subjective feelings, quickly analyze and distinguish the odor of complex samples, and has the advantages of simple operation, quick detection, and high sensitivity [14,15]. It can detect the overall information of samples and form odor fingerprints and has been widely applied in many fields such as food, medicine, environment, and agriculture [16–18]. In terms of antibacterial activity, essential oil of silver wormwood has certain inhibition activity on *Escherichia coli* and *Staphylococcus aureus* and the antibacterial activity of silver wormwood essential oil is also different in different places [19]. In this paper, two G-negative bacteria and one Gram-positive bacteria were used as test bacteria to determine the antibacterial activity of the essential oils from silver wormwood.

This study used E-nose to detect the odor of essential oil samples of silver wormwood from different habitats, analyzed the volatile components in silver wormwood from different habitats by GC-MS combined with chemometrics, and researched the antibacterial activity of essential oils in silver wormwood from different habitats, thus providing a reference for quality control, variety breeding, and comprehensive development of silver wormwood from different habitats.

2. Materials and Methods

2.1. Instruments and Reagents

A 7890A/5975C gas chromatography–mass spectrometer (Agilent Technology Co., Ltd., Beijing, China); SuperNose Electronic Nose (Shanghai Ruixuan International Trade Co., Ltd., Shanghai, China); BT25S Electronic Analytical Balance (Beijing Sai Dolis Instrument Co., Ltd., Beijing, China); essential oil extractor (Sichuan Shuniu Glass Instrument Co., Ltd., Chongzhou, China); 80KCS vertical steam sterilization pot (Shanghai Shen'an Medical Device Factory, Shanghai, China); and Model SPX-150F Biochemical Incubator (Shanghai Yue Long Instrument and Equipment Co., Ltd., Shanghai, China) were used.

Reagents such as *n*-hexane and anhydrous sodium sulphate were analytical reagents; C7–C32 *n*-alkanes (Chengdu Croma Co., Ltd., Chengdu, China); *Staphylococcus aureus* (ATCC 25923), *Escherichia coli* (ATCC 25922), and *Bacillus subtilis* (ATCC 6633) were all ordered from American TypeCulture Collection (ATCC).

2.2. Materials

The seven habitats of silver wormwood are shown in Table 1. The materials were all identified as *Artemisia argyi Levl.* et Vant by Professor Yang Ming of the Jiangxi University of Chinese Medicine.

Table 1. Silver wormwood habitat information.

No.	Sample Name	Habitat
1	HA	Jingxi Village, Quzhou, Zhejiang Province, China
2	PA	Tuanzhang Village, Poyang, Jiangxi Province, China
3	QA	Qizhou Town, Qichun, Hubei Province, China
4	CZA	Guichi District, Chizhou, Anhui Province, China
5	SYA	Longhui Village, Shaoyang, Hunan Province, China
6	BGA	Dacheng Village, Poyang, Jiangxi Province, China
7	LJA	Linjiang Town, Kaixian, Chongqing Municipality, China

2.3. Isolation of Essential Oil

Steam distillation is usually used to extract essential oil [20]. A total of 200 g of fresh silver wormwood leaves were weighed, cut into pieces, placed in a round-bottomed flask, added with 2000 mL double-distilled water, shaken and mixed, and soaked for 1 h. Then, essential oils were isolated for 3.5 h according to the A determination method of essential oils in the four general rules of Chinese Pharmacopoeia (2020 edition), stood, and layered. After that, their volumes were read and their yields were calculated; then, they were added to anhydrous sodium sulphate, dried, put in a brown glass bottle, and sealed at 4 °C for later use. The parallel experiment was repeated three times, and the average was taken.

2.4. Odor Analysis by E-Nose

The analysis method of E-nose comes from the research of other students in our research group [9]. A 50 μL sample was sucked into a 40 mL autosampler, sealed, and left for 1 h before the headspace vial was filled with sample gas for autosampler analysis. The sampling parameters of the electronic nose are set as follows: pure air was used as a carrier; carrier air flow rate was 0.6 L/min; cleaning time was 180 s; and detection time was 120 s. The sample amount was 10 μL, and each sample was measured three times in parallel.

2.5. GC-MS Analysis

The essential oils in silver wormwood were qualitatively and quantitatively analyzed by GC-MS. A total of 50 μL volatile oil was precisely sucked from silver wormwood, dissolved in *n*-hexane and constant-volumed into a 10 mL volumetric flask, filtrated with a 0.22 μm microporous filter membrane to obtain a volatile oil sample solution.

Gas chromatographic conditions: HP-5 MS quartz capillary column (30 m × 0.25 mm × 0.25 μm). The programmed temperature was adopted: the initial temperature was 40 °C, kept for 1 min, then running at 70 °C, then heating at 10 °C/min to 220 °C, kept for 0 min, and then heating at 25 °C/min to 280 °C, and kept at 280 °C for 9 min. The carrier gas was high-purity helium with a flow rate of 1.0 mL/min. The inlet temperature was 280 °C. The split ratio was 40:1. Solvent was delayed for 3 min. The sample amount was 1.0 μL.

Mass spectrometry conditions: quadrupole temperature 150 °C. The ionization source was standard EI source, and the ion source temperature was 230 °C. Electron multiplier tube voltage was 2447.06 V. The mass scanning range m/z was 50~650.

2.6. Antibacterial Activity Determination

The antibacterial effects on *Bacillus subtilis*, *Staphylococcus aureus*, and *Escherichia coli* of the essential oils from silver wormwood were determined by the measurement of inhibition zones [21]. A total of 200 μL of various bacteria suspensions were sucked by a pipette on a clean worktable and uniformly coated on the surface of the plate culture medium to make a plate containing bacteria. A 6 mm round filter paper was clamped with sterile

tweezers, and 10 µL essential oil was sucked by a pipette gun and added to the filter paper. The dripping side faced down and was attached to a flat plate with three filter papers and stood upright for 20 min. Sterile water was used as a negative control and gentamicin drug-sensitive paper (10 µg/plate) was used as positive control. All the plates were put upside down in a constant temperature incubator at 37 °C for 24 h. The diameters of the inhibition zones were measured by the cross method, and the average was taken after three parallel experiments.

2.7. Data Analysis

The electronic nose used its software to perform principal component analysis (PCA) and linear discriminant analysis (LAD) on the data. The relative content of components was determined by the peak area normalization method. By searching the standard mass spectrometry library of the NIST20 chemical workstation and the comparison of their GC Kováts retention indices (RI) and referring to relevant literature, the chemical structure of components was identified.

3. Results and Discussion

3.1. Color and Content of Essential Oil

The silver wormwood essential oils from different habitats were isolated by steam distillation. The color and content of the essential oils are shown in Table 2. The results showed that the color and content of silver wormwood essential oils from different habitats were quite different. The content of essential oils from seven different habitats ranged from 0.30% to 1.09%. The content of essential oil in QA samples was the highest (1.09%), which was significantly higher than that in other samples, and the content of essential oil in BGA samples was the lowest (0.30%). Among the essential oil samples, the essential oil color of HA samples was dark blue, which was easy to distinguish; LJA samples were grass green, BGA samples were yellow, essential oils from other habitats showed different degrees of blue–green, and SYA samples were the lightest. The blue color of essential oil in silver wormwood was mainly related to the chemical components of azulene and davanone with unique color in the samples [22].

Table 2. Content and color of silver wormwood essential oils from different habitats.

Sample Name	Color	Yields (%)
HA	Dark blue	0.60 ± 0.03
PA	Blue–green	0.57 ± 0.04
QA	Blue–green	1.09 ± 0.02
CZA	Blue–green	0.69 ± 0.07
SYA	Light blue–green	0.74 ± 0.06
BGA	Yellow	0.30 ± 0.00
LJA	Grass green	0.33 ± 0.03

3.2. Odor Analysis by E-Nose

3.2.1. Radar Chart of Odor Information

E-noses are very sensitive to odor information from a sample, and slight changes in volatile compounds may cause differences in sensor response. Therefore, E-noses are widely used in analysis fields such as food, beverages, cosmetics, medicine, and agriculture. In this study, an E-nose equipped with 14 sensors was used to detect and analyze the comprehensive odor components of silver wormwood essential oil samples from different origins. The sensors of the E-noses are equivalent to the olfactory cells of the human nose. The sensors respond to the odor components by the sample, and each sensor has a different response focus. The difference in sensor response not only depends on the different odor components but also depends on the concentration of the odor components [23].

The average response value of each sensor of the E-noses was selected to draw the radar map of the odor information of the E-noses of silver wormwood samples, as shown

in Figure 1. The response values of 14 sensors to volatile components of different samples were different, and the response values of S1 and S8 sensors to the odor of samples were larger. According to the performance of the sensor, it is sensitive to terpenes, alcohols, and ketones. So, it is speculated that the differences in odor components in silver wormwood from different habitats are mainly affected by terpenes, alcohols, and ketones.

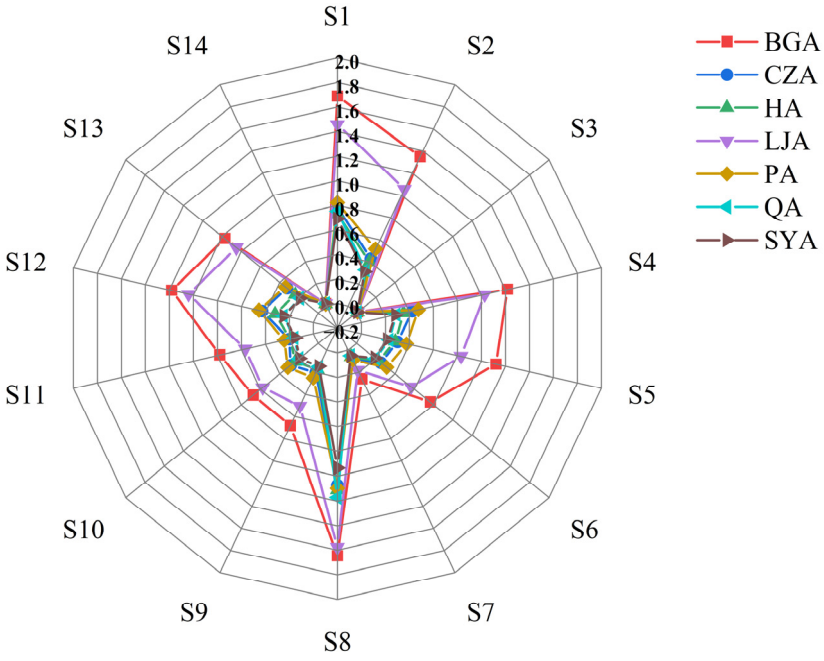

Figure 1. Radar map of silver wormwood essential oils from different habitats.

3.2.2. Principal Component Analysis (PCA)

PCA is an unsupervised statistical method that generates a new set of variables, called principal components, each of which is a linear combination of defined original variables [24]. Siger studied 418 vegetable oil samples through PCA and HCA, showing the usefulness of statistical tools [25]. Wei used electronic nose combined with PCA and linear discriminant analysis to measure peony seed oil doping [26].

PCA analysis of E-nose detection data of silver wormwood samples from different habitats is shown in Figure 2. The contribution rates of PC1 and PC2 were 99.10% and 0.59%, respectively, and the cumulative variance contribution rate was 99.69%, which could represent all the information of samples. The recognition index (DI value) was 96.83%, and the discrimination between different data was obvious, which indicates that there are apparent differences in the odor of silver wormwood samples from different habitats and that the electronic nose can distinguish silver wormwood samples from different habitats well.

3.2.3. Linear Discriminant Analysis (LDA)

Different from the PCA variance maximization theory, the idea of the LDA algorithm is to project the data into a low-dimensional space to make the same type of data as compact as possible and different types of data as dispersed as possible. Therefore, the LDA is a supervised pattern-recognition learning algorithm [27].

It can be seen from Figure 3 that the DI value was 99.91% and each sample was distributed in different areas without overlapping each other, indicating that the E-noses can effectively distinguish silver wormwood from different habitats. The model had a good distinguishing effect, and the trend and results obtained were the same as those obtained by PCA, which further verified the results of PCA.

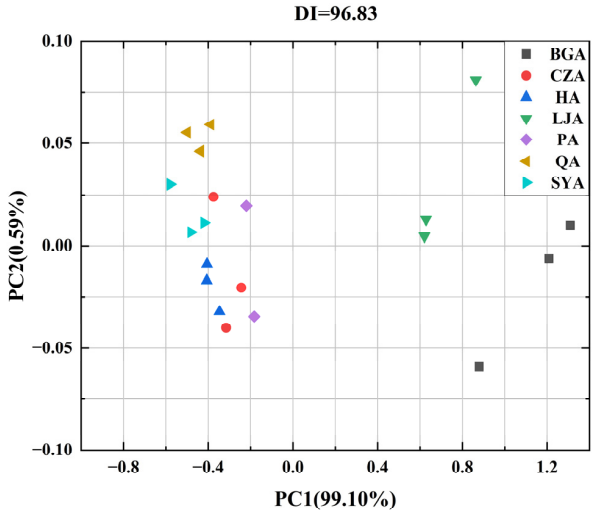

Figure 2. PCA of E-noses of silver wormwood from different habitats.

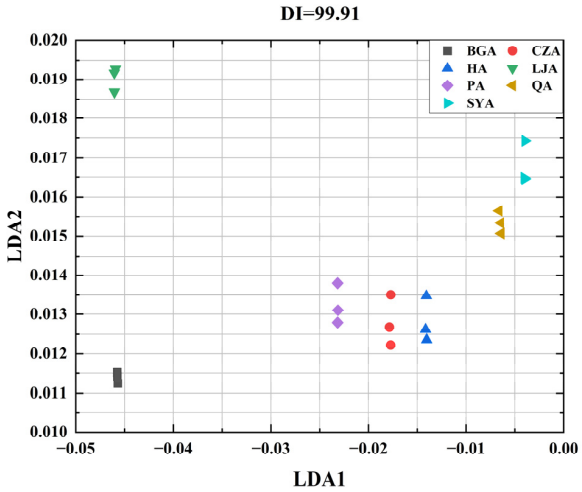

Figure 3. LDA of E-noses of silver wormwood from different habitats.

3.3. GC-MS Analysis

3.3.1. GC-MS Analysis of Volatile Components

According to the above GC-MS conditions, the volatile components in silver wormwood samples from different habitats were analyzed, and the total ion flow diagram of essential oils in silver wormwood from different habitats was obtained, as shown in Figure 4.

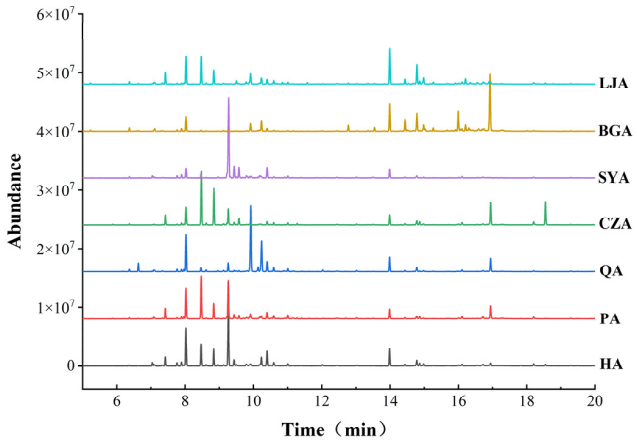

Figure 4. Total ion chromatogram of volatile components in silver wormwood from different habitats.

According to the obtained mass spectrometry data, the chromatographic peaks in the total ion flow diagram were analyzed, and the relative contents of each component were calculated by peak area normalization method after NIST20 mass spectrometry library retrieval and artificial auxiliary analysis, as shown in Table 3.

Table 3. Volatile components of silver wormwood essential oils from different habitats.

No.	Compounds	RI from Experiment	RI from Literature	Relative Content (%)						
				HA	PA	CZA	QA	SYA	BGA	LJA
1	1-Hexanol	892	-	- *	-	-	-	-	0.48	0.53
2	Santolina triene	936	-	-	-	0.23	-	-	-	-
3	α-Tricyclene	953	-	-	-	-	0.14	-	-	-
4	α-Pinene	965	977	0.30	0.38	0.40	0.81	0.56	1.16	0.95
5	Camphene	980	988	-	-	-	3.13	-	-	-
6	α-Sabinene	1002	1013	1.17	-	-	-	0.94	-	0.23
7	3-Octenol	1006	1019	0.79	0.89	0.32	0.93	0.67	-	-
8	β-Pinene	1007	1018	-	-	-	-	-	1.22	1.23
9	Dehydrocineole	1022	1041	0.39	-	-	0.34	-	-	0.67
10	Yamogi alcohol	1028	-	2.94	4.23	3.54	-	-	-	4.36
11	α-Terpinene-	1049	1067	1.17	0.45	0.28	0.87	1.00	0.47	0.36
12	Cymene	1057	1071	1.40	0.78	0.71	0.78	1.56	0.99	0.39
13	Limonene	1062	1074	0.42	0.92	0.31	0.93	0.64	-	0.55
14	Eucalyptol	1065	1076	13.81	13.02	7.31	14.46	4.81	5.37	11.57
15	g-Terpinene	1090	1097	-	-	-	1.41	1.76	0.35	-
16	Atemisia ketone	1091	1098	7.96	18.09	22.45	-	-	-	10.89
17	4-Thujanol	1099	1171	1.10	0.44	1.42	1.23	-	0.31	1.00
18	Artemesia alcohol	1114	-	5.65	6.30	14.97	-	-	-	5.43
19	Thujone	1142	-	25.88	18.05	8.22	3.84	58.44	-	-
20	2-Cyclohexenol	1158	1109	0.57	-	-	-	0.90	-	2.37
21	*trans*-Verbenol	1181	1192	0.40	-	-	-	0.69	-	-
22	Methyl methacrylate	1182	-	-	-	0.22	-	-	-	-
23	2-Bornanone	1183	-	0.93	3.17	-	28.65	-	2.92	5.41
24	Photocitral B	1196	-	-	-	-	-	0.60	0.42	-
25	*cis*-Chrysanthenol	1197	-	-	-	-	2.17	-	-	-
26	Lavandulol	1199	-	-	-	-	-	-	0.42	0.56
27	3-Methyl-3-nitro-1-butene	1199	-	-	-	0.18	-	-	-	-
28	4-Methyl-1, 4-heptadiene	1199	-	-	0.75	-	-	-	-	-
29	Artemisia triene	1269	-	0.51	-	-	-	-	-	-
30	1,5-Dimethyl-6-methylenespiro [2.4]heptane	1200	-	-	-	-	-	1.34	-	-
31	*p*-Menthaol	1202	-	-	-	-	-	1.00	-	-
32	Borneol	1203	1218	3.56	1.82	-	14.79	-	4.32	2.97
33	4-Terpineol	1215	1236	5.47	2.96	1.32	3.98	5.31	1.07	1.99
34	α-Terpineol	1229	1265	1.57	1.54	0.88	1.82	-	0.36	1.56
35	*cis*-Piperitol	1246	-	-	-	-	-	-	-	0.61
36	1,4-Dimethyl-cyclohex-3-enyl methyl ketone	1249	-	-	-	-	0.55	-	-	-
37	Verbenone	1251	-	-	0.41	-	-	0.51	-	-
38	Carveol	1257	1274	0.94	1.77	0.86	1.19	1.01	0.33	0.74
39	*cis*-*p*-mentha-1 (7), 8-dien-2-ol	1267		-	0.60	-	-	-	-	-
40	1-Imidazole-1-yl-3-methylbut-2-en-1-one	1275	-	-	0.36	0.52	-	-	-	-
41	3-Carvomenthenone	1295	-	-	-	-	-	-	-	0.69
42	2-Butylphenol	1327	-	0.33	-	--	-	-	-	-
43	Bornyl acetate	1328	1364	-	-	-	0.75	-	-	-
44	α-Elemene	1382	-	-	-	-	-	-	2.22	0.41
45	3-Allylguaiacol	1398	-	0.38	-	-	0.50	0.51	-	-
46	Eugenol	1398	1418	-	0.45	0.43	-	-	-	-
47	Copaene	1425	-	-	-	-	-	-	-	0.33
48	Crysanthenone	1436	-	-	-	-	-	0.70	-	-
49	β-Elemene	1440	-	-	-	-	-	-	1.41	-
50	Caryophyllene	1473	1487	6.27	4.35	4.42	5.67	4.52	10.62	15.12
51	Humulene	1508	-	0.75	-	-	0.46	-	-	-
52	1, 4, 7,-Cycloundecatriene, 1, 5, 9, 9-tetramethyl-	1508	-	-	0.48	0.55	-	0.44	4.50	2.15
53	Alloaromadendrene	1516	1516	-	-	-	-	-	-	0.33
54	g-Muurolene	1529	-	-	-	-	-	-	-	0.53
55	Germacrene	1537	1551	2.45	1.51	1.95	1.93	1.30	7.13	8.60
56	1,2,3,4,4a,5,6,8a-octahydro-4a,8-dimethylnaphthalene	1544	-	1.10	1.05	1.33	-	-	-	1.30
57	Bicylogermacrene	1553	1573	0.92	-	0.53	0.42	-	2.86	3.32
58	g-Elemene	1553	-	-	-	-	-	0.43	-	-
59	Davana ether	1556	-	-	-	-	-	-	0.82	-
60	α-Amorphene	1569	-	-	-	-	-	-	1.17	0.76
61	Nerolidol	1609	1630	-	-	-	-	-	0.55	-
62	Virdiflorol	1630	-	-	-	-	-	-	3.11	2.96
63	Spatulenol	1638	1595	0.43	0.34	0.41	-	-	-	0.60

Table 3. *Cont.*

No.	Compounds	RI from Experiment	RI from Literature	Relative Content (%)						
				HA	PA	CZA	QA	SYA	BGA	LJA
64	Isolongifolen-5-one	1643	1602	-	-	-	-	-	0.85	-
65	Caryophyllene oxide	1647	-	0.82	1.15	1.09	0.75	0.75	1.06	1.26
66	Umbelliferone	1660	-	-	-	-	-	-	0.31	-
67	2-Norprezizene	1664	-	-	-	-	-	-	1.52	-
68	γ-Gurjunene	1667	-	-	-	-	-	-	-	0.93
69	Ledol	1667	1666	-	-	-	-	-	0.76	-
70	Junenol	1684	-	-	-	-	-	-	-	0.94
71	Isospathulenol	1688	-	-	-	-	-	-	1.77	-
72	3-Ethyl-2-methyl-1,3-hexadiene	1696	-	-	-	-	-	-	0.82	-
73	Cariophylladienol	1698	-	1.00	1.21	0.59	0.48	0.61	-	-
74	τ-Cadinol	1699	-	-	-	-	-	-	1.80	1.83
75	α-Cadinol	1715	-	-	-	-	-	-	-	1.09
76	Longiverbenone	1717	-	-	-	-	-	-	26.02	-
77	Neointermedeol	1719	-	1.12	6.12	10.06	5.35	-	-	0.95
78	Isoaromadendrene epoxide	1750	-	-	-	-	-	-	1.01	-
79	Silane, diphenylisobutoxy (2-methodyethoxy)-	1835	-	-	-	-	0.39	-	-	-
80	3, 4, 5-Trimethoxy-β-methyl-β-nitrostyrene	1835	-	-	-	1.46	-	-	-	-
81	2,3-Dphenyl-1H-1,2,4-triazole-5-thion	1835	-	1.00	-	-	-	-	-	-
82	1-Naphthylamine	1835	-	-	0.99	-	-	-	-	-
83	1, 5-Diphenyl-2H-1, 2, 4-triazoline-3-thione	1867	-	-	-	-	-	0.28	0.39	-
84	Methyl oleate	2053	-	0.57	-	10.05	-	-	-	0.43
85	4-[(Dimethylamino)methyl]-2,5-dimethylphenol	2170	-	-	-	-	0.21	-	-	-
86	Octasiloxane, 1, 1, 3, 3, 5, 5, 7, 7, 9, 9, 11, 11, 13, 13, 15, 15-hexadecamethyl-	2190	-	1.29	-	-	-	-	-	-
87	Cyclotrisiloxane, hexamethyl	2209	-	-	0.85	-	-	-	-	-
	Total			95.36	95.43	97.01	98.93	91.28	90.89	98.90

RI: retention indices. *—not detected.

A total of 87 compounds were identified in the silver wormwood essential oils from seven different habitats, accounting for 90.89~99.83% of the total volatile components. Huang detected more than 30 chemical components from silver wormwood essential oil by GC-MS [28].

Significant differences existed in the types and relative contents of volatile components in silver wormwood from different habitats. The components with high content were α-terpinene, cymene, eucalyptus, *p*-terphenyl, carvinol, caryophyllene oxide, camphor, borneol, etc. These compounds may constitute the main components of the basic flavor characteristics of silver wormwood essential oil.

Eucalyptol and borneol are the index components for the content determination of silver wormwood in Chinese Pharmacopoeia (2020 edition), which have many pharmacological effects, such as antibacterial, anti-inflammatory effects, etc. Their contents had certain significance for the quality evaluation of silver wormwood [29]. The content of eucalyptol in samples from different habitats all met the standard, among which the relative content of QA samples was as high as 14.46%, followed by HA samples (13.81%) and PA samples (13.02%), and the relative content of eucalyptus in SYA samples was the lowest, with a content of 4.81%. Except for CZA samples and SYA samples, borneol was found in silver wormwood from other habitats. The relative content of PA samples was as high as 14.79%, and the content of PA samples was 1.82%. Limonene, terpinene, α-terpineol, borneol, and other components have a strong aroma, and the differences in these components' content will lead to differences in aroma and curative effect of silver wormwood from different habitats.

3.3.2. PCA of Volatile Components

To further compare the differences of volatile components in silver wormwood from different habitats and determine the volatile components that play a key role in aroma intensity, PCA was carried out with the relative content of common peaks as eigenvalue. The eigenvalue and contribution rate of principal components are the basis for selecting

principal components [30]. It can be seen from Table 4 that the eigenvalue of the first two principal components were all greater than 1. The contribution rate of the first principal component was 55.80%, the contribution rate of the second principal component was 23.30%, and the cumulative contribution rate of total variance was 79.10%, indicating that the first two principal components can reflect the main characteristics of silver wormwood samples from different provenances; so, the first two principal components were extracted for analysis. Among them, *α*-terpinene, cymene, cucalyptus, *p*-terpineol, and carvingol contributed more to PC1, while cucalyptus, carvingol, and caryophyllene contributed more to PC2.

Table 4. Eigenvalue and contribution rates of principal components of silver wormwood from different habitats.

Principal Component	Eigenvalue	Variance Contribution (%)	Cumulative Variance Contribution (%)
1	3.91	55.80	55.80
2	1.63	23.30	79.10

The quality of silver wormwood samples was evaluated by adding the sum of factor scores of principal components and their weights (weight = contribution rate of principal components/cumulative contribution rate of two principal components) and calculating the total score F of each factor of principal components. The higher the F, the better the quality. It can be seen from Table 5 that, among silver wormwood from seven different habitats, HA samples had the highest comprehensive score and the best quality, followed by SYA samples, and LJA samples had the worst quality.

Table 5. Scores of principal component factors of silver wormwood from different habitats.

Sample Name	F1 [1]	F2 [2]	F [3]
HA	2.186	−0.506	1.392
PA	0.208	2.295	0.824
CZA	−0.952	0.495	−0.525
QA	1.301	0.769	1.144
SYA	2.376	−1.366	1.272
BGA	−2.001	−1.715	−1.917
LJA	−3.119	0.028	−2.190

[1] F1: principal component factor 1; [2] F2: principal component factor 2; [3] F: total score of principal component factors.

After PCA, the principal component analysis scores of silver wormwood samples from different habitats were obtained, as shown in Figure 5. According to the score chart, all samples of silver wormwood could be effectively distinguished, indicating that there are apparent differences in volatile odor components and contents of silver wormwood from different habitats, and there are great differences between PA samples and BGA samples.

3.3.3. Hierarchical Cluster Analysis (HCA)

The purpose of performing HCA is to classify data into specific groups by considering similarity criteria, and distance measures such as Euclidean distance [31].

SPSS 21.0 software was used to conduct cluster analysis, taking the relative percentage data of common peaks as variables and adopting the method of inter-group connection and taking the square Euclidean distance as the measurement standard interval [32]. The systematic cluster analysis was carried out on silver wormwood samples from seven producing areas, as shown in Figure 6. It can be seen from the figure that the samples of silver wormwood are divided into two categories by cluster analysis: the first category is HA samples, QA samples, PA samples, CZA samples, and SYA samples and the second category is BGA samples and LJA samples. This result was consistent with that of PCA.

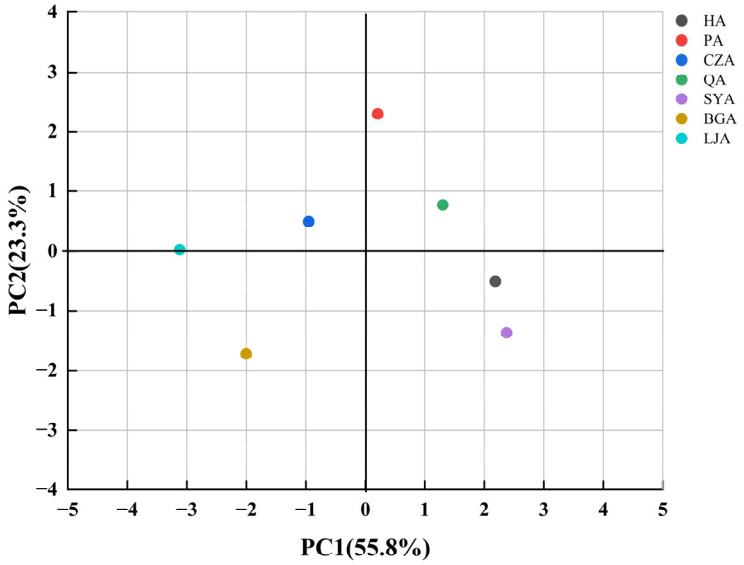

Figure 5. PCA diagram of volatile components in silver wormwood from different habitats.

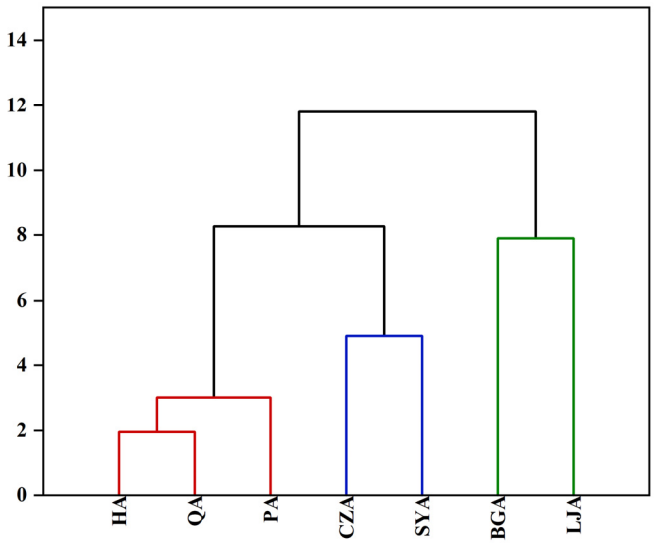

Figure 6. HCA of silver wormwood from different habitats: The same color represents the same category.

3.4. Antibacterial Activity Determination

The diameter of the inhibition zones of silver wormwood essential oils was determined by the paper disc diffusion method [33]. The results are shown in Table 6. The silver wormwood essential oil had a good inhibitory effect on *Bacillus subtilis*, *Staphylococcus aureus*, and *Escherichia coli*. The diameter of the inhibition zone of CZA samples against *Bacillus subtilis* was 21.22 ± 1.05 mm and the inhibitory effect of CZA samples on *Bacillus subtilis* was the strongest. The diameter of the inhibition zone of the QA samples against *Staphylococcus aureus* was 18.20 ± 0.17 mm and the inhibitory effect was the strongest. The diameter of the inhibition zone of HA samples was 17.93 ± 1.04 mm and the inhibitory effect of HA samples on *Escherichia coli* was the strongest.

Table 6. The antimicrobial dimension of silver wormwood essential oils from different habitats.

Essential Oil	Gram-Positive Bacteria (mm)		Gram-Negative Bacteria (mm)
	Bacillus Subtilis	**Staphylococcus Aureus**	***Escherichia coli***
HA	13.89 ± 0.75	11.48 ± 0.12	17.93 ± 1.04
PA	20.32 ± 0.82	6.29 ± 0.41	16.09 ± 0.91
CZA	21.22 ± 1.05	4.47 ± 0. 08	15.67 ± 0.27
QA	13.80 ± 1.74	18.20 ± 0.17	13.78 ± 0.48
SYA	15.42 ± 0.66	15.28 ± 0.33	16.07 ± 0.45
BGA	17.27 ± 0.56	7.67 ± 0.71	8.21 ± 0.16
LJA	16.30 ± 1.01	7.76 ± 0.22	11.50 ± 0.45
Positive control	19.57 ± 0.63	16.55 ± 0.35	23.32 ± 0.45
Negative control	- [1]	-	-

[1]—Not detected.

4. Conclusions

Silver wormwood essential oil has a strong aroma and complex components, and it is an important index affecting its quality [34]. Due to the influence of climate, planting methods, and harvesting, the quality of silver wormwood from different habitats is quite different, so it is difficult to judge the best variety of silver wormwood. Wang combined HS-SPME with GC-MS, studied the essential oils of silver wormwood that come from five habitats, and explored the influence of climatic factors on the main components of the essential oils [35]. The combination of E-nose technology and GC-MS technology can be used to analyze the volatile components of silver wormwood from different habitats from two aspects: the overall odor information and the specific types and contents of essential oil components.

In this study, silver wormwood essential oils from seven different habitats were isolated by steam distillation, the volatile components were analyzed by E-nose, GC-MS, and chemometrics, and the antibacterial effects of essential oils from seven habitats were studied. The results of E-nose showed that the samples of silver wormwood from seven different habitats were distributed in different regions and did not overlap with each other, which indicated that there were obvious differences in odor characteristics among samples of silver wormwood from different habitats. GC-MS was used to detect the volatile components of silver wormwood samples from different habitats qualitatively and quantitatively. PCA and HCA analysis were consistent with the results of E-nose detection. Analysis showed that the HA samples had the best quality and the LJA samples had the worst quality. The results of antibacterial activity showed that the silver wormwood essential oils from seven habitats had a good inhibitory effect on *Bacillus subtilis*, *Staphylococcus aureus*, and *Escherichia coli*.

This study shows that the E-nose combined with GC-MS can effectively distinguish silver wormwood from different habitats, which provides a new idea for studying the odor substance basis and rapid identification of silver wormwood and provides a scientific basis for breeding superior varieties, quality control, and comprehensive development of silver wormwood resources.

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