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compounds

Article

Volatile Compound Profiling and Antibacterial Efficacy of Heyang Fragrance: Bridging Cultural Heritage with Modern Scientific Analysis

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Abstract

Heyang Fragrance, a traditional incense dating back to the Eastern Han Dynasty (25–220 AD), was recently inscribed on China's national list of intangible cultural heritage. This study aimed to systematically analyze three variants of Heyang Fragrance (Aicao, Qinqiang, and Jianjia) through integrated methodologies including electronic nose analysis, headspace solid-phase microextraction coupled with gas chromatography–mass spectrometry (HS-SPME-GC-MS), and antimicrobial activity assays. We selected *Escherichia coli*, *Bacillus subtilis*, and *Candida glabrata* for the antimicrobial activity assays. Comparative analysis revealed significant compositional differences between pre- and post-combustion volatile profiles. Upon ignition, sensor response values increased by 50–100% relative to baseline measurements, with sulfides, terpenes, and short-chain alkanes emerging as dominant components. Qinqiang demonstrated the highest odor activity values (OAVs), particularly through carvacrol (OAV = 6676.60) and eugenol (OAV = 2720.84), which collectively contributed to its complex aromatic characteristics. Antimicrobial assessments revealed concentration-dependent efficacy, with Qinqiang exhibiting broad antimicrobial activity against *Escherichia coli* (11.33 mm inhibition zone) and *Bacillus subtilis* (15.00 mm), while Jianjia showed maximal effectiveness against *Bacillus subtilis* (17.67 mm). These findings underscore the dual significance of Heyang Fragrance in cultural conservation and its prospective applications in aroma therapeutic and antimicrobial contexts.

Keywords: Heyang fragrance; electronic nose; GC-MS; OAV; antibacterial efficacy

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1. Introduction

Heyang Fragrance, a traditional incense from Heyang County (Shaanxi Province, China), has been crafted using techniques dating to the Eastern Han Dynasty (25–220 CE). Its precursor, "Shenming Ointment" (a medicinal incense for epidemic control), is recorded on the 185 CE Cao Quan Stele, noting its use of aromatic materials like mugwort for both medicinal and ceremonial purposes [1,2]. The modern Heyang Fragrance retains these ancient formulas and medicinal properties, and in 2023, its production was inscribed on Weinan City's Intangible Cultural Heritage list, highlighting its cultural and therapeutic value.

In ancient Chinese history, traditional incense held a significant role, used for burning as fragrance, in rituals, or for medicinal purposes. The Wei and Jin Dynasties (220–420 CE) witnessed intensified Buddhist acculturation, during which resin-based (*Aquilaria* spp.) and ligneous (*Santalum album*) incense materials gained ritual prominence. The Southern Dynasties (420–589 CE) saw the emergence of "Bai Ke Incense," crafted into seal script shapes for burning, serving both practical and esthetic functions [3]. Tang-Song aristocrats (618–1279 CE) formulated composite "Ya Xiang" (Elegant Fragrance) blends for domestic fumigation and textile impregnation, reflecting Neo-Confucian sensibilities. Li Shizhen's *Bencao Gangmu* (1593 CE) records moxa (*Artemisia argyi*) and thuja (*Platycladus orientalis*) incense rods for epidemic prevention, with contemporary GC-MS analyses confirming their antimicrobial volatiles [4,5].

The current research on traditional incense primarily focuses on agarwood, sandalwood, borneol, and other similar substances. Chen et al. systematically cataloged agarwood's phytoconstituents and pharmacodynamic properties, highlighting two main classes of compounds: sesquiterpenes and 2-(2-phenylethyl)chromone derivatives. Over 300 compounds have been isolated and identified from agarwood, exhibiting diverse biological activities such as central nervous system regulation and antibacterial, antitumor, and antioxidant effects [6–8]. Tu et al. utilized thermal desorption–gas chromatography/mass spectrometry (TD-GC/MS) to systematically analyze the volatile components of sandalwood (*Santalum album* L.) and its combustion smoke. They identified 27, 24, and 37 compounds in reference samples, commercial samples, and smoke, respectively. α-Santalol and β-santalol were common major components, while phenolic substances significantly increased post-combustion, reaching 4.5 times their pre-combustion levels, revealing dynamic changes in chemical composition during burning [9]. Zhu et al. analyzed the volatile organic compounds (TVOCs) emitted from the combustion of five natural incenses, including Xiaogang incense, agarwood, and sandalwood. Their research provides data supporting the safety assessment of natural incenses, although there is a lack of toxicological and pharmacological studies on the related compounds [10].

Existing studies primarily analyze volatile components of burning incense but neglect sensory evaluation and pharmacological effects. Notably, Heyang Fragrance—an Eastern Han intangible cultural heritage—has not been investigated for its aromatic or pharmacologically active components. In this experiment, we will use headspace solid-phase microextraction coupled with gas chromatography–mass spectrometry (HS-SPME-GC/MS) and an electronic nose (a device for detecting and quantifying volatile organic compounds via sensor arrays) to analyze the volatile components of three types of Heyang Fragrance. Additionally, we will assess their antibacterial effects. This study elucidates aroma dynamics pre- and post-combustion, identifies volatile compounds, and confirms Heyang Fragrance's medicinal value, supporting its intangible cultural heritage preservation.

2. Methods and Materials

2.1. Fragrance Materials

In this experiment, three types of Heyang Fragrance—Aicao, Qinqiang, and Jianjia—as shown in Figure 1 (the unground samples) were crushed and ground until the particles could pass through a No. 3 sieve (as specified by the Chinese Pharmacopoeia, with a pore size of 355 µm, 50 mesh). After grinding, the Heyang Fragrance particles were collected in transparent sealed bags and stored at room temperature in a dry environment.

2.2. Research Methodology

2.2.1. Electronic Nose

The electronic nose (e-nose) is a device that detects and quantifies volatile organic compounds (VOCs) using a sensor array. The samples were placed in 20 mL bottles at room temperature. Wearing gloves and masks, a small number of researchers were allowed to stand in a quiet environment with no odors. In this study, experiments were conducted using both unburned granular fragrance (solid group) and the gas collected after combustion (gas group). For each type of Heyang Fragrance, 0.2 g were used per experiment, with each type undergoing three parallel repetitions. In the solid group, 0.2 g of granular fragrance was placed at the bottom of a vial and left undisturbed for 20 min to allow the volatile substances to be fully released. In the gas group, 0.2 g of granular fragrance was placed on an aluminum foil spoon, ignited, and allowed to burn completely within the vial to collect the smoke. The aroma compositions were determined by the PEN3 electronic nose from AIRSENSE, Germany. The parameters of the electronic nose were set as follows: gas flow rate 150 mL/min, cleaning time 120 s, zeroing time 5 s, preparation time 5 s, and measurement time 120 s. The built-in WinMuster software on the PEN3 was used for data collection, measurement, and analysis. In the preliminary experiments, it was observed that the curve of the G/G0 ratio tends to stabilize toward the end of the injection period, and the data were analyzed by averaging the response values of the electronic nose from 116 s to 118 s. Three sets of replicates were made for each kind of fragrance, and each experiment was conducted to ensure that the height and relative position of the two probes in the bottle were as consistent as possible [11]. In this context, the electronic nose response value is defined as the dimensionless ratio of the resistance value, G, of each sensor to the air resistance value, G0. The response of electronic nose sensors to different types of compounds is shown in Table 1.

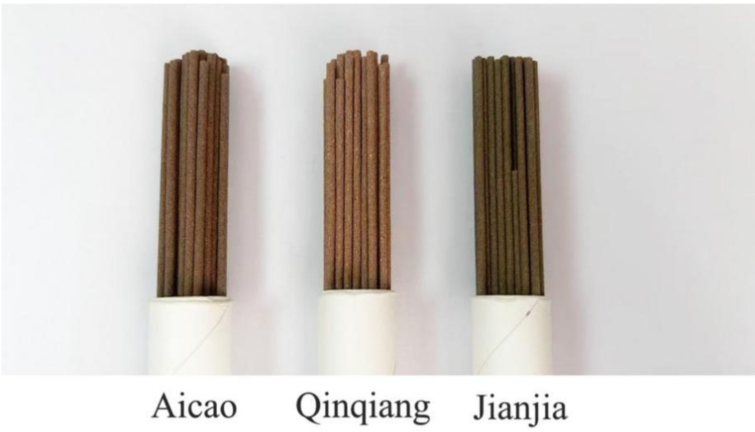


Figure 1. Three types of Heyang Fragrance: Aicao, Qinqiang, and Jianjia.

Table 1. Response characteristics of each sensor of PEN3 electronic nose.

Array Serial No.	Sensor Name	Performance Description
1	W1C	Aromatic
2	W5S	Broad range
3	W3C	Aromatic
4	W6S	Hydrogen
5	W5C	Arom-aliph
6	W1S	Broad-methane
7	W1W	Sulfur-organic
8	W2S	Broad-alcohol
9	W2W	Sulf-chlor
10	W3S	Methane-aliph

2.2.2. SPME-GC-MS Analysis

For each type of Heyang Fragrance, four parallel experiments were performed. A 0.2 g fragrance stick was ignited, and smoke was collected in a 20 mL headspace vial for a duration of 60 s. Following this, the samples were equilibrated in a water bath at 70 °C for 20 min. To immerse the sample, 15 mL of saturated saline solution was added. The vial was then sealed with aluminum foil and the cap tightened. Subsequently, 10 μL of 2-nonanone at a concentration of 0.008 μL/mL was added using a micro-adjustable pipette along the wall at the bottom of the extraction bottle. The mouth of the extraction bottle was then sealed with tin foil and capped.

The SPME extraction head from the German company Supelco, model DVB/CAR/PDMS, with a diameter of 50/30 μm, was utilized. The extraction vial was placed in the center of a preheated constant-temperature extraction apparatus set to 40 °C. After an equilibration period of 5 min, the extraction head support was adjusted to the appropriate position, and the extraction head was inserted into the vial. The knob was slid to extend the fiber head 1 cm above the liquid surface, allowing for a 20 min adsorption period at 45 °C. Once the time elapsed, the fiber head was retracted, and the adsorbed extraction head was swiftly removed in preparation for injection into the gas chromatograph.

GC-MS Analysis (Thermo Fisher Scientific Inc., Waltham, MA, USA; English name: SQ and TRACE ISQ; Model: ISQ and TRACE ISQ): The manual headspace injector was inserted into the GC-MS injection port. After a 2.5 min equilibration period at 250 °C, the aroma components were fully released into the GC-MS system. The chromatographic column employed was an Rtx-1MS (30 m × 0.32 mm × 0.25 μm) with the following temperature settings: the inlet temperature was maintained at 250 °C, while the initial column temperature was set at 40 °C for 2 min. This was followed by a linear increase at a rate of 8 °C/min to reach 130 °C for 4 min and a further increase at 15 °C/min to 250 °C for 3 min. Nitrogen (99.999%) served as the carrier gas, with a flow rate of 2.41 mL/min and a split ratio of 1:5. The conditions for mass spectrometry included electron impact (EI) ionization mode with an electron energy of 70 eV, an ion source temperature of 200 °C, and an interface temperature of 230 °C. Mass spectra were recorded over a scanning range from 45 to 450 atomic mass units (amu) [12].

2.2.3. Extraction of Active Compounds and Antibacterial Activity Assay

Heyang Fragrance was ground into a fine powder. Active compounds were extracted using an ethanol extraction method. Briefly, 1 g of powder was dissolved in 10 mL absolute ethanol through vortex mixing (final concentration: 100 mg/mL). The mixture was extracted with absolute ethanol for 24 h, extracted overnight in a 28 °C shaking incubator. We measured the antibacterial activity using the disk diffusion method. *Escherichia coli*, *Bacillus subtilis*, and *Candida glabrata* (Beijing Solarbio Science & Technology Co., Ltd., Beijing, China) were activated and inoculated onto LB media homogeneously. The concentration gradients of the Heyang Fragrance extract obtained via ethanol extraction were 12.5, 25, 50, and 100 mg/mL. Use a microbiological well punch to cut out 5 mm diameter filter paper disks. Disks were individually immersed in 20 μL of each concentration extract corresponding to each microbial strain and then placed onto the culture medium. Filter paper disks treated with absolute ethanol were used as the control group. Each group included three parallel replicates. The plates were incubated at 28 °C for 48 h. Antibacterial effects were measured by assessing inhibition zone diameters using the cross method [13–16].

2.2.4. Identification and Analysis Methods

Qualitative method: Each individual component was retrieved and compared against the NIST 2017 mass spectrometry and standard information database, and their identification was cross-verified with the relevant literature. They were identified and analyzed together using the related literature. By using the carbon standard method and the same column as well as rising and cooling procedures such as GC-MS, the mixed standard of C7–C30 normal alkane was used as the standard to calculate the linear retention index

(Formula (1)) of various aroma components of Heyang Fragrance samples. The results were compared with those of the NIST spectrum database. Starting from the first stable peak according to the ion peak diagram, the stable peak corresponds to the first place of a variety of substances under the same RT value, and the selected substances should appear under the same RT value in the three basic biological repeats and rank in the top three.

$$LRI = 100\,z + 100\,(RT - RT_z)/(RT_{(z+n)} - RT_z) \quad (1)$$

Quantitative method—Internal standard substance method: First, 2-nonone was selected as the internal standard material, and the density was 0.82 g/mL. The volume of the internal standard substance added was 2 μL. All kinds of aroma components in the Heyang Fragrance sample were quantized (Formula (2)), and the average value was obtained after four parallel repetitions.

$$Mi = C_0 \times V_0 \times A_i \div (A_0 \times M) \quad (2)$$

In Formula (2), Mi is the content of each aroma component (μg/g), and C_0 is the internal standard substance concentration (μg/μL), while V_0 is the internal standard substance volume (μL). A_i is the peak area of the desired aroma component; A_0 is the internal standard substance peak area; and M is the sample mass (g).

OAV value: the ratio of the mass concentration of the substance (Formula (3)) to the threshold value of the substance in water (Formula (4)) is regarded as a standard to evaluate the contribution of the substance to the overall aroma profile of the sample, thus selecting out the standard sample for aroma reconstruction, in which the substance with OAV ≥ 1 is the characteristic aroma component [17].

$$C_i = C_0 \times A_i \div A_0 \quad (3)$$

In Formula (3), Ci is the mass concentration of each aroma component (μg/mL), and C_0 is the internal standard substance concentration (μg/μL). A_i and A_0 are the peak area and internal standard substance peak area of the aroma component, respectively.

$$OAV = C_i \div OT_i \quad (4)$$

In Formula (4), OT_i is the threshold value of the aroma component in water (μg/mL) [18]. If there is no numerical value for the threshold of a compound in water, then the value of the closest medium by properties to water should be selected.

2.2.5. Statistical Analysis

All experiments were performed in triplicate (for electronic nose and antibacterial assays) or quadruplicate (for GC-MS analysis). Data were expressed as mean ± standard deviation (SD). Statistical significance was determined using one-way analysis of variance (ANOVA) followed by Tukey's post hoc test. A p-value < 0.05 was considered statistically significant. Statistical analyses were performed using SPSS 26.0 software (IBM Corp., Armonk, NY, USA).

3. Results

3.1. Electronic Nose Response Characteristics and Multivariate Analysis Results

Figure 2 presents the radar charts of the electronic nose response values for volatile compounds of three types of Heyang Fragrance before and after ignition, showing significant differences in sensor response ratios between these two states. Before ignition, the response values ranged from 0 to 16, while, after ignition, they increased to the range of 0–35, indicating a substantial increase in the content of volatile compounds post-ignition.

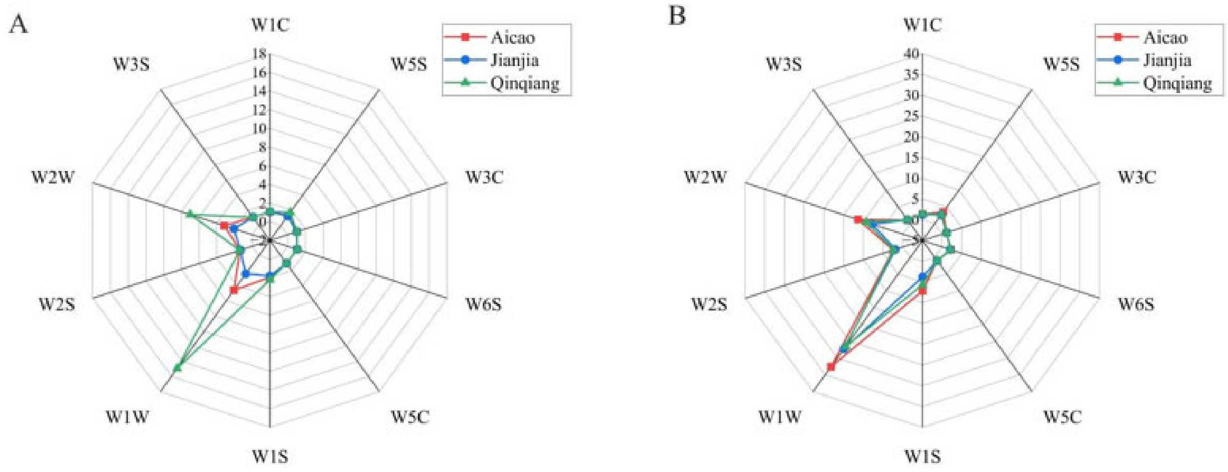
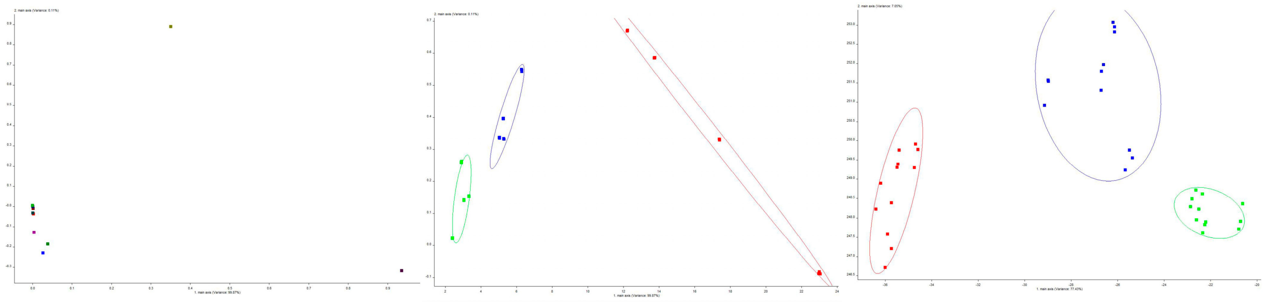


Figure 2. Radar map of the characteristic response of the volatile components in Heyang Fragrance. (**A**): Radar map of the response of the solid-group Heyang Fragrance electronic nose before ignition. (**B**): Radar map response of gas-group Heyang Fragrance electronic nose after ignition.

Before ignition, the sensor response values of volatile compounds varied among different types of fragrance. The W1W (sulfur-organic) and W2W (sulfur-chlorine) sensor responses of Aicao and Jianjia were between 2 and 6, whereas Qinqiang exhibited remarkable values of 15 and 7 for W1W and W2W, respectively. The response values of the other eight sensors for the unignited fragrances were all below 2. This suggests that, before ignition, these fragrances were mainly rich in sulfides, terpenes, and aromatic compounds, and Qinqiang contained significantly higher levels of volatile compounds than the other two.

After ignition, the sensor responses of volatile compounds became more similar among the three types of fragrance. The response values of the W1W and W2W sensors were significantly higher than those of other sensors, approximately 25–35 and 10, respectively, and the response value of the W1S (broad-methane) sensor also increased to about 5–10. Notably, Aicao consistently had higher sensor values than the other two types. This indicates that the contents of sulfides, terpenes, aromatic compounds, and short-chain alkanes increased significantly after ignition, and Aicao showed the strongest sensor responses.

Figure 3 presents the loading analysis, principal component analysis (PCA), and linear discriminant analysis (LDA) of the three types of Heyang Fragrance before ignition. In the loading analysis and PCA, the first principal component accounts for 99.87% of the variance, while the second accounts for 0.11%, totaling 99.98%. In the LDA, the first principal component contributes 77.43% and the second 7.05%, with a combined contribution of 84.48%, indicating effective dimensionality reduction. Both analyses effectively capture the primary characteristics of the samples. As shown in Figure 3A, Sensor W1W (sensitive to sulfides and terpenes) and Sensor W2W (sensitive to organic sulfides and aromatics) are significantly distant from the origin. This indicates that these two sensors are crucial for distinguishing the volatile compounds of the three types of Heyang Fragrance before ignition. The primary factors contributing to the aromatic differences are sulfides, terpenes, and aromatics. Both PCA and LDA successfully differentiate the volatile compounds of the incense types before ignition, confirming distinct pre-ignition aromatic profiles.

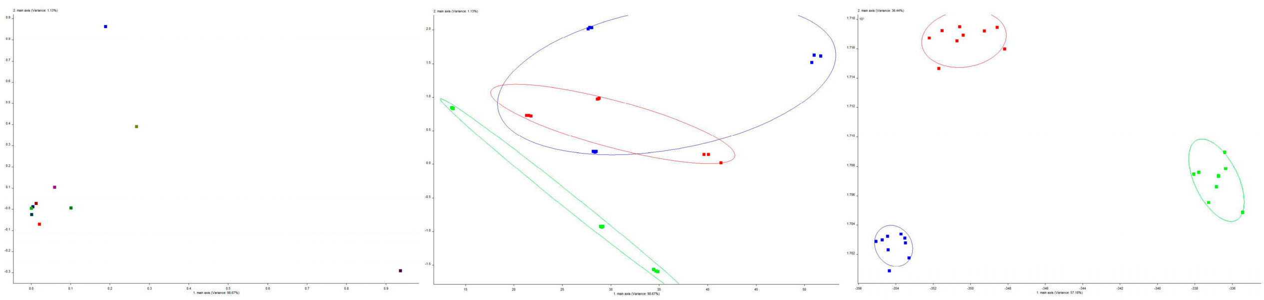


(**A**) (**B**) (**C**)

Figure 3. PCA result of the volatile components in Heyang Fragrance before ignition. (**A**): Loading result of the volatile components, where different colored dots represent different sensors. (**B**): PCA result of the volatile components. (**C**): LDA result of the volatile components. With blue dots representing jianjia, red dots representing qinqiang, and green dots representing aicao.

3.2. Electronic Nose Analysis of Volatile Compounds

Figure 4 shows the loading analysis, PCA, and LDA of the gaseous volatile compounds of the three types of Heyang Fragrance after ignition. In the loading analysis and PCA, the first principal component accounts for 98.67% of the variance and the second for 1.13%, totaling 99.8%. In LDA, the first principal component contributes 57.18% and the second 36.44%, with a combined contribution of 93.62%, indicating effective dimensionality reduction.



(**A**) (**B**) (**C**)

Figure 4. PCA result of the volatile components in Heyang Fragrance after ignition. (**A**): Loading result of the volatile components, where different colored dots represent different sensors. (**B**): PCA result of the volatile components. (**C**): LDA result of the volatile components with blue dots representing Jianjia, red dots representing Qinqiang, and green dots representing Aicao.

These analyses effectively capture the primary characteristics of the samples. As seen in Figure 4A, Sensor W1S (sensitive to methane), Sensor W1W (sensitive to sulfides and terpenes), and Sensor W2W (sensitive to organic sulfides and aromatics) are significantly distant from the origin. This indicates that these three sensors are crucial for distinguishing the volatile compounds of the incense after ignition. The main factors contributing to the aromatic differences are sulfides, terpenes, aromatics, and short-chain alkanes. Compared with the pre-ignition state, the presence of short-chain alkanes significantly affects the post-ignition aroma differences.

However, PCA cannot distinguish between the aromas of Qinqiang and Jianjia after ignition, whereas LDA can completely differentiate them. This demonstrates that, while there are significant differences in post-ignition aromas, LDA is more effective than PCA in distinguishing them.

3.3. GC-MS Analysis of Volatile Compounds

The volatile compounds of Heyang Fragrance were qualitatively and semi-quantitatively analyzed using headspace solid-phase microextraction coupled with gas chromatography–mass spectrometry (GC-MS). A total of 71 compounds were identified across the three types of Heyang Fragrance. Among them, 69 compounds were structurally identified (NIST library match). Specifically, 37 compounds were identified in Aicao, 36 in Jianjia, and 29 in Qinqiang. Figure 5 presents a Venn diagram of the volatile compounds found in the different types of Heyang Fragrance. Aicao and Jianjia each have 19 unique compounds, while Qinqiang has 9 unique compounds not found in the other two fragrances.

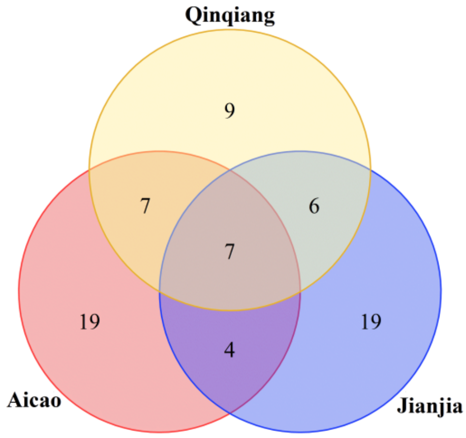


Figure 5. The Venn diagram of the numbers of volatile components in Heyang Fragrance after ignition.

Figures 6 and 7 illustrate the number and mass fractions of volatile compounds in the three types of Heyang Fragrance. Among all the compound categories, terpenes stand out as the most prominent both in quantity and mass fraction, playing a dominant and irreplaceable role in defining the volatile profiles of Heyang Fragrance. Specifically, 17 terpenes were detected in Aicao, 15 in Jianjia, and 12 in Qinqiang, with mass fractions of 67.49%, 46.03%, and 56.20%, respectively. Esters and ethers were rarely identified in the three types of Heyang Fragrance. The quantities and mass fractions of other compounds, such as alcohols, aromatics, phenols, aldehydes, ketones, and alkenes, were relatively similar across the fragrances, but none approached the magnitude of terpenes in either count or mass contribution.

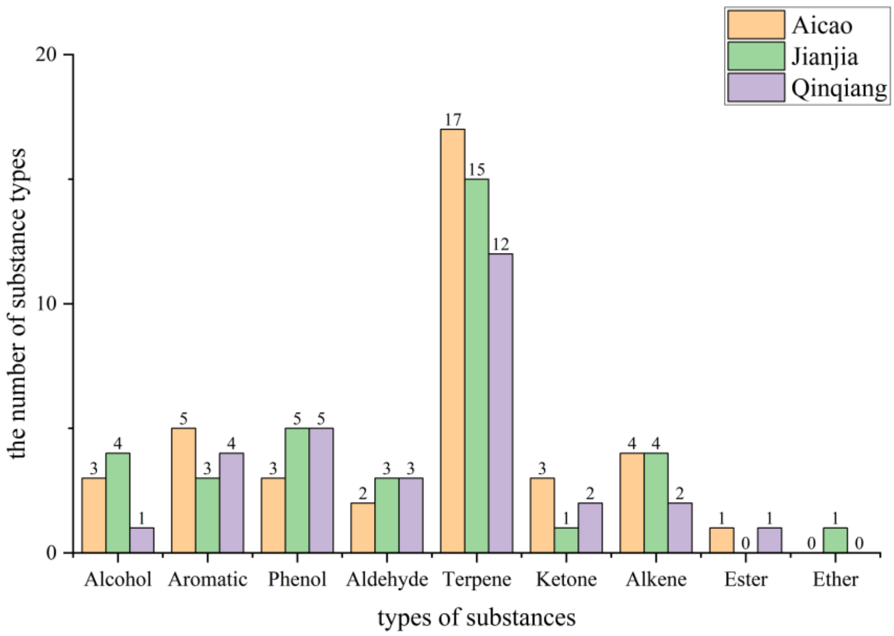


Figure 6. The number of volatile compounds in Heyang Fragrance after ignition.

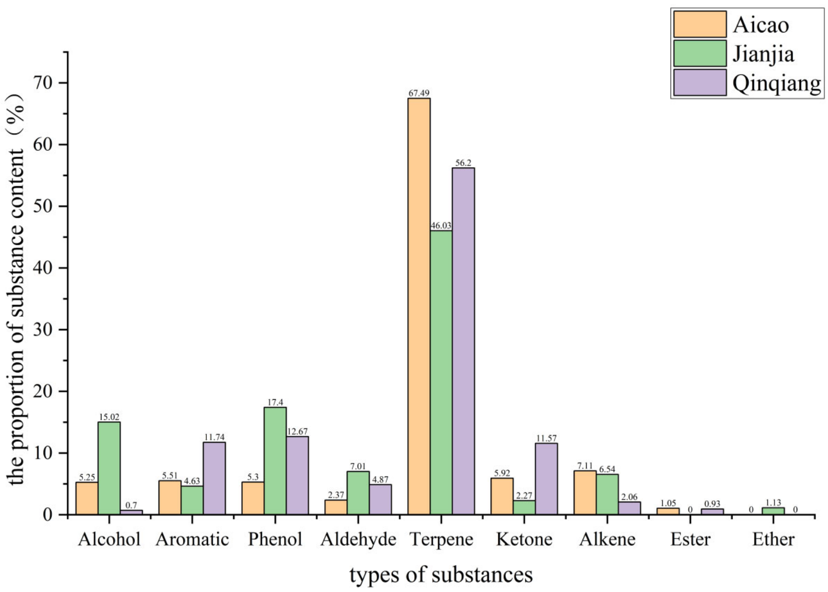


Figure 7. The mass fraction of volatile compounds in Heyang Fragrance after ignition.

Table 2 meticulously details the nomenclature and mass fractional distributions of volatile compounds across the Aicao, Jianjia, and Qinqiang variants of Heyang Fragrance, offering critical insights into their chemical architectures. Seven shared compounds, including 3-furaldehyde and caryophyllene oxide, form the cornerstone of the fragrance's volatile matrix, influencing its olfactory profile. Terpenoids dominate each fragrance but vary significantly: thujopsene enriches Qinqiang (12.43%) and Aicao (4.98%) but is absent in Jianjia, while 8-epicedrol prevails in Aicao (10.03%) and Jianjia (4.22%) yet is undetected in Qinqiang. Further, (+)-borneol ranks second in Aicao at 8.18%. By compound class, (3E)-3-prop-2-enylidenecyclobutene exists only in Jianjia (1.59 ± 0.66%) and Qinqiang (0.64 ± 0.45%); α-cadinol acetate is unique to Aicao (0.87 ± 0.14%); and γ-asarone is exclusive to Jianjia (0.84 ± 0.18%). Other compound classes, like aldehydes with 3-furaldehyde showing different mass fractions (1.3 ± 0.46% in Aicao, 1.73 ± 0.73% in Jianjia, 1.03 ± 0.66% in Qinqiang), also exhibit quantitative fluctuations. Collectively, these findings comprehensively elucidate the conserved and divergent chemical features of Heyang Fragrance variants.

Table 2. The mass fraction of the compound in Heyang Fragrance after ignition (%).

No.	CAS	RI	Compound	Aicao	Jianjia	Qinqiang
Alkene						
1	52097-85-5	715	(3E)-3-Prop-2-enylidenecyclobutene		1.59 ± 0.66	0.64 ± 0.45
2	544-25-2	717	Cyclohepta-1,3,5-triene	2.4 ± 0.68		
3	611-15-4	1074	2-Ethenyltoluene		0.93 ± 0.35	
4	2867-05-2	1086	α-Thujene	0.79 ± 0.11		
5	124-11-8	1196	Non-1-ene	1.21 ± 0.67		
6	25679-28-1	1383	(Z)-Anethole	1.52 ± 0.16		
7	19903-72-1	1615	(Z)-α-Santalol		1.25 ± 0.62	
Ester						
8	149197-48-8	1551	α-Cadinol acetate	0.87 ± 0.14		
9	92691-77-5	1637	Gossonorol			0.54 ± 0.06
Ether						
10	5353-15-1	1563	γ-Asarone		0.84 ± 0.18	
Aldehyde						
11	498-60-2	838	3-Furaldehyde	1.3 ± 0.46	1.73 ± 0.73	1.03 ± 0.66
12	620-02-0	1029	5-Methyl-2-furaldehyde		2.81 ± 0.14	0.45 ± 0.19
13	16933-18-9	1420	2,3-Dimethyltricyclo[2.2.1.02,6]heptane-3-propanal		1.41 ± 0.19	

Table 2. *Cont.*

No.	CAS	RI	Compound	Aicao	Jianjia	Qinqiang
14	98093-94-8	1561	2-Hydroxy-3-methoxy-4-methylbenzaldehyde			1.32 ± 0.37
15	470-41-7	1632	Borneol	0.68 ± 0.04		
Ketone						
16	464-48-2	1249	Camphor	3.2 ± 0.36		4.99 ± 0.19
17	585-74-0	1285	3-Methylacetophenone		1.69 ± 0.88	
18	80-57-9	1312	Verbenone	0.98 ± 0.1		
19	50657-30-2	1583	Allocedrol			3.35 ± 1.11
Alcohol						
20	98-00-0	884	2-Furanmethanol		2.19 ± 0.82	
21	60-12-8	1218	2-Phenylethanol		3.44 ± 0.16	
22	40607-48-5	1327	3,7-Dimethyloct-2-en-1-ol		3.24 ± 2.75	
23	28400-11-5	1602	β-Acorenol		0.98 ± 0.04	0.4 ± 0.1
24	78339-53-4	1633	(2Z)-2-Methyl-6-(4-methylphenyl)hept-2-en-1-ol		2.32 ± 0.96	
Phenol						
25	108-95-2	1064	Phenol	0.75 ± 0.21	2.66 ± 0.25	1.15 ± 0.08
26	108-39-4	1179	3-Methylphenol		1.11 ± 0.62	
27	90-05-1	1191	2-Methoxyphenol	2.7 ± 0.55	3.73 ± 1.32	2.41 ± 2.05
28	7786-61-0	1406	2-Methoxy-4-vinylphenol		3.7 ± 1.47	2.66 ± 2.46
29	97-53-0	1438	Eugenol			1.55 ± 0.69
30	1941-12-4	1438	3-Allyl-2-methoxyphenol	0.97 ± 0.29		
31	97-54-1	1506	Isoeugenol		1.8 ± 0.98	
Aromatic						
32	100-41-4	887	Ethylbenzene	0.64 ± 0.01		2.77 ± 0.05
33	108-38-3	900	1,3-Dimethylbenzene	0.69 ± 0.1	1.12 ± 0.09	4.34 ± 0.32
34	100-42-5	929	Styrene	0.35 ± 0.3		3.36 ± 0.03
35	620-14-4	1030	3-Ethyltoluene		1.58 ± 0.16	
36	271-89-6	1072	Benzofuran	0.66 ± 0.29		
37	527-84-4	1111	1-Isopropyl-4-methylbenzene	2.25 ± 0.62	1.04 ± 0.16	0.73 ± 0.39
Terpene						
38	16609-28-2	1099	3-Methylene-1,5,5-trimethylcyclohexene	0.67 ± 0.05		
39	1461-27-4	1114	Sylvestrene	4.33 ± 0.52	1.39± 0.24	0.55 ± 0.19
40	37665-99-9	1186	2,7-Dimethylocta-2,6-dien-4-ol	3.49 ± 0.59		
41	29803-82-5	1245	1-Methyl-4-(propan-2-yl)cyclohex-2-en-1-ol	0.74 ± 0.02		
42	507-70-0	1269	Borneol	8.18 ± 0.63	2.06 ± 1.79	
43	20126-76-5	1284	4-Terpineol	5.12 ± 1.16		
44	98-55-5	1297	α-Terpineol	5.96 ± 0.05	3.57 ± 0.69	
45	106-24-1	1351	Geraniol		1.81 ± 0.53	0.75 ± 0.09
46	89-81-6	1355.	Piperitone	0.91 ± 0.03		
47	499-75-2	1388	Carvacrol		7.6 ± 3.77	3.82 ± 4.89
48	469-61-4	1485	α-Cedrene	2.21 ± 0.7		
49	79120-98-2	1489	β-Funebrene			2.99 ± 0.48
50	87-44-5	1490	β-Caryophyllene	2.33 ± 0.71		4.25 ± 0.78
51	470-40-6	1498	Thujopsene	4.98 ± 2.72		12.43 ± 4.27
52	28973-97-9	1510	(E,E)-β-Farnesene		0.93 ± 0.16	
53	51446-91-4	1517	Phosphatidylserine			1.34 ± 0.13
54	18431-82-8	1526	β-Chamigrene			0.72 ± 0.29
55	17066-67-0	1532	β-Selinene	1.02 ± 0.06		
56	16982-00-6	1541	Cuparene	2.51 ± 0.4		4.66 ± 5.29
57	18319-35-2	1541	α-Cedrenol		0.84 ± 0.18	
58	142-50-7	1569	(3E,7E)-Nerolidol		2.44 ± 0.73	
59	77171-55-2	1578	(3E,7E)-Nerolidol	0.68 ± 0.04	1.12 ± 0.21	
60	1139-30-6	1579	β-Caryophyllene oxide	3.5 ± 0.14	1.01 ± 0.5	0.69 ± 0.28
61	19903-73-2	1588	8-Epicedrol	10.03 ± 1.82	4.22 ± 5.19	
62	28400-11-5	1599	β-Acorenol		0.98 ± 0.04	0.4 ± 0.1
63	88-84-6	1604	β-Guaiene			0.59 ± 0.06
64	34413-94-0	1605	Squamulosone	0.75 ± 0.05		0.98 ± 0.05
65	5945-72-2	1608	Neointermedeol		3.47 ± 0.16	
66	22451-73-6	1615	Bulnesol	2.14 ± 0.01		
67	515-69-5	1619	α-Bisabolol		2.67 ± 1.07	
68	66512-56-9	1619	Khusiol		1.20 ± 0.08	1.20 ± 0.08
69	504-96-1	1667	Neophytadiene		1.04 ± 0.45	

The odor activity value (OAV) is a crucial criterion for assessing the contribution of individual volatile compounds to a sample's aroma. Based on previously reported threshold values of volatile compounds in water, the OAV of volatile compounds in Heyang Fragrance were calculated as seen in Table 3. Eleven compounds with OAVs greater than 1 were identified across the three types of Heyang Fragrance. These include five terpenes, one aldehyde, one ketone, three aromatic compounds, and two phenols. Terpenes, in particular, exhibited high OAVs, significantly contributing to the aroma profile. Among these compounds, Aicao contained seven, Jianjia five, and Qinqiang nine. Phenol was the only compound with an OAV greater than 1 common to all three fragrances, contributing to their distinct aromatic profiles.

Table 3. The OAV of volatile compounds in Heyang Fragrance.

	CAS	Compound	Odor Descriptions	Aicao	Jianjia	Qinqiang
1	100-41-4	Ethylbenzene	Gasoline	502.65		1741.23
2	100-42-5	Styrene	Sweet floral	203.41		1533.38
3	106-24-1	Geraniol	Rose, geranium		2904.00	2151.43
4	108-38-3	m-Xylene	Sweet	1.60		7.92
5	108-95-2	Phenol	Sweet, spicy	31.45	49.84	38.49
6	464-48-2	Camphor	Mothball, wood, mint	1.1		
7	499-75-2	Carvacrol	Thyme, spicy, oregano		4275.60	6676.60
8	620-02-0	5-Methyl-2-furaldehyde	Burnt		236.04	154.62
9	87-44-5	Caryophyllene	Spicy	184.04		266.69
10	97-53-0	Eugenol	Sweet spicy			2720.84
11	98-55-5	α-Terpineol	Floral, citrus, woody	251.40	49.98	

For Aicao, ethylbenzene, α-terpineol, styrene, and caryophyllene had high OAVs of 502.65, 251.40, 203.41, and 184.04, respectively, imparting aromas described as gasoline, sweet floral, spicy, citrus, and woody. In Jianjia, carvacrol and geraniol had high OAVs of 4275.60 and 2904, respectively, contributing to rose, geranium, thyme, spicy, and oregano scents. For Qinqiang, carvacrol, eugenol, geraniol, ethylbenzene, and styrene had exceptionally high OAVs of 6676.60, 2720.84, 2151.43, 1741.23, and 1533.38, respectively, imparting aromas described as thyme, spicy, oregano, sweet spicy, gasoline, sweet floral, rose, and geranium. It is evident that Qinqiang has a more diverse range of aromatic variations.

The threshold values are from *Compilations of Flavor Threshold Values in Water and Other Media* (second enlarged and revised edition) written by V. Gemert.

3.4. Antibacterial Activity Research of Heyang Fragrance Extracts

Table 4 shows the results of the inhibition diameters of Heyang Fragrance extracts against three types of bacteria. The results indicated significant variations in antibacterial activities among different types and concentration-dependent differences. Aicao exhibited antibacterial effects against Bacillus subtilis only at high concentrations, with an inhibition zone diameter of 11.33 ± 1.53 mm against Bacillus subtilis at a concentration of 100%. Jianjia showed strong antibacterial activities against all three bacterial strains, especially against Bacillus subtilis, achieving an inhibition zone diameter of 17.67 ± 0.58 mm at a concentration of 100%. Qinqiang demonstrated the broadest effective concentration range against Bacillus subtilis, with an inhibition zone diameter of 2.67 ± 1.15 mm even at a concentration of 12.5%. Its effective concentrations against Escherichia coli and Candida glabrata were 100% and 50%, respectively. Overall, the antibacterial activities of most samples decreased significantly with decreasing concentration. Compared with other samples, Jianjia exhibited superior antibacterial effects against Bacillus subtilis and Candida glabrata ($p < 0.01$), and Qinqiang showed a unique and relatively strong inhibitory capacity against Bacillus subtilis even at low concentrations ($p < 0.05$). The standard deviations of inhibition diameters at various concentrations were generally small, indicating that the inter-assay precision met the requirements of ISO 5725 [19].

Table 4. The inhibition diameter of the Heyang Fragrance extracts against three types of bacteria.

Sample	Bacteria	Sample Concentration	Diameter (mm)
Aicao	*Escherichia coli*	100%	6.00 ± 2.00 a
		50%	2.67 ± 0.58 d
		25%	0.00 ± 0.00 e
		12.5%	0.00 ± 0.00 e
	Candida glabrata	100%	2.33 ± 0.58 d
		50%	0.00 ± 0.00 e
		25%	0.00 ± 0.00 e
		12.5%	0.00 ± 0.00 e
	Bacillus subtilis	100%	11.33 ± 1.53 b
		50%	6.33 ± 1.15 c
		25%	5.67 ± 0.58 c
		12.5%	0.00 ± 0.00 e
Jianjia	*Escherichia coli*	100%	10.00 ± 1.00 cd
		50%	3.33 ± 1.53 f
		25%	0.00 ± 0.00 g
		12.5%	0.00 ± 0.00 g
	Candida glabrata	100%	10.67 ± 0.58 c
		50%	6.67 ± 0.58 e
		25%	0.67 ± 1.15 g
		12.5%	0.00 ± 0.00 g
	Bacillus subtilis	100%	17.67 ± 0.58 a
		50%	13.33 ± 0.58 b
		25%	8.33 ± 0.58 de
		12.5%	0.00 ± 0.00 g
Qinqiang	*Escherichia coli*	100%	11.33 ± 0.58 b
		50%	8.00 ± 1.00 c
		25%	1.67 ± 0.58 ef
		12.5%	0.00 ± 0.00 f
	Candida glabrata	100%	8.00 ± 1.00 c
		50%	2.67 ± 0.58 e
		25%	0.00 ± 0.00 f
		12.5%	0.00 ± 0.00 f
	Bacillus subtilis	100%	15.00 ± 1.00 a
		50%	11.67 ± 0.58 b
		25%	5.00 ± 1.00 d
		12.5%	2.67 ± 1.15 e

Note: The values in the table are the mean ± standard deviation. The same letter within each row indicates no significant difference ($p > 0.05$), while different letters indicate significant differences ($p < 0.05$).

4. Discussion

This study utilized electronic nose, GC-MS, and antibacterial tests to systematically characterize the volatile compound characteristics and antibacterial performance of three types of Heyang Fragrance. The findings provide significant insights into the aromatic properties and potential applications of these traditional fragrances.

The electronic nose analysis revealed notable differences in the volatile characteristics of the fragrances before and after ignition. Prior to ignition, Qinqiang exhibited significantly higher levels of sulfides, terpenes, and aromatic compounds than Aicao and Jianjia ($p < 0.05$), suggesting a potentially more intense aromatic experience in its unignited state. PCA and LDA analyses effectively distinguished these features, highlighting the importance of sensors W1W and W2W in detecting key aromatic compounds. After ignition, the increased sensor response indicated a significant rise in volatile compound content, with Aicao showing the highest concentration ($p < 0.01$). This suggests a more pronounced aromatic release post-ignition, with the presence of short-chain alkanes adding complexity to the fragrance profile. LDA was able to differentiate the aromas of Qinqiang and Jianjia whereas PCA could not, underscoring LDA's effectiveness in capturing subtle differences in aromatic characteristics post-ignition.

The GC-MS technique identified 71 volatile compounds across the three fragrances. There were significant differences in the types and concentrations of volatile substances among the fragrances. The presence of key aromatic compounds, such as terpenes and aromatic hydrocarbons, was consistent with the electronic nose analysis results. The changes in compound concentrations before and after ignition indicate that the thermal process significantly altered the chemical characteristics of the fragrances. This transformation may be the reason for the enhanced aromatic properties post-ignition. Aicao and Jianjia each possess 19 unique compounds, while Qinqiang has 9, emphasizing their individual contributions to the overall fragrance profile.

The presence of ethers and esters in spices depends on the availability of precursor substances and the plant's metabolic priorities. Some spices naturally accumulate other types of compounds, leading to lower levels of ethers and esters even when environmental conditions change or the material is processed [20,21]. Growth environment and genetic factors can limit the synthesis of these precursors, further reducing the potential for ether and ester formation [20].

The abundance of ethers and esters in some plants is linked to the specific regulation of secondary metabolism. This includes the activation of certain metabolic pathways at key developmental stages, high activity of enzymes involved in ether and ester synthesis, and strong expression of regulatory genes [21]. In spices with low ether and ester content, these regulatory mechanisms are less active or absent, resulting in lower production of these compounds [20,21].

The high odor activity values (OAVs) of certain terpenes, such as thujopsene and 8-epicedrol, indicate their significant impact on the aroma. Qinqiang, with the highest diversity of aromatic compounds, demonstrates a complex scent profile, influenced by compounds like carvacrol and eugenol. These findings suggest that Qinqiang may offer a richer and more varied aromatic experience than Aicao and Jianjia.

The study also reveals that certain compounds with high OAVs, such as ethylbenzene and α-terpineol in Aicao, contribute distinct scents described as gasoline, sweet floral, and woody. In Jianjia, carvacrol and geraniol impart rose and thyme notes. The diversity of aromatic compounds and their contributions to scent profiles highlight the potential applications of Heyang Fragrance in aromatherapy and perfumery. Future research could explore the therapeutic benefits and consumer preferences associated with these unique aromatic profiles.

We measured the antibacterial activity using the filter paper disk method. The antibacterial tests demonstrated significant concentration-dependent antibacterial activity in the extracts of the three Heyang Fragrance types. Qinqiang extract exhibited excellent antibacterial activity, particularly against *Escherichia coli* and *Candida glabrata* at full concentration, and remained effective against *Bacillus subtilis* at lower concentrations, indicating broad-spectrum potential. Jianjia was most effective against *Bacillus subtilis*, while Aicao showed significant activity against *Bacillus subtilis* at higher concentrations.

The differences in antibacterial effects among the fragrance types may be attributed to variations in their volatile compound profiles, consistent with GC-MS results. The strong antibacterial effect of Qinqiang could be due to its high content of thujopsene, camphor, and caryophyllene. Jianjia's effectiveness might stem from its carvacrol, guaiacol, geraniol, and phenol content. These high-activity compounds likely work synergistically to enhance antibacterial effects. Aicao, containing only terpineol and sylvestrene in low amounts, showed less-effective antibacterial properties.

These findings indicate that these fragrances not only serve as aromatics but also possess functional properties that could be utilized in antibacterial treatments. This underscores the importance of understanding the chemical composition of these ancient intangible cultural heritage fragrances in developing effective antibacterial solutions.

5. Conclusions

This study comprehensively analyzed the aromatic and antibacterial characteristics of Heyang Fragrance, an intangible cultural heritage with over 2000 years of history. Electronic nose and GC-MS revealed that combustion significantly enhanced volatile compound release, particularly sulfides and terpenes, with Aicao showing the highest post-ignition concentration. Qinqiang's aroma complexity, driven by high-OAV compounds like carvacrol and eugenol, underscores its potential in perfumery and aromatherapy. Antibacterial assays demonstrated functional diversity: Qinqiang showed broad-spectrum activity (inhibition zones of 11.33 mm against Escherichia coli and 15.00 mm against *Bacillus subtilis*), while Jianjia exhibited the strongest efficacy against *Bacillus subtilis* (17.67 mm inhibition zone at 100% concentration).

Notably, this study has limitations: antimicrobial assays were restricted to only three microorganisms (*Escherichia coli*, *Bacillus subtilis*, and *Candida glabrata*), with no evaluations of efficacy against other clinically relevant pathogens (e.g., *Staphylococcus aureus*, *Aspergillus* spp.). Additionally, despite characterizing antibacterial activity and aromatic components, no assessments of environmental risks (e.g., volatile organic compound emissions) or toxicological safety (e.g., long-term inhalation effects) were conducted, which are critical for therapeutic or daily use. The findings of this study provide chemical validation of historical medicinal claims with modern science, emphasizing Heyang Fragrance's medicinal and cultural significance. Future research should explore synergistic compound interactions, long-term safety, and conduct hedonic scaling with trained sensory panels to unlock its full potential in heritage preservation and therapeutic applications.

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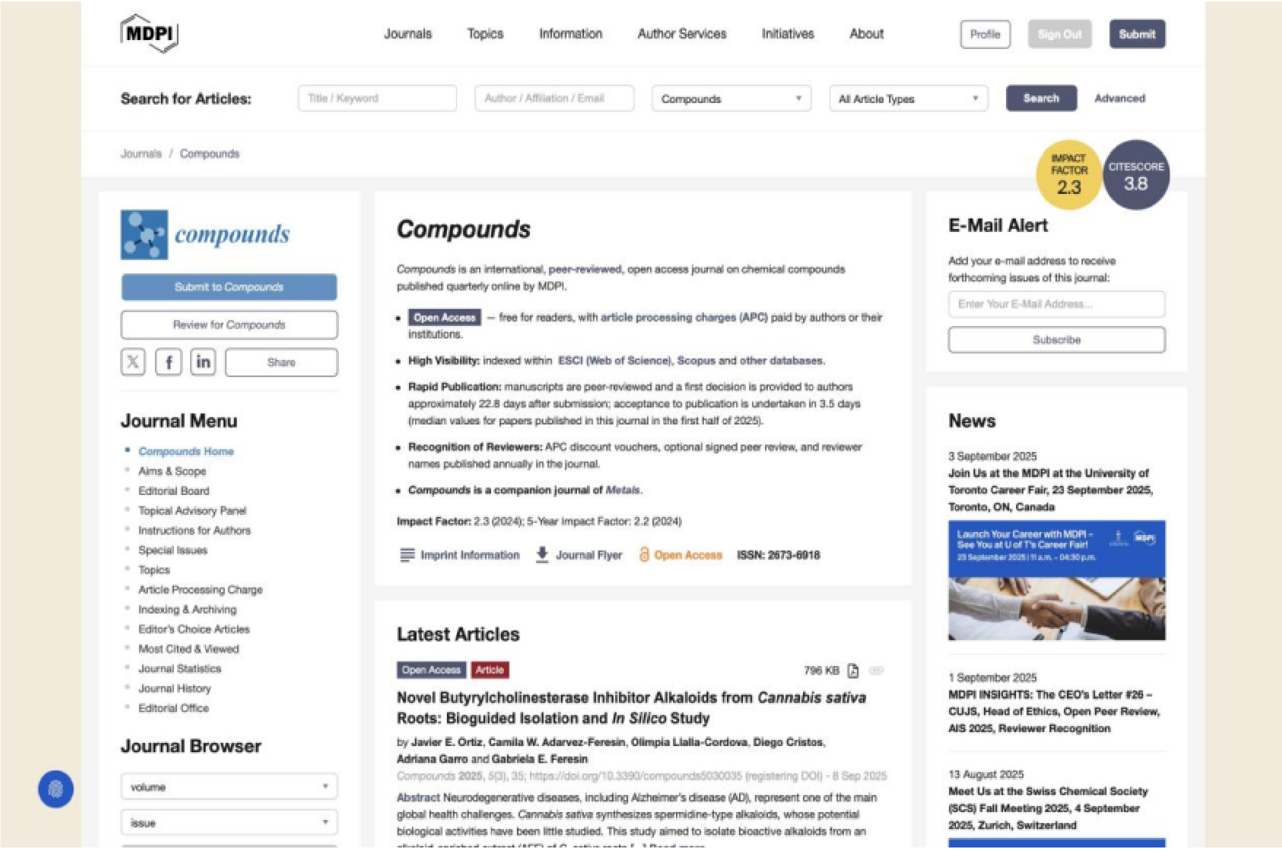

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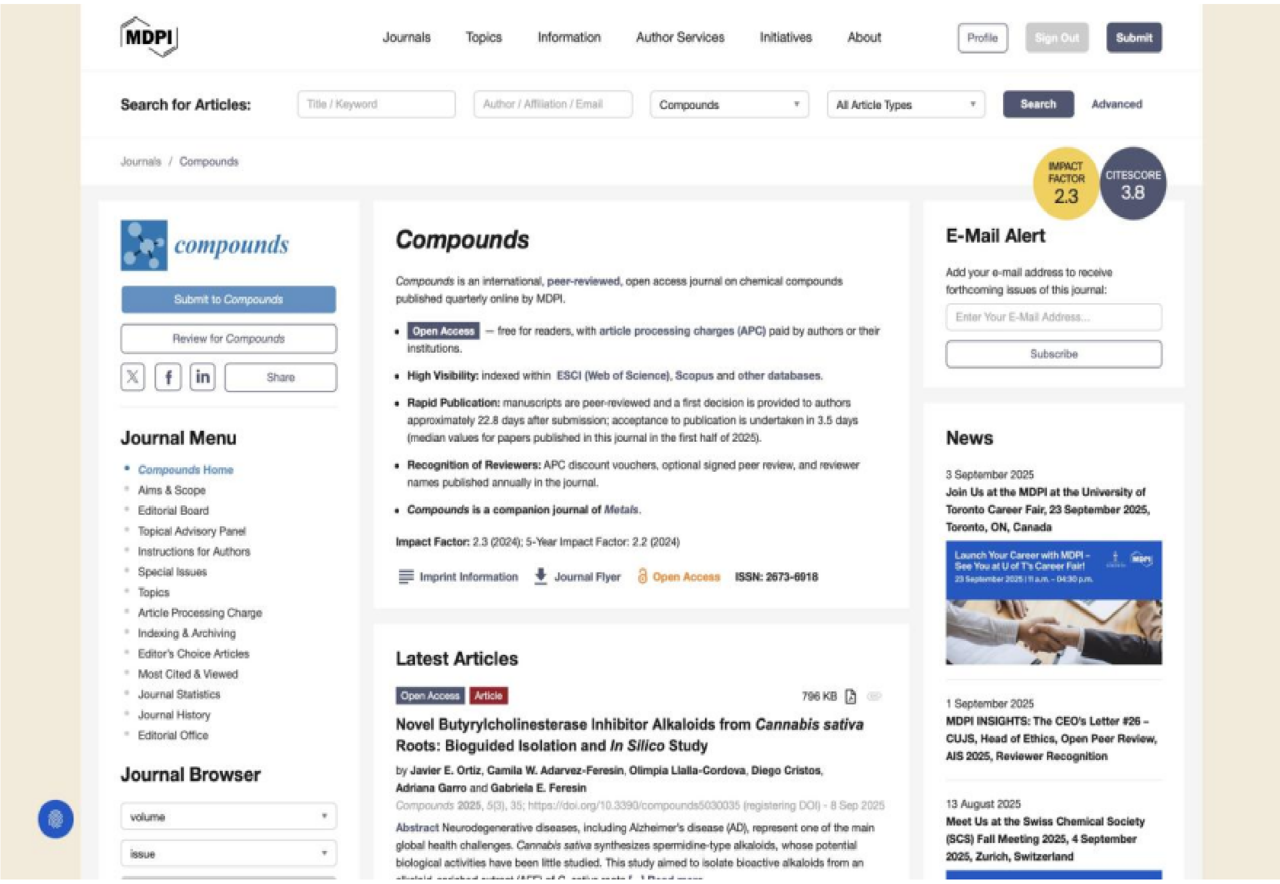

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