

ORIGINAL RESEARCH

Composition of Chinese Medicine in Hanhou Anshen Incense Based on Gas Chromatography-Mass Spectrometry

Chunlin Li, MMed; Zhen Sun, BS; Hui Liu, BM; Bowen Li, BM

ABSTRACT

Objective • Our aim was to analyze and study the medicinal components of Hanhou Anshen incense by headspace static injection gas chromatography-mass spectrometry (GC-MS).

Methods • After sample preparation, the results were analyzed by chromatography, and the drug components in Hanhou Anshen incense were analyzed according to the total ion flow pattern.

Results • A total of 11 drug components of Hanhou Anshen incense were analyzed.

Conclusion • This method is applicable to the analysis of the drug composition of Hanhou Anshen incense—which contains a wide variety of drug ingredients—that can provide a theoretical and material basis for the study of improving insomnia. (*Altern Ther Health Med*. 2023;29(2):186-190)

Chunlin Li, MMed, Associate Chief Physician; Department of Encephalopathy, Affiliated Hospital of Shandong University of Traditional Chinese Medicine, Jinan, Shandong, China. **Zhen Sun, BS**, Third Level Chief Clerk; Infectious Disease Supervision Section, Jinan Health and Health Supervision Institute, Jinan, China. **Hui Liu, BM**, Student; Department of Traditional Chinese Internal Medicine, First School of Clinical Medicine, Shandong University of Traditional Chinese Medicine, Jinan, China. **Bowen Li, BM**, Student, Department of Traditional Chinese Internal Medicine, College of Traditional Chinese Medicine, Shandong University of Traditional Chinese Medicine, Jinan, China.

Corresponding author: Li Chunlin, MSc
E-mail: 37673261@qq.com

INTRODUCTION

Insomnia has become one of society's most common public health problems. It is defined as the inability to fall asleep or maintain a normal state of sleep, and affects normal social function during the day, a subjective experience¹ manifested as difficulty falling asleep and insufficient sleep depth. Long-term persistent insomnia has different degrees of impact on the circulatory, digestive, immune and nervous systems, such as hypertension, irritable bowel syndrome, stroke and depression. Insomnia results in fatigue, inattention, poor mental condition and other symptoms, putting both physical and psychological pressure on patients.

Currently, to treat insomnia, Western medicine relies on benzodiazepines, melatonin receptor agonists, antihistamines

and tricyclic drugs. However, long-term use of drugs is prone to lead to addiction, drug resistance, dependence and other problems;^{2,3} for example, long-term use of dixi Pan drugs can easily result in addiction. If they are stopped suddenly, this can lead to emotional agitation, irritability, depression and other withdrawal symptoms,⁴ drug treatment brought about by adverse events cannot be properly dealt with.

In Traditional Chinese Medicine (TCM) insomnia is called “sleepless”; clinical treatment is dialectical according to the specific condition, and drug treatment has more significant effects. In the study of sleeplessness, many doctors have their own treatment preference. Zhu Weiju, known as “Zhu Zhizi,” uses Wen Qian, believing that people take Yang Qi as the weight. Yang decline is one point of the illness, positive qi is one part, evil qi is decreased by one point, often using the method of Warm Kidney Yang To help the right. Sleeplessness treatment should be the same; Shi Jinmo will not The sleep classification is 9: heart and kidney non-intercourse, blood is not honorable, hyper-yang, brain and kidney insufficiency, yang deficiency sleepless, yin deficiency sleepless, stomach sleepless, bile fever sleepless, liver suffering disease. Medication is divided into 3 categories: those that nourish the mind and sleep, result in pure heart and tranquility and result in heavy calm and tranquility. Huang Wendongzhi does not sleep much with plain medicine to take effect, and the evidence is divided into virtual and real, believing that it is mostly real fire and virtual fire, and often uses the method of “shocking people to be flat.” Fang Yizheng believes that yin and yang “must not be from its way” as the basis of the disease, qi, blood and liquid can block blood circulation without following the usual path,

blood stasis obstruction, heart loss and blood loss and cause sleeplessness, so it is mostly from stasis. Yang Yun Hanzhi is not sleepy, often moist and dry, clearing heat, and reconciling yin and yang. In addition, there are Shi Guanqing for liver treatment, Zhao Shaoqin Fang with a small chai hu soup from the liver and gallbladder treatment.⁵ In addition to medication, acupuncture, tuina massage, eight-dan brocade and ear acupuncture pressure, umbilical patch and other external treatment therapies all have a certain therapeutic effect on sleeplessness.

Rhinotherapy is one of the most distinctive external TCM treatments and includes nose drops, nose sprays, nose blowing, nose stuffing, etc, as well as aromatherapy. As early as the Shang and Zhou Dynasties, a large number of inhalation therapies was recorded. In the "Fifty-Two Sick Formulas," there is a record of using the aromatic *Artemisia annua* to cure "hemorrhoids:" take the weak 5 buckets to boil the 2 large numbers of *artemisia annua*, and the carp is like 7 in the hand and 6 inches of yegui. Dried ginger 2 fruits, 10 boiling. Lyrically placed in the middle of the altar, under the seat to smoke hemorrhoids. With the use of baizhi, clove, Xinyi, Peilan, acacia flower and other aromatic drugs being recorded in large quantities, the use of aromatherapy is more and more extensive. Some foreign herbs such as dragon brain, benzoin and Suhexiang have also been widely used. During the Song Dynasty incense reached the peak of The history of Chinese incense, and is mostly used for sleeping and calming the spirit or meditation that involves burning incense.

Aromatic drugs enter the body through the respiratory tract, act on the mouth and nose, pass through the skin and spread through the meridians of the whole body into the internal organs. They play a role in adjusting yin/yang balance, encouraging healthy qi, defending against external evils and other effects, for the purpose of treating diseases. The holistic view is that man himself is a whole, and the physiology and pathology between the organs are closely related and affect each other, are connected to each other; "The five qi have their own masters, only the aroma plays the spleen." The Suqin Jin Kuai Zhenyan says: "The central yellow enters the spleen, opens the mouth, and hides the essence in the spleen, so the disease is in the tongue." Its sweet taste, its soil-like, its cattle, its millet, its should be 4 hours, on the town star. It is to know the disease in the flesh also. Its sound palace, its number five, its smelly fragrance. "The heart and lungs within the nasal knots." Su Qing's *Five Visceral Theory* says: "The five qi enter the nose and are hidden in the heart and lungs." The god, the lord of the 5 internal organs, is located in the heart; the main qi of the lungs, the main body qi machine; the aroma takes the nose into the heart and lungs, smooths the qi machine, and calms the mind.

In addition, from the perspective of meridian theory, the Lingshu Hai Theory states that "...the twelve meridians belong to the internal organs and the outer networks are in the limbs." The meridians of the human body are all over the body. The nose is located on the head surface, and the head is the meeting of the yangs, of the 12 meridians, 7 through the nose, 6 into the brain, and there are meridians through the nose into the brain, such as the stomach meridian circulation,

which starts next to the nose, goes up to the root of the nose and then down along the outside of the nose into the gums, around the lips, down to the pulp, and then back along the jaw to the big ying acupoint, along the angle of the lower jaw to the cheek car point and up the ear anterior meridian through the upper point to the forehead. Yang Ming is known as the "Yang Ming Void, then the evil qi is easy to enter," and "the transformation of qi and blood, due to the water valley, and the sea of the water valley lies in Yang Ming. Yang Ming is also the origin of the pulse. Yang Ming is full of pulses, healthy spleen and stomach, and the gods are safe and wise." The pulse starts in the lower abdomen, descends out of the perineum, goes up along the spine, reaches the Wind House Acupoint and enters the brain, ascends to the top, and descends along the forehead to the column of the nose, which is also the meridian of entering the brain through the nose. Holographic biology believes that the nose is a highly developed holographic embryo, containing all the information of the human body, the nose is the exposed part of the brain and aromas can directly stimulate the brain to respond.

"Lingshu The Great Confusion Theory": Wei Qi must not enter the yin, often stay in the yang, stay in the yang is full of yang, yang is full of yin, and cannot enter the yin, so the eyes are not blind. Zhang Jiebin has in the "Jingyue Quanshu": "Although there are differences in illness without sleeping, only the word evil and righteousness is known, and it is all done." Gai sleep is yin, God and his Lord are also, God is peaceful and sleepy, and God is restless and does not sleep." The onset of sleepless illness is in the heart and is closely related to the liver, spleen and kidneys. The heart is the god, and the god is restless. In addition, if the diet is not moderate, the stomach and qi are out of harmony, which also leads to restlessness. The emotions are chaotic, the anger hurts the liver, the depression and fire disturbs the mind and can also lead to restlessness. The total disease mechanism of sleeplessness is mainly based on mental restlessness. Wei qi cannot enter the yin, and the restless mind is an important disease mechanism that causes insomnia. The treatment should adjust the yin and yang qi and blood, and the adjustment of qi and calm is an important treatment.

There are many TCM methods to tone the qi and calm the spirit, such as acupuncture, moxibustion, medicine pillows, etc. This article mainly concerns toning the qi and calming the spirit with aromatherapy as put forward in the Rear Han Dynasty. The *Shennong Materia Medica* (classic herbal text; one of the 3 pillars of TCM) says: "[In] Those who [use] incense, the qi is righteous, and righteous qi is strong; then the evil is removed and the impurities are also removed." China's use of incense has a long history. As early as the end of the Eastern Han Dynasty, there are records of Hua Tuo's use of incense to treat diseases. In addition to single scent incense, there are more and more applications of incense, such as Zheng Xuan's "Han Harem and Xiangfang." A large number of incense recipes specifically introduced its use, and there are numerous ancient works concerning incense medicine and the exploration of the best application methods, including musk "burning to ward off the epidemic." Agarwood and sandalwood

“burn smoke, ward off evil qi, cure plague sores”, lower true incense “bring it”; benzoin “burn” incense can promote yang qi, remove evil and dispel impurities, live blood and qi, open up the scriptures, not only can prevent diseases and maintain health, but also treat diseases. This theory has been confirmed by Western medical research: odor molecules can promote the production of immunoglobulins in the human body, while regulating systemic metabolism and balancing autonomic nerve function.

Incense therapy is a green, healthy effective way to avoid drug dependence and does not cause adverse events. On the basis of their predecessors and combined with modern technology, modern Chinese physicians have inherited and developed this safe and effective external treatment method, and the development of aromatherapy has gradually improved. The methods of administration are diversified and include homemade incense, improved essential oils with specific effects; traditional incense, which is still widely used; and wearing incense sachets and using medicine pillows. Aromatic drugs are made into certain dosages that transmit information to the cerebral cortex through olfactory stimulation, so that the cerebral cortex responds and provides disease treatment. Aromatherapy is economical, easy to use, quick-acting and eliminated from the body without gastrointestinal absorption, so there is no liver first-pass effect.

This incense is mainly composed of agarwood, sandalwood, benzoin, incense, tulips and so on. The medicinal use of Fangzhong agarwood was first published in the *Catalogue of Famous Doctors* and listed as a top product. Gong can relieve the liver and reach depression, lower the qi and calm the spirit, and communicate with the heart and kidneys. For example, the *Outline of the Medical Forest* says: “All the unsympathetic qi can be adjusted.” *Materia Medica Renewed* states: “Cure liver depression, lower liver qi, and spleen and stomach, eliminate moisture, and open up water.” Sandalwood is a medicine for the rational qi, and can adjust the qi of the spleen and stomach, such as in the *Materia Medica Truth-seeking*: “The smoke is refreshing and lovely, where the cold air is knotted, the diet is not entered, the qi is reversed and vomited, the depression is not comfortable, and the service can cause the stomach qi to rise.” The spleen and stomach are the hub of the lifting and descending of the qi machine, so it can be used to make “the qi up and down, the five internal organs are stable, and the spirit is the dwelling.” In the *Materia Medica*, it is recorded that: “Summer moon sac incense, can dispel the odor.” Benzoin opens the way to avoid impurities, proclaims qi and blood, and can especially calm the gods. “*Materia Medica Reads*” records: aromatic enlightenment. There is the merit of Wen Xuan Qi and Blood. Hard work to ward off evil spirits, excelling in the power of the heart and spleen. Or calm the gods and rest in the charm. Or fry and burn incense. The *Materia Medica Harms* also expounds that benzoin can calm the gods, because it “spicy incense and bitterness into the heart sutra, study and practice blood and qi, calm the spirit, and poison can be eliminated.” The heart is the lord of the god, the god is evil, the heart is the main blood, the blood stagnation is not fast, and the god of peace is also cured by blood. And its to

start with less yin sutra. The lord of the yin hides the gods, and the gods are evil and inviolable, and the fragrance is theosophical and the evils are also repelled. “Descending true incense can open the breath and harmonize the spleen and stomach.” Because it “burns the true descent, the interpretation of the name descends to the truth.” The one who covers the truth, the immortals change the heavens, lift the heavens and the earth, grasp the yin and yang, independently guard the gods, and destiny the true gods. Therefore the Lord is angry when he walks in heaven, and his house is strange, and he repels evil spirits.” In the *Introduction to Medicine*, it is said: And the incense burns, straight up to the heavens, summoning cranes to hover above. The taste is warm and non-toxic. The Lord’s qi is strange when he travels, and he burns to avoid the evil qi. *Materia Medica* is easy to read: “Burn it, break the sky and the weather, and the house is weird.” Incense attached to the liver and rational qi: *Materia Medica Justice* says: Xiangfu, spicy taste is very strong, the aroma is quite strong, all use qi to do things, so it is special to cure qi as a disease. However, although this substance contains a gentle flow effect, and the substance is firm, it can be kept although it is good at walking, not dry and not scattered, all of which are unique, so it can be used frequently without any drawbacks. It is not impossible to reach the fur, but it is absolutely different from the solution of wind medicine. It is not impossible to untie the knots, and it is not comparable to the upward work. Yujin can open the Yujing God. In the “*Introduction to Medicine*”, it is said: “Tulips are not very fragrant, but their breath is light, and they can reach the height of wine and séance.” Just like ambergris, it has no fragrance and can disperse the fragrance of the ears. The ancients used it to cure depression. “The combination of all medicines plays the effect of harmonizing qi and calming the gods.”

Good work does not abolish external treatment, and Wu Shiji of the Qing Dynasty proposed in the *Li Yi Pian Wen* that “the principle of external treatment is the principle of internal treatment, and the medicine of external treatment is also the medicine of internal treatment,” which is an important theoretical basis for the remarkable effect of Hanhou calming the gods. “Smelling incense can get rid of diseases”;⁶ before sleeping, the fragrance and active ingredients form an environment that makes it easy to fall asleep and relax the body, which has a good effect on improving insomnia.

Based on this, our study adopted headspace static injection gas chromatography-mass spectrometry analysis (GC-MS analysis) method to conduct in-depth research on the various pharmaceutical ingredients contained in Hanhou tranquilizing incense, determine the material basis for its tranquilizing and tempering effect, and lay out the basis for the study of its pharmacological relevance.

MATERIALS AND METHODS

Reagents and Instruments

Instruments: GC6890 Gas Chromatograph, MS5975 Mass Spectrometer, 7694E Headspace Injector, Chromatographic Data Processing System (MSDCHEMSTATION D.03.00.611) (Agilent, Santa Clara, California USA), Mass

Spectrometry Search Database: NISTMS search 2.0. Analyze Sample of Hanhou Anshen Incense.

Sample preparation

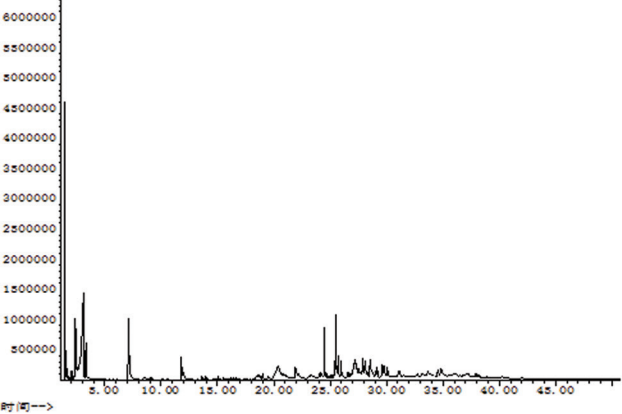
Take 0.5 g of freshly crushed Hanhou Ashen incense and place in a 10-mL headspace bottle, seal and place in the headspace injector at 160°C. Sample ring temperature 180°C; transmission line temperature 20°C. Heat vials for 30 minutes. Sample ring equilibration time is 0.05 min, and the sample is injected in 1 minute.

GC-MS analysis

Chromatographic conditions. Column Agilent HP-5MS (30 m × 250 μm, 0.25 μm); inlet temperature 250°C; helium is the carrier gas, column flow rate is 1.0 mL/min-1.

Heating conditions. 40°C initial temperature, hold for 5 minutes at 5°C/min-1 liter to 140°C, raise to 165°C at 4°C/min-1 and finally raise to 250°C at 3°C/min-1.

Figure 1. GC-MS total ion flow diagram of volatile components contained in Hanhou Anshen incense.



Abbreviations: GC-MS, chromatography-mass spectrometry.

Mass spectrometry conditional tuning method: Standard spectrogram tuning. Electron ionization mode, electron energy 70 eV; ion source temperature: 230°C; 4-stage rod temperature: 150°C.

Data acquisition scanning mode. Full scan; solvent delay time: 0.1 minutes.

RESULTS

Experimental

In the above conditions, the volatile components contained in Hanhou Anshen incense were analyzed by GC-MS; total ion flow diagram is shown in Figure 1.

The mass spectrometry of each peak was retrieved by a chemical workstation, and the chemicals with high degree of mass spectrometry matching were selected based on the National Institute of Standards and Technology (NIST) standard library criteria in order to determine the content of Hanhou Anshen incense and the structure of volatile chemical components. The results are shown in Table 1.

Analysis of the Composition of Hanhou Anshen Incense

In the GC-MS analysis of Hanhou Anshen incense, the characteristic peaks were shown according to the KI value, including B-cypressene and cedarene delta-juniperene, reflecting the main characteristics of the medicinal ingredients of Hanhou Anshen incense.

DISCUSSION

Establishment of GC-MS Analysis Atlas

The GC-MS analysis method used in this study is both sensitive and reliable, and the sample pre-processing process is simple and fast enough to meet the needs of clinical drug component analysis.

Table 1. GC-MS Analysis of Volatile Chemical Components in Hanhou Anshen Incense

Peak	Time	Molecular formula	CAS	Chinese name	English name	KI
1	2.068	C4H6O2	431-03-8	2,3-Butanedione	butane-2,3-dione	558
2	7.125	C5H4O2	498-60-2	3-Furfural	3-Furaldehyde	801
3	24.487	C15H24	3856-25-5	(-)-Alpha-Pinene	a-Copaene	1397
4	25.478	C15H24	469-61-4	alpha-Cypressene	(-)-Cedrene	1405
5	25.701	C15H24	546-28-1	beta-Cypressene	(+)-beta-CEDRENE	14236
		C15H24	11028-42-5	Cedarene	Cedrene	1415.1
6	25.968	C15H24	470-40-6	Rohan berry	(-)-thujopsene	1435
7	27.887	C15H24	31983-22-9	1,2,4a,5,6,8a-hexahydro-4,7-dimethyl-1-(1-methylethyl)naphthalene	1,2,4a,5,6,8a-Hexahydro-4,7-dimethyl-1-(1-methylethyl)naphthalene	1490
8	25.959	C15H24	470-40-6	Rohan berry	(-)-thujopsene	1435
9	28.078	C15H22	16982-00-6	Cypress on the side of the flower	cuparene	1687
10	28.549	C15H24	483-76-1	delta-Juniperene	(+)-delta-Cadinene	151411
11	30.073	C15H24O	1139-30-6	Caryophyllum	Caryophyllene oxide	1576

Abbreviations: GC-MS, chromatography-mass spectrometry; KI, inhibition constant.

Selection of Active Ingredients

In this study, the volatile components contained in Hanhou Anshen incense were analyzed by GC-MS, and 11 chemical components including B-Cypressene, Cedarene and Rohan berry were determined by degree of mass spectrum matching. According to the characteristic peak shown by the Ki value, the chemical constituents that reflect the characteristics of the medicinal ingredients of Hanhou Anshen incense were obtained, including B-cypressene and cedarene delta-juniperene. The medicinal effect of Hanhou tranquilizer incense is inseparable from its specific chemical composition: 2,3-butanedione, also known as diacetyl, is yellow to light green; because of its strong cream flavor, it is considered to be an important substance in dairy products, and is also an important flavor substance in cream, cheese and other non-dairy products that require milk flavor.⁷ It is widely present in essential oils such as lavender, cardamom and cloves, and can also be used as an intermediate in the synthesis of fine chemicals such as medicine and pesticides to prepare fragrance substances.⁸ 3-furfural is a flavor and fragrance component found in high levels in foods such as ice cream, milk, tea, jelly and green beans. An important organic chemical ingredient, present in flue gasses; (-)-alpha-pinene is one of the main components of turpentine, with a volatile and aromatic odor. It is an important raw material for synthetic fragrances, and is also used as a synthetic lubricant, plasticizer, etc. α -cypressene, β -cypressene is derived from cypress plants with cypress and sandalwood aromas. Longjing tea, a typical geographical indication tea products in China, is one of the historically famous teas, an important spice, and a 3-ring sesquiterpene. It is mainly used for synthetic spices⁹⁻¹⁰ and essential oils and is also widely used in the cosmetic and pharmaceutical fields. Cedarene, the main ingredient in cypress oil, is a commonly used spice ingredient, but is also present in tea and some wines. Roacherpene has a relaxing spirit, stabilizes mood, improves immunity, etc.; it is extracted from cypress essential oil rich in content, and the older the tree, the higher the content. Cypress essential oil is widely used in food and cosmetics.¹¹ 1,2,4a,5,6,8a-hexahydro-4,7-dimethyl-1-(1-methylethyl) naphthalene is found in tobacco leaves and mainstream flue gas.

Cypress can soothe nerves, reduce physical and mental stress, calm the mind, and effectively promote sleep; delta-juniperene is present in flue-cured tobacco leaves, burley tobacco leaves and spiced tobacco leaves; caryophyllum is an effective local anesthetic and can be used for anxiety, depression, pain sedation and nerve protection.^{9,12-13} Luohan cypressene, flower side cypressene, and caryophyllids are closely related to the effect of the smelling incense and sleep aid of Hanhou incense. The 11 drug ingredients selected in this study are closely related to the treatment of insomnia-based diseases, and the active ingredients contained in them are used as indicators of quality control, and the intrinsic quality of Hanhou anshen incense. A more intuitive evaluation was made.

Compared with previous studies, our study analyzed the volatile components in Hanhou Anshen incense via GC-MS, verified the material basis of its function by modern technical means and provided a scientific basis for its clinical application.

CONCLUSION

In summary, the GC-MS analysis method used by our institute is sensitive and reliable, which is suitable for the analysis of the drug composition of Hanhou Anshen incense; the determination of the pharmaceutical composition of Hanhou Anshen incense can meet the needs of clinical assessment of its effectiveness. Hanhou Anshen incense contains a wide variety of medicinal ingredients, which can provide an objective material basis for the study of improving insomnia with its use.

Study Limitations

This study had certain limitations. (1) The source and production process standards of the medicinal tablets for making incense are not clear, which may affect the quality of the fragrance, thereby affecting the determination of the active ingredient at this time. (2) The concentration of each active ingredient in different batches of incense may be unstable if it is not measured repeatedly.

In subsequent research, these limitations can be eliminated by standardizing the tablet source, formulating standardized incense-making processes, and measuring the medicinal components in the incense before the determination of the active ingredient of Hanhou Anshen incense. The concentration of the active ingredient in the incense can be further studied and it can be stabilized. The concentration of each active ingredient in the incense could be one of the criteria for controlling the quality and quantity of incense, providing a future for the next market application of Hanhou Anshen incense. The concentration of the active ingredient can be increased by adjusting the dosage of different Chinese medicines, thereby improving the incense. By adjusting the drug composition to control the rate of combustion, and further explore the relationship between the combustion rate and the release of active ingredients, we can improve the competitiveness of the application of Hanhou Anshen incense in the market.

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