Comparison between Different Sampling Methods on Gas Chromatography–Mass Spectrometry Analysis of *Pterocarpus santalinus*

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Abstract

Gas chromatography–mass spectrometry (GC-MS), along with other chromatography and spectroscopy techniques, has been used to provide convenient, rapid, and accurate information for identifying wood species. However, different sampling methods are capable of affecting the final results. Therefore, two different sampling methods, namely, direct injection of liquid wood extractives (DIWE) and head space of wood powder (HSWP), of GC-MS were compared in this work when typical Hongmu *Pterocarpus santalinus* was selected as the material. It was found that the number of chemical compounds produced by HSWP is less than the number produced by DIWE, and the boiling points of the HSWP compounds were also lower than the counterparts of DIWE because their retaining time in GC-MS spectrum is longer. It should be mentioned that the procedure of sample preparation for HSWP is simpler and more convenient than for DIWE, which gives HSWP an advantage over DIWE when many wood samples need to be identified by GC-MS. In addition, fingerprints of GC-MS spectra in both methods have been established as a histogram according to the main peaks in the spectra.

Most people like the smell of wood, which is one of the reasons that wood is the most common material for construction and furniture (Sjostrom 1993, Barnet and Jeronimidis 2003, Bowyer et al. 2003). Assorted wood extractives are the main sources of odor emissions from wood-based materials, especially for trees grown in the tropic zone (Hillis 1962, Jurd and Manners 1980). Furthermore, several treatments to wood can enhance the quantity of natural odor emission and produce other new volatile compounds by degradation of the original chemical composition (Bozalongo et al. 2007, Vichi et al. 2007). In order to clarify and analyze odor emitted from wood extractives, several techniques including gas chromatography–mass spectroscopy (GC-MS; Kaur et al. 2001, Balaban 2004), high-performance liquid chromatography (HPLC; Surowiec et al. 2004), and nuclear magnetic resonance (NMR; Fors et al. 2011) were used to elucidate and identify their chemical structure. GC-MS is considered to be a very useful technique to provide information about the possible chemical groups in materials, especially for the volatile compounds (Kaur et al. 2001, Balaban 2004). Because some dried traditional Chinese medicines have a group of unique volatile compounds, GC-MS has been applied to differentiate true medicine from imitations (Ruan and Li 2007).

Hongmu is the general name of a series of precious commercial wood products coming from five genera, including *Peterocarpus, Dalbergia, Diospyros, Millettia,* and *Cassia* (Standardization Administration of P.R. China 2009) and is famous because of its unique appearance, texture, and smell. Because these species of wood have

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Forest Prod. J. 65(5/6):226–231.
doi:10.13073/FPJ-D-14-00044

long been preferred for use in furniture or crafts, natural forests of Hongmu species have been sharply declining since the beginning of the Ming dynasty (AD 1368 to 1644). In order to protect the natural resource, most Hongmu species have been listed in the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITS; Jiao et al. 2013). So, first of all, we need to differentiate each type of wood from the others, determine their use in the timber trade, combat illegal logging, ensure wood certification, and instill forensic know-how. However, it is quite difficult to realize the target by observing their macroscopic or microscopic structures because there is little variation among them and only a few typical features can be helpful (Jiao et al. 2013). New techniques including HPLC (Shen et al. 2012), near infrared spectroscopy (Yang et al. 2012), and DNA bar-coding (Jiao et al. 2013) have been selected to give more convenient, rapid, and accurate information for wood identification. It is well known that some wood species have a specific smell because of volatile extractives. This indicates the possibility that GC-MS can be used to identify wood species based on chemical compounds emitted from wood (Zhou et al. 2006, Havelcová et al. 2013). We also first reported that GC-MS is successful in discriminating *Phoebe zhennan* from *Machilus pingii* (Xu et al. 2013). Nevertheless, because of the complexity of wood extractives, spectra of GC-MS are inconsistent among different preparation and sampling methods, such as injection of liquid, head space, solid-phase sorption, and so on (Balaban 2004, Flamini et al. 2007, Liu et al. 2012, Havelcová et al. 2013, Pellati et al. 2013). This complexity raises the question of which method is suitable for wood identification. Therefore, in this study we attempted to find a better sampling method between direct injection of solvent extraction and head space of wood powder. Furthermore, when the spectrum of GC-MS is too complicated to give quick identification or evaluation of specific chemical substances, we can extract the main information of the spectrum through a mathematical method and form a simpler spectrum than the former one. Therefore, a fingerprint based on the GC-MS spectrum emerges accordingly (Surowiec et al. 2004, Cardeal et al. 2008, Pongsuwan et al. 2008). *Pterocarpus santalinus,* which is a typical Hongmu wood species and mainly grown in the tropical forests of Asia, including India, Malaysia, and China, was chosen as the material in this work. When the mass spectral data and linear retention indices of both sampling methods were acquired, the corresponding fingerprints were constructed and compared with each other for selecting the better sampling method for wood identification by GC-MS.

Materials and Methods

Preparation of materials

A small wood block of *P. santalinus* was collected from the wood samples room (registered no. 006500) at the Anhui Agricultural University. The wood block was first sliced into thin sections by a microtome and then put into a frozen grinding mill (Beijing DHS Life Science and Technology Ltd.) under the following conditions: rotation rate of 1,700 rpm for 7 minutes with each sample weighing 1.0 g. Wood powder was put into a sealed polyethylene bag and refrigerated before use.

Sampling methods

Two different sampling methods, namely, static head-space of crude wood powder that was not extracted (marked HSWP) and direct injection of solvent extractives from wood powder (marked DIWE), were preset for GC-MS analysis. Direct injection liquid solvent extractives were prepared by 150 mL of benzene–ethanol (2:1) mixed solvent extracting 1.0 g of wood powder in a Soxhlet vial at 90°C for 6 hours and then condensed into 10 mL by rotary evaporators. Only 1.0 mL of condensed solvent was sucked into the sampling tube (Agilent Technologies, USA) and directly injected into the column for the GC-MS test. Meanwhile, 1.0 g of wood powder was laid into a 20-mL headspace sampling vial (Agilent Technologies) for GC-MS testing. In order to ensure the result is repeatable, more than four GC-MS tests with refreshed sample were executed for the two sampling methods, respectively.

GC-MS test procedure

All tests were performed on an Agilent 7890GC-5975C System (Agilent Technologies) following the temperature program shown in Table 1. HP-5MS (30 m length by 0.25 μm inside diameter by 0.25 μm film thickness) was selected as the separating chromatographic column, and the carrier gas was helium serving a speed of 1.0 mL/min. Sampling dosage, split ratio, injection temperature, and quadrupole temperature were set to 1 μL, 1:1, 250°C, and 150°C, respectively. The mass spectroscopy system was operated in selected ion monitoring mode. The mass scan range was 50 to 550 amu. The ionizer voltage of the MS detector was set at 70 eV. Agilent's Chem Station Software and database (American NIST05) were used to collect spectra and identify chemical compounds.

Results and Discussion

Comparison of GC-MS spectrum between two sampling methods

Total ion chromatogram spectra of both sampling methods of *P. santalinus* are presented at Figures 1 and 2, respectively. Apparent differences between the two spectra were found at the retaining time between 30 and 36 minutes. DIWE shows several peaks in the area, while HSWP displays an elevated baseline. This means that chemical compounds detected by GC from solvent extractives are more complicated than the volatized ones from crude wood powder. Liu et al. (2012) also reported similar results when the direct head space method was compared with the solid-phase microextraction head space method. It is suggested that extraction is very useful to increase both sensitivity and precision of GC-MS tests (Liu et al. 2012, Culleré et al.

Table 1.—Temperature program setting for gas chromatography–mass spectrometry test.[a]

Temp. (°C)	Temp. increasing rate (°C min^{-1})	Hold time (min)		Total time (min)	
		DIWE	HSWP	DIWE	HSWP
60		2	2	2	2
200	8	5	5	24.5	24.5
280	10	10	2	42.5	34.5

[a] DIWE = direct injection of liquid wood extractives method; HSWP = head space of wood powder method.

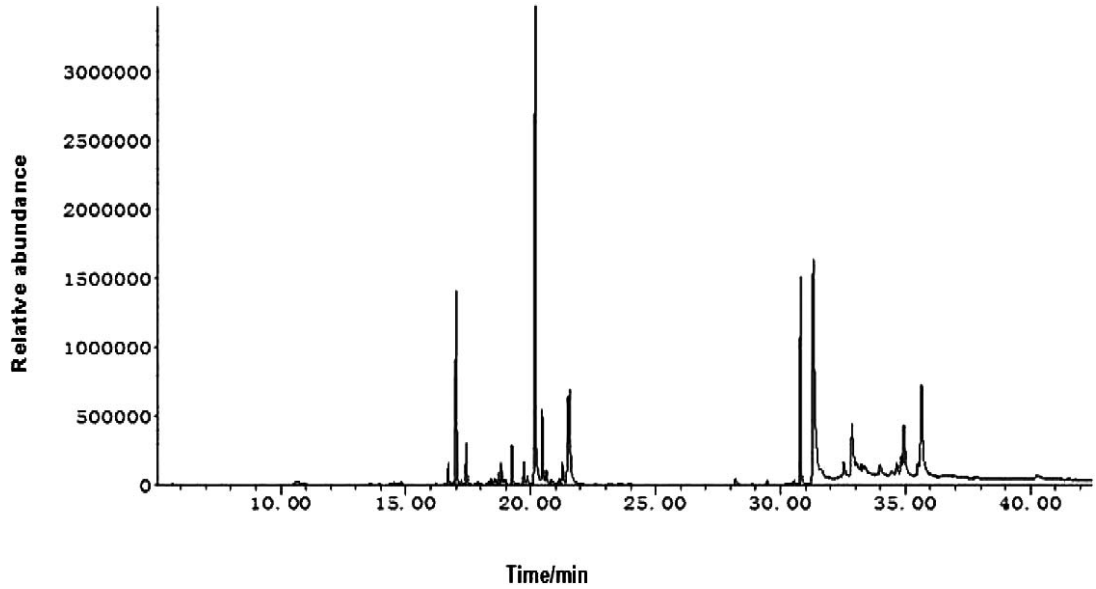

Figure 1.—Total ion chromatogram of Pterocarpus santalinus *(benzene–ethanol extraction sampling).*

2013). In order to further clarify and calculate peaks of GC-MS, 5975C Agilent's Chem Station software from GC-MS was used to calculate the relative peak area by integrating the relative abundance on certain retaining times. Only the peak whose relative area is more than 1 percent can be considered as a single peak vector. Otherwise, the peak cannot be included in the calculation and is omitted accordingly (Zhou et al. 2006, Ruan and Li 2007). Therefore, each of the peaks with its corresponding area is listed in Table 2 for solvent extractives and Table 3 for crude wood powder. It was found that for HSWP, eight peaks exist in the GC-MS spectrum of *P. santalinus,* and two typical peaks appear at 17.01 ± 0.19 and 17.44 ± 0.07 minutes, with corresponding relative areas of 45.73 ± 2.06 and 13.59 ± 1.32 percent, respectively. However, for DIWE, the number of peaks increased to 15 and the typical peaks moved to 20.17 ± 0.02 and 31.31 ± 0.10 minutes, with areas of 20.83 ± 2.02 and 20.45 ± 1.32 percent, respectively. Peaks at 17.01 ± 0.02 minutes and 17.43 ± 0.10 minutes also can be detected with the spectrum of the solvent extractives method, but the relative areas decreased sharply to 6.54 ± 0.12 and 1.64 ± 0.05 percent. It is suggested that several volatile components were released from the crude wood powder during the GC-MS test, and these compounds also can be found in the solvent extractives but with relatively low content compared with the total released compounds from extractives. Solvent extraction is able to release more chemical compounds because of the interactions between the organic reagent and

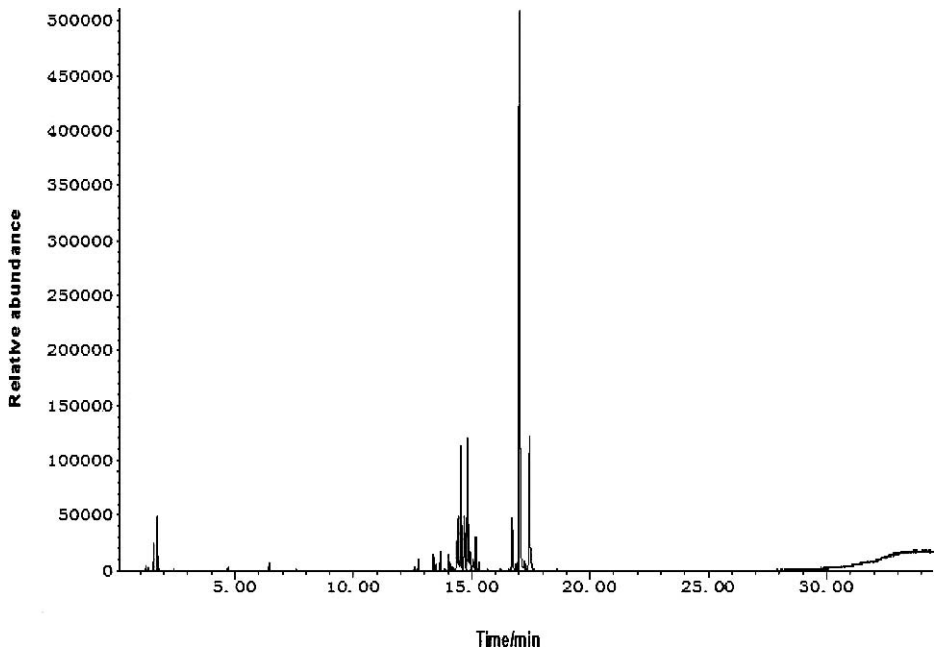

Figure 2.—Total ion chromatogram of Pterocarpus santalinus *(static headspace sampling).*

Table 2.—Relative peak areas of Pterocarpus santalinus *by direct injection of liquid wood extractives method.*

No. of peaks	Retention time (min)	Relative peak area (%)
1	17.01 ± 0.02	6.54 ± 0.12
2	17.43 ± 0.10	1.64 ± 0.05
3	19.24 ± 0.21	1.39 ± 0.06
4	20.17 ± 0.02	20.83 ± 2.02
5	20.46 ± 0.03	2.91 ± 0.12
6	21.27 ± 0.12	1.11 ± 0.06
7	21.52 ± 0.23	9.78 ± 0.15
8	30.80 ± 0.06	7.63 ± 0.12
9	31.31 ± 0.10	20.45 ± 1.32
10	32.52 ± 0.11	1.29 ± 0.03
11	32.86 ± 0.15	4.42 ± 0.32
12	33.03 ± 0.07	1.22 ± 0.08
13	34.82 ± 0.05	1.34 ± 0.09
14	34.94 ± 0.15	4.86 ± 0.14
15	35.64 ± 0.12	10.04 ± 0.82

Table 3.—Relative peak areas of Pterocarpus santalinus *by head space of wood powder method.*

No. of peaks	Retention time (min)	Relative peak area (%)
1	14.43 ± 0.01	5.38 ± 0.73
2	14.55 ± 0.04	8.84 ± 1.32
3	14.68 ± 0.10	4.43 ± 0.19
4	14.86 ± 0.23	11.68 ± 1.09
5	15.18 ± 0.12	2.45 ± 0.02
6	16.71 ± 0.05	4.85 ± 0.02
7	17.01 ± 0.19	45.73 ± 2.06
8	17.44 ± 0.07	13.59 ± 1.32

wood extractives (Jurd and Manners 1980, Ruan and Li 2007, Culleré et al. 2013).

Comparison of volatile chemical components between two sampling methods

Although the GC-MS testing procedure had been optimized before the implementation of this work, there were still several peaks merged together, which was attributed to the small differences in retention time of adjacent peaks. Therefore, for the sake of convenience, the adjacent peaks will only be accepted as two individual peaks when the difference between two adjacent retention times is more than 0.1 minute; otherwise, they should be treated as a whole peak. Mass spectroscopy is capable of estimating compounds in testing samples by comparing the m/z position in the spectrum with the related information in the database (Flamini et al. 2007). Main specific peaks of each sampling method were matched with the data in NIST2005 from the work station to present possible chemical compositions in the volatility of both sampling methods in this work (Stein et al. 2005). Results of retrieval are listed in Tables 4 and 5, and the detailed chemical structures of compounds detected in the GC-MS test are shown in Figure 3. Most of the chemicals from HSWP were accurately identified as terpenes with low boiling points, for example (+)-aromadendrene and l-β-bisabolene. They were also found by other published articles in which the HSWP method was used directly (Balaban 2004, Zhou et al. 2006, Culleré et al. 2013). However, DIWE presented more complicated and illogical retrieval results owing to its relatively low matching rate (Culleré et al. 2013). Nevertheless, it is still suggested that chemicals of relative higher boiling points were found by the DIWE method because of their longer retaining times and higher atomic mass compared with HSWP.

Construction of fingerprints of both sampling methods

During GC-MS analysis, a pattern of chemical components is formed by a set of retaining times and intensities of spectrometry peaks. Fingerprinting of GC-MS spectra is a method to classify chemical components based on their patterns and can provide a quick and convenient comparison among different samples. In order to obtain a more operable and accurate fingerprint of GC-MS spectra, there are several prerequisites (Surowiec et al. 2004, Ruan and Li 2007, Cardeal et al. 2008). First of all, the testing sample should have detailed information and precise

Table 4.—Chemical composition of Pterocarpus santalinus *tested by the direct injection of liquid wood extractives method.*

Retention time (min)	Compound	Chemical formula	Chemical structure[a]	Matching rate (%)	Relative peak area (%)
20.17	Espatulenol	$C_{15}H_{24}O$	a	41	20.83
21.52	Dimethyl 2,3-dicyano-2-butenedioate	$C_8H_6N_2O_4$	b	42	9.78
31.31	*trans*-4-Fluoro-4′-methoxychalcone	$C_{16}H_{13}FO_2$	c	72	20.45
35.64	1,1′-Biphenyl, 2,3,4,4′-tetramethoxy-5,6′-diformyl-	$C_{18}H_{18}O_6$	d	49	10.04

[a] Letters correspond to the lettered panels in Figure 3.

Table 5.—Chemical composition of Pterocarpus santalinus *tested by the head space of wood powder method.*

Retention time (min)	Compound	Chemical formula	Chemical structure[a]	Matching rate (%)	Relative peak area (%)
14.55	(+)-Aromadendrene	$C_{15}H_{24}$	e	99	8.84
14.82	l-β-Bisabolene	$C_{15}H_{24}$	f	96	11.68
17.01	H-Indene, 1-ethylideneoctahydro-7a-methyl-, (1Z,3a. α.,7a. β.)-	$C_{12}H_{20}$	g	95	45.73
17.44	Di-epi-α-cedrene	$C_{15}H_{24}$	h	47	13.59

[a] Letters correspond to the lettered panels in Figure 3.

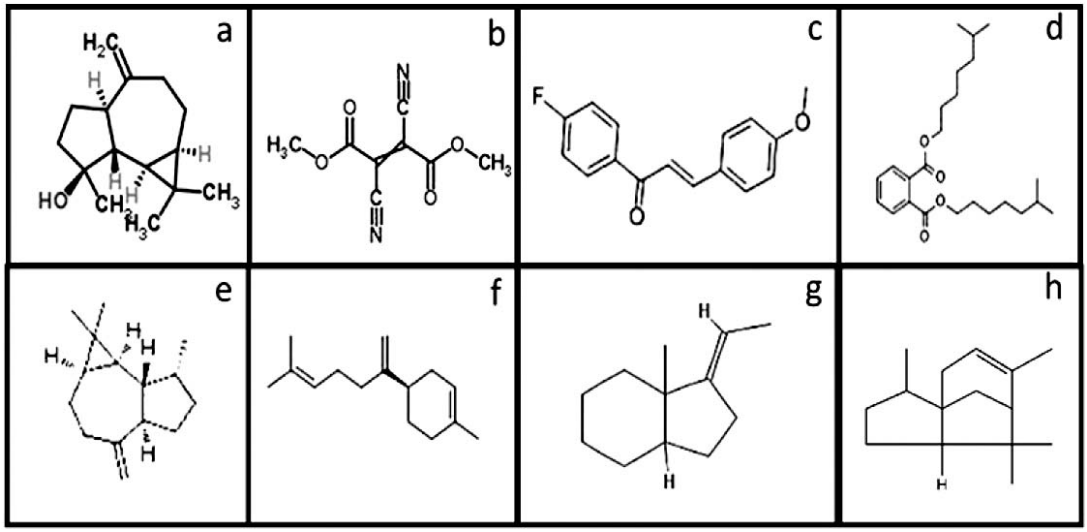

Figure 3.—Chemical structures of compounds detected in gas chromatography–mass spectrometry. See Tables 4 and 5 for details on these structures.

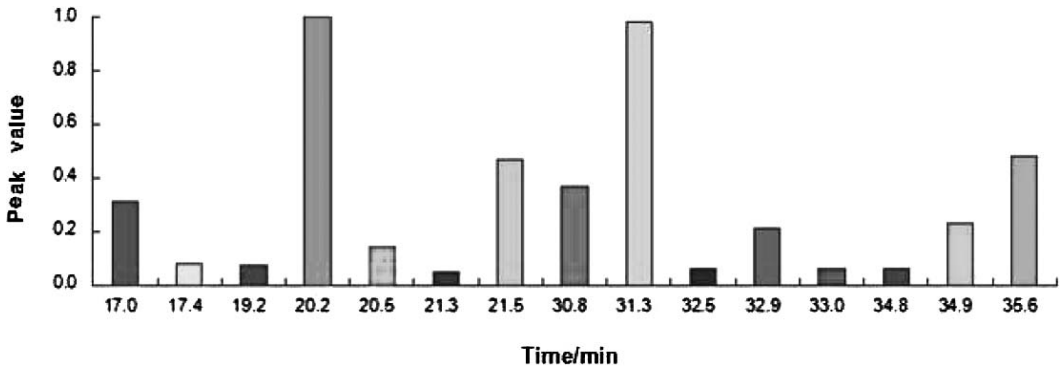

Figure 4.—Histogram of fingerprint based on gas chromatography–mass spectrometry spectrum by the direct injection of liquid wood extractives method.

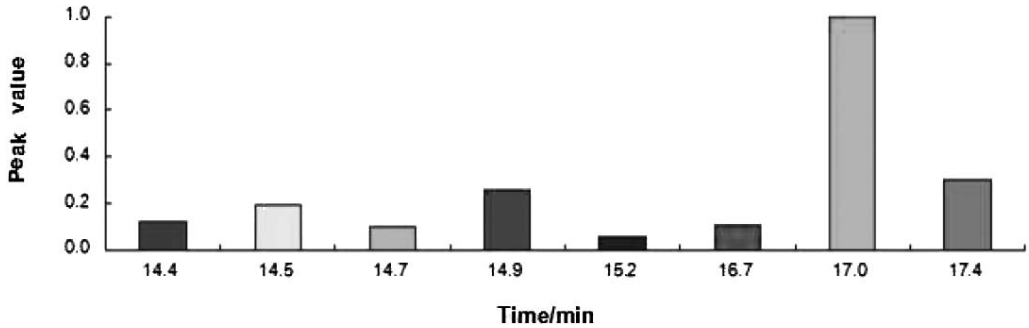

Figure 5.—Histogram of fingerprint based on gas chromatography–mass spectrometry spectrum by the head space of wood powder method.

records. Second, only the main peaks are included for establishing a fingerprint, especially the peak whose matching rate with chemical compounds in the database is relatively higher. Third, the relative peak area will be standardized again based on the highest peak of all suitable peaks. Therefore, the fingerprints of both sampling methods were established according to the procedure above. A histogram is selected to form a fingerprint where the average retention time is set as the horizontal axis and standardized peak area is set as the vertical axis. As shown in Figures 4 and 5, the DIWE method has a more complex fingerprint than the HSWP method because of its variable chemical composition.

Conclusions

Based on two different sampling methods of GC-MS, namely, DIWE and HSWP, two different total ion chromatograms were obtained. After integrating the peak

area at a certain retention time, large differences were found between the two methods including total peak number and position of typical peaks. The number of chemical compounds produced by HSWP was less than by DIWE, and the boiling points of HSWP compounds were also lower than those of DIWE because DIWE retention times in GC-MS spectra were longer. It should be mentioned that the procedure of sample preparation for HSWP is simpler and more convenient than for DIWE, which provides an advantage over DIWE when a large number of wood samples need to be identified by GC-MS. In addition, a fingerprint of GC-MS spectra in both methods was established as a histogram according to the major peaks in spectra. However, it should be noted that the fingerprints of GC-MS spectra tend to be affected by many factors, such as the sampling site, age of sample, different batches, and so on. So, a fingerprint presented in this work is just a preliminary trial; a large number of repeatable and systematic GC-MS experiments are still required.

Acknowledgments

This study was kindly supported by the Key Laboratory of Biology Center of Anhui Agricultural University in China under the ''Primary study on wood identification based on GC-MS injected directly and the fingerprint construction of Hongmu (NSFC, No. 31270599).'' Thanks are due to Professor Song-Ling Fu for reviewing the manuscript.

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