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Distinguish the Wild from Cultivated Agarwood by Using HPLC Combined with PCA

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ABSTRACT

Agarwood is the dark resinous and aromatic wood harvested from Aquilaria trees belong to Thymelaeaceae. It has great value as a raw material for perfume and traditional Chinese medicine. Agarwood is wildly accepted from natural forests and plantations. The former has more highly valued. This paper focused on distinguishing the wild agarwood from sustainably produced cultivated agarwood using high performance liquid chromatography (HPLC) combined with statistical analysis. 10 cultivated and 30 wild agarwood samples were used to respectively establish agarwood HPLC characteristic chromatograms by multivariate analysis. By the retention time and peak area analyses of two types of agarwood, characteristic chromatograms and characteristic peaks (i.e., 10 characteristic peaks of cultivated agarwood (CPC1-10) and 9 characteristic peaks of wild ones (CPW1-9)) were established. The principal component analysis (PCA) and sample validation further verified the accuracy and feasibility of the characteristic chromatograms and characteristic peaks. The difference of the HPLC characteristic chromatograms of cultivated and wild agarwood helps to differentiate these two types and provides a reliable method for ensuring wild agarwood used for high value products.

Keywords: HPLC, Agarwood, Characteristic chromatograms, Metabolite

INTRODUCTION

Agarwood is the dark resinous and aromatic wood harvested from the tree of Aquilaria species (Thymelaeaceae) [1]. In China, agarwood is a valued raw material used as perfume and traditional medicine [2-4]. In addition, agarwood also has special significance in local religious culture. The formation of agarwood is a lengthy process. It is wildly accepted that agarwood forms as a response of the tree wounding or injury (e.g. lighting striking, burning, fungus inoculation or microbial invasion [4]). Insect attack can also activate the immune system of the trees [5-9], which slowly results in the formation of oleoresin compounds to heal the wound [10-12]. The typical wild agarwood owes decades or even centuries of history. Due to strong market demand and slow resin formation process, agarwood resource has been in short supply. As the endangered species of wild flora [13,14], it is becoming increasingly rare. In 2004, all Aquilaria were listed on Appendix A of the Convention on the International Trade in Endangered Species (CITES) [15]. In order to satisfy the commercial demand and reduce the harvest of wild agarwood, cultivated agarwood has become increasingly common [16]. The formation time of cultivated agarwood is about several months, which is far less than wild agarwood. Hence, the identification of wild agarwood and cultivated agarwood is essential to protect the endangered wild agarwood. In addition, the availability of cultivated type can eliminate the great need from natural forests. It will provide a new economy growth point [17,18].

Agarwood contains over 70 unique sesquiterpene compounds [19] and more than 80 types of 2-(2-phenylethyl) chromone and its derivatives, more than 30 types of volatile aromatic compounds [5-9,20,21] and many others. Among all compounds, the oxidized 2-(2-phenylethyl) chromones is present only in *Imperata cylindrica*, *Bothriochloa ischaemum*, *Cucumis melo* L. *varreticulatus* in addition to agarwood [22-24]. Based on these compounds, some methods were reported to differentiate the qualities of agarwood products. Lancaster [21] and Espinoza [25] used

time-of-flight mass spectrometry to identify the agarwood based on oxidized 5,6,7,8-tetrahydro-2-(2-phenylethyl)-chromones and verify the commercial products. However, this method is limited to identify the same m/z diagnostic ions. Some researchers reported the gas chromatography-mass spectrometry (GC-MS) method to identify agarwood [26-28]. A GC-MS chromatographic fingerprint analysis combined with chemometric method had been reported to identity natural and cultivated agarwood [29]. GC-MS is an excellent method to identify the sesquiterpene compounds, while the chromatogram characteristic is barely satisfactory and it is constrained to identify the chromones. Fourier transform infrared (FTIR) has been proved to be a powerful technique for carbonyl groups identification [30]. Unfortunately, only a few samples conformed to the rules of good analytical practice. In addition, the quantities and diversity of samples quality are important facts to make sure the accuracy of the results of the study. Therefore, it is still a challenge to distinguish wild from cultivated agarwood using the above methods due to the limited availability of wild grown agarwood. Based on the specificity of 2-(2-phenylethyl) chromones and plant chemotaxonomy [31-33], HPLC is a powerful method to identify the wild and cultivated agarwood. Wild and cultivated agarwood have resin formation time differences, which results in differences in chemical compounds [34]. Similarity Evaluation System for Chromatographic Fingerprint of Tradition Chinese Medicine (SESCFTCM) was used to analyze the HPLC chromatograms. The software could automatically match peak positions, peak values and peak areas. And it was recommended by the State Food and Drug Administration (SFDA) of China to evaluate fingerprint chromatograms of traditional Chinese medicine. Therefore, HPLC with SESCFTCM software can distinguish the two types of agarwood by quantity and relative content of 2-(2-phenylethyl) chromones and its derivatives.

To distinguish wild and cultivated agarwood, we explored a HPLC method combined with statistical analysis (retention time and peak area), which differentiated the wild (n=30) and cultivated agarwood (n=10). The characteristic chromatograms and characteristic peaks were respectively established to identify these two types of agarwood. The PCA analysis and sample validation further verified the accuracy. This identification method is economical, rapid, robust and efficient. It will help to stop agarwood trafficking, preserve wild agarwood and develop cultivated agarwood trade.

EXPERIMENT AND METHOD

Materials

10 samples of cultivated agarwood were harvested from different provinces throughout P.R. China, including Guangdong, Taiwan. Partial optical images of the cultivated agarwood samples are shown in Figure 1a. Additional sample detail is provided in Table 1. The cultivated agarwood (C1-C10) samples were induced by different artificial methods, including holing, injection, nailing, cauterization, and artificial liquid transfusion technology. 30 samples of wild agarwood (W1-W30) were obtained from eight countries in Southeast Asia (i.e., Laos, Indonesia, China, Cambodia, Vietnam, Malaysia, Burma, and Brunei). Figure 1b shows the optical images of partial wild agarwood examples. The specific geographic information is in Table 2 cultivated and 5 wild validation samples are used to prove the accuracy of research results. 10 cultivated and 30 wild agarwood were all positively identified as *Aquilaria* sp. by the Research Institute of Wood Industry, Chinese Academy of Forestry.

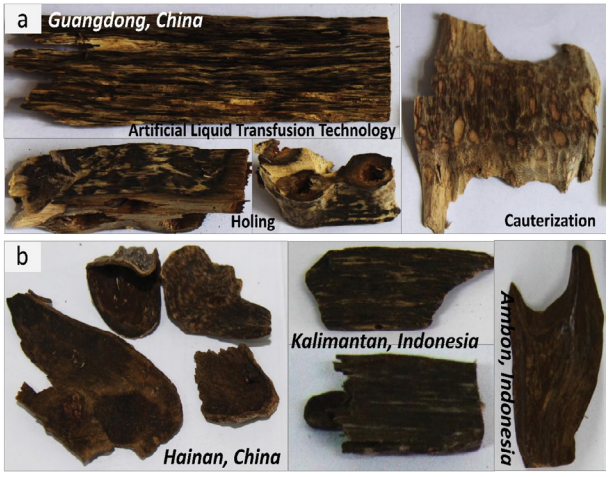

Figure 1: Cultivated and wild agarwood sample images. a) Cultivated agarwood samples and b) wild agarwood samples. There are obvious black resins in the samples

Table 1: Cultivated agarwood sample reference. C1-C10 was the cultivated agarwood samples. The reference included the resin formation method, resin formation time, geographic landscape and morphology description of 10 cultivated samples

No.	Resin formation method	Resin formation time	Geographic landscape	Morphology description
C1	Holing-chilling-nailing	10 months	Guangdong	Uneven woodchips, light aroma, black brown resin
C2	Artificial liquid transfusion	16 months	Guangdong	Smooth woodchips, light aroma, black brown resin
C3	Cauterization	10 months	Unknown	Smooth woodchips, light aroma, red brown resin
C4	Artificial liquid transfusion	15 months	Guangdong	Woodsticks, light aroma, light brown resin
C5	Holing	Unknown	Unknown	Woodbrick with hole, light aroma, black brown resin
C6	Cauterization and holing	12 months	Unknown	Smooth woodchips, light aroma, red brown resin
C7	Holing	Unknown	Guangdong	Woodbrick with hole, light aroma, black brown resin
C8	Injection	8 months	Tai Wan	Woodbrick, light aroma, light brown resin
C9	Nailing	10 months	Guangdong	Smooth woodchips, light aroma, black brown resin
C10	Injection	4 months	Guangdong	Woodbrick with groove, light aroma, black brown resin

Table 2: Wild agarwood sample reference. Wild agarwood samples were numbered W1-W10. The reference provided the geographic landscape detail of wild agarwood. 30 samples of wild agarwood were obtained from different regions in south-east Asia

No.	Geographic landscape	No.	Geographic landscape	No.	Geographic landscape
W1	Laos	W11	Hainan, China	W21	Papua, Indonesia
W2	Ambon, Indonesia	W12	Hainan, China	W22	Indonesia
W3	Huian, China	W13	Ma Chen, Kalimantan, Indonesia	W23	Vietnam
W4	Papua, Indonesia	W14	Ilian, Indonesia	W24	Cambodia
W5	Jayapura, Indonesia	W15	Ilian, Indonesia	W25	Vietnam
W6	Cambodia	W16	Hainan, China	W26	Hong Kong, China
W7	Vietnam	W17	Malaysia	W27	Hainan, China
W8	Hue, Vietnam	W18	Malaysia	W28	Papua, Indonesia
W9	Vietnam	W19	Burma	W29	Ambon, Indonesia
W10	Vietnam	W20	Malaysia	W30	Brunei

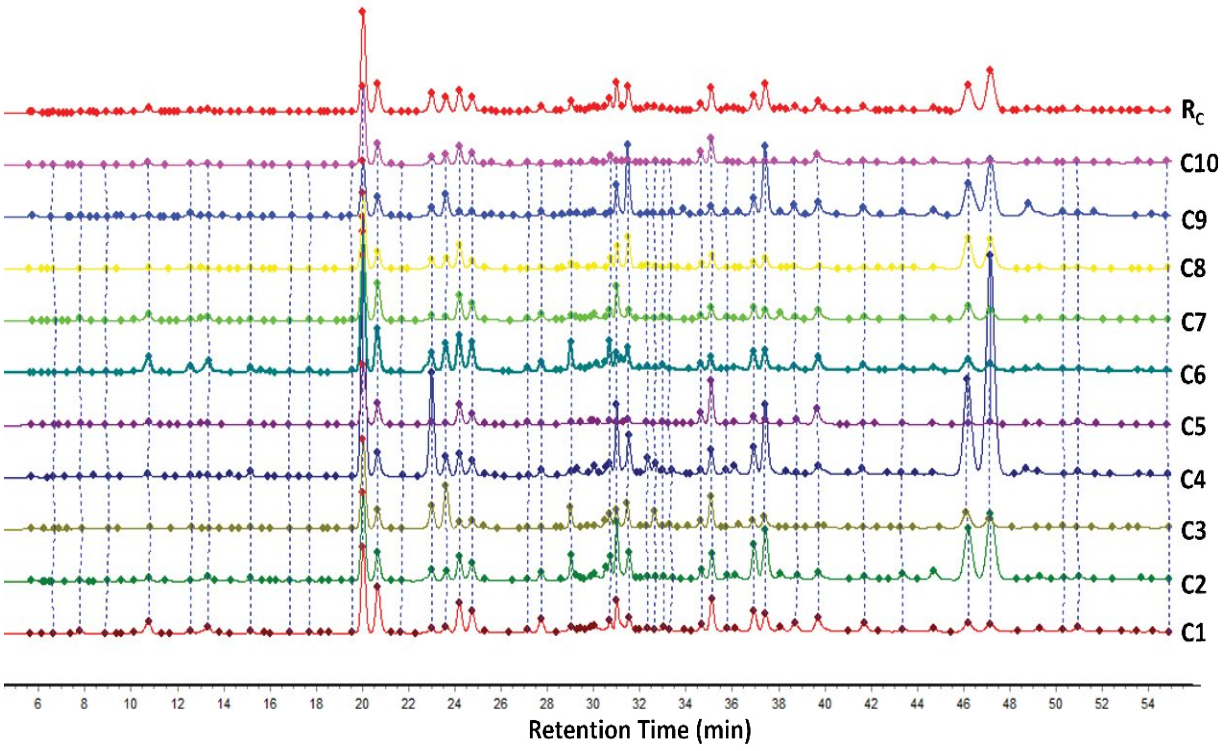

Figure 2: The HPLC chromatograms of 10 samples of cultivated agarwood C1-C10. The first red chromatogram (R_c) was the characteristic chromatogram

Table 3: The characteristic peaks area percent in each chromatogram of 10 cultivated and 30 wild agarwood. a) Characteristic peak area percent of cultivated agarwood samples (%); b) Characteristic peak area percent of wild agarwood samples (%)

	CPC1	CPC2	CPC3	CPC4	CPC5	CPC6	CPC7	CPC8	CPC9	CPC10
C1	20.02	5.81	2.22	2.96	5.33	3.27	6.29	1.19	0.89	2.21
C2	11.82	7.33	1.04	1.29	4.63	3.58	4.67	3.30	2.61	2.61
C3	9.29	3.4	1.65	1.30	2.95	2.45	2.80	6.29	9.86	13.25
C4	16.65	3.77	4.99	8.85	1.58	1.97	5.59	3.08	5.60	3.62
C5	14.78	1.87	6.54	1.38	1.53	1.23	1.59	5.13	10.14	23.58
C6	16.23	6.32	1.23	0.90	6.20	3.43	10.73	2.17	1.00	1.29
C7	31.45	8.84	3.17	3.81	4.65	5.28	1.93	3.30	2.60	1.75
C8	10.16	9.23	1.22	1.04	4.96	3.7	2.44	2.38	4.91	3.33
C9	16.96	4.32	2.60	2.77	6.04	2.12	2.87	3.13	12.37	12.04
C10	7.39	3.07	1.39	3.40	0.78	0.87	1.53	10.45	9.69	13.57
Average Value (%)	14.22	4.86	2.61	2.77	3.87	2.79	4.04	4.04	5.97	7.73
RSD (%)	25.5	36.2	66.7	81.8	48.9	44.8	67.6	62.9	67.4	91.9

	CPW1	CPW2	CPW3	CPW4	CPW5	CPW6	CPW7	CPW8	CPW9
W1	2.30	4.52	0.16	0.99	0.87	2.30	2.76	2.08	2.87
W2	3.16	1.49	0.92	0.65	0.63	0.57	2.66	2.39	3.49
W3	12.34	7.06	1.94	2.04	1.52	1.39	2.93	1.83	1.17
W4	1.24	0.95	0.39	0.57	0.57	0.81	3.48	3.39	3.70
W5	0.52	0.47	0.26	0.58	0.72	1.09	3.6	4.45	6.04
W6	4.40	1.61	1.90	1.66	0.43	0.44	3.76	1.54	2.39
W7	2.74	2.53	0.35	1.12	1.21	1.34	4.09	2.00	3.34
W8	3.52	5.17	0.62	1.67	1.09	2.46	2.88	2.68	1.60
W9	3.43	2.79	0.62	1.17	0.79	1.18	3.81	1.88	1.27
W10	2.02	2.08	0.28	0.82	0.89	0.73	4.14	2.52	1.69
W11	1.85	2.64	0.31	1.10	0.59	1.44	3.64	3.16	3.94
W12	3.97	2.15	0.39	1.45	1.45	1.32	2.76	2.96	1.70
W13	12.49	4.93	0.98	1.80	2.99	2.54	1.76	1.12	2.31
W14	8.18	4.37	0.57	1.82	2.17	2.54	2.50	1.33	2.04
W15	3.03	1.81	0.35	0.79	0.75	1.31	2.23	3.55	3.74
W16	2.33	2.95	0.34	1.15	0.93	1.93	1.73	5.36	1.17
W17	29.82	1.22	4.13	0.87	1.53	0.40	3.18	8.40	0.66
W18	27.52	1.66	2.97	1.05	3.15	0.74	6.86	4.15	1.93
W19	20.04	2.91	1.48	1.02	4.62	1.05	4.25	3.00	2.69
W20	21.89	2.95	2.03	1.04	5.27	1.23	0.80	2.63	5.43
W21	13.63	8.79	4.83	5.45	3.03	3.83	0.75	1.24	1.24
W22	8.91	7.14	1.58	1.87	2.42	4.01	1.99	3.38	0.79
W23	10.73	4.47	1.17	0.93	3.81	2.14	2.42	1.68	8.24
W24	34.57	4.59	9.43	5.55	6.69	2.27	4.44	0.52	1.01
W25	16.12	3.46	0.35	0.74	1.23	1.49	2.46	1.84	1.54
W26	9.73	6.62	3.87	4.59	3.56	4.22	6.00	5.07	4.29
W27	12.86	4.03	1.70	0.82	4.43	2.18	2.35	4.82	2.50
W28	19.33	10.43	1.98	2.64	1.61	1.62	1.09	2.32	1.32
W29	19.9	16.37	2.23	5.38	1.99	3.55	2.50	2.14	1.37
W30	29.08	3.87	2.98	4.64	2.88	2.02	0.56	3.84	0.76
Average Value (%)	11.39	4.20	1.70	1.87	2.13	1.80	2.95	2.91	2.54
RSD (%)	85.3	76.9	111.5	82.5	74.2	57.0	47.3	54.1	67.6

Instruments and chemistry reagent

A portable high-speed universal kibbler (Model: DFT-50A, Wenling City Forest Machinery Co., Ltd.) was used to mechanically reduce the agarwood to particles. A No. 2 mesh screen was used to filter the crushed agarwood powder to produce particles of ~850 μm ± 29 μm. The high performance liquid chromatography (LC-20A, SHIMADZU, Japan) was used samples identification. The ethanol extraction solution was prepared by the Chinese Pharmacopeia method [35]. 0.2 g of agarwood powder and 10 ml of 95% ethanol was put in a glass centrifuge tube. The ultrasonic extraction was performed for 60 min (frequency 20 kHz and power 250 W) and the ultrasonic temperature of water kept below 40℃. The supernatant was filtered through a 0.45 μm organic microporous membrane filter.

Apparatus, chromatographic condition and HPLC data analysis

Chromatographic separation was carried out on a Diamonsil C_{18} column (250 mm × 4.6 mm × 5 μm). The mobile phase A was acetonitrile and B was composed of 0.1% formic acid in water. The injection volume was set with 10 μL. Column temperature was 32℃ and the flow rate was 0.7 ml/min. Detection wavelength was 252 nm. The following gradient program: 0～10 min, linear gradient 15%-20% A; 10～19 min, linear gradient 20%-23% A; 19～21 min, linear gradient 23%-33% A; 21～39 min, isocratic 33% A; 39～40 min, linear gradient 33%-35% A; 40～50 min, isocratic 35% A; 50.1～60 min, isocratic 95% A.

Data and statistical analyses

All the data analyses were performed with SESCFTCM (version 2004 A, National Committee of Pharmacopoeia, China). The PCA analysis was obtained by Unscrambler X10.0 software. PCA scores plot always provides a visual determination of the same classes of samples. And the samples with unexpected features will be excluded from the model and diagnosed as a different one. The software performed PCA according to cross-validation.

RESULTS AND DISCUSSION

Characteristic chromatogram analysis of cultivated agarwood

The resulting chromatograms of 10 cultivated agarwood samples were fed into the SESCFTCM software and the data were analyzed. All the characteristic peaks were counted and analyzed and 41 common peaks were screened out based on the HPLC retention time. The relative standard deviation (RSD) of retention time ranges from 0.02-1.13% (all<3%). The results showed that these common characteristic peaks were present in all cultivated agarwood samples. Using the analysis software, C1 sample was set as the reference chromatogram. The characteristic chromatogram of cultivated agarwood was automatically matched by setting 0.1 of time window and median method. The characteristic chromatogram was used to evaluate the similarity of all the samples by calculation of the correlation coefficient from the original HPLC data. Figure 2 shows the characteristic chromatogram by one point correction (at 19.8 min) and automatically matching. There were 41 common peaks. The first red one (R_c) was the characteristic chromatogram. The similarity index was 0.876 (0.771-0.965) and RSD was 0.055% by contrast of standard characteristic chromatogram, in which only C6 was lower than 0.8. The index of similarity could reflect the overall similarity of each chemical component of different batch samples in their relative amounts. The analysis illustrated that the similarity was higher, which showed cultivated agarwood owned the more similar chemical compositions. The inducing methods used for cultivated agarwood formation affected little on the chemical components.

Figure 3a showed the 41 common peaks (NO.1-NO.41). Based on the analyses including the relative retention time, reference chromatogram peak area, RSD of relative retention time and peak area, we chose top 10 common peaks (RSD of retention time <0.05%) as the characteristic peaks of cultivated agarwood (CPC1-CPC10) (Figure 3b). In Figure 3a, the characteristic peaks in orange dashed boxes were chosen by the peak areas and the yellow dots were their corresponding relative retention times.

Characteristic chromatogram analysis of wild agarwood

The results of wild agarwood chromatogram were fed into the same analysis software with the cultivated agarwood. We chose 16 common peaks from all the peaks by using statistical methods and analyzed the 30 samples of wild agarwood HPLC retention time. The RSD of retention time ranged from 0.32-1.80% (all <3%). Using the analysis software, sample W1 was selected as the reference chromatogram. It was set as 0.1 time window and median method. Due to the greater variance of wild agarwood and excessively rigorous of software analysis, a multi-point correction was used to analyze for similarity. After 16 common peaks multipoint correction, the software provided a wild agarwood characteristic chromatogram. The index of similarity of wild agarwood ranged from 0.382-0.911 with an average value of 0.698 and RSD was 0.126%. Compared with the cultivated agarwood, different samples of wild agarwood had great disparities due to the similarity statistics (less than cultivated agarwood), which suggested that wild agarwood was more complex in chemical components than cultivated agarwood.

Figure 4 presented the chromatogram of wild agarwood and characteristic chromatograms (R_W) by multipoint correction and automatic matching. Based on analyses of the relative retention time, reference chromatogram peak area and their RSD, it was obviously different on peak areas and peak position between 35.0-55.0 min. In order to address this situation, we chose 9 common peaks as the characteristic peaks of wild agarwood (CPW1-CPW9) by comprehensive analyses of peak area, degree of peak separation and RSD (Figure 5a). 30 samples of wild agarwood

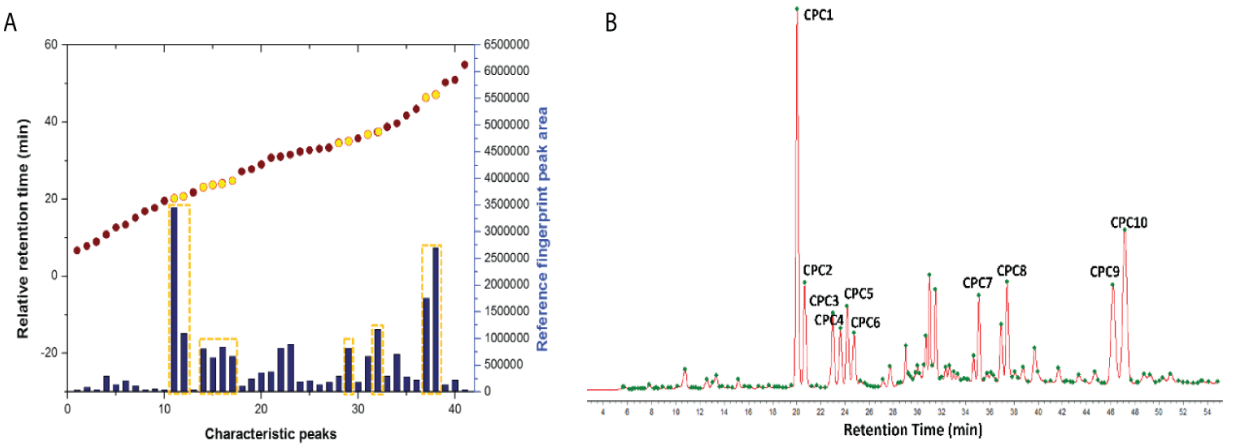

Figure 3: The data analyses of 41 common peaks of cultivated agarwood and characteristic chromatogram. (a) 10 obvious characteristic peaks (orange dashed frame) were chosen based on the peak area and RSD optimization data. The yellow dots were their corresponding relative retention time. (b) The cultivated agarwood characteristic chromatogram was established by analysis software of 10 cultivated samples

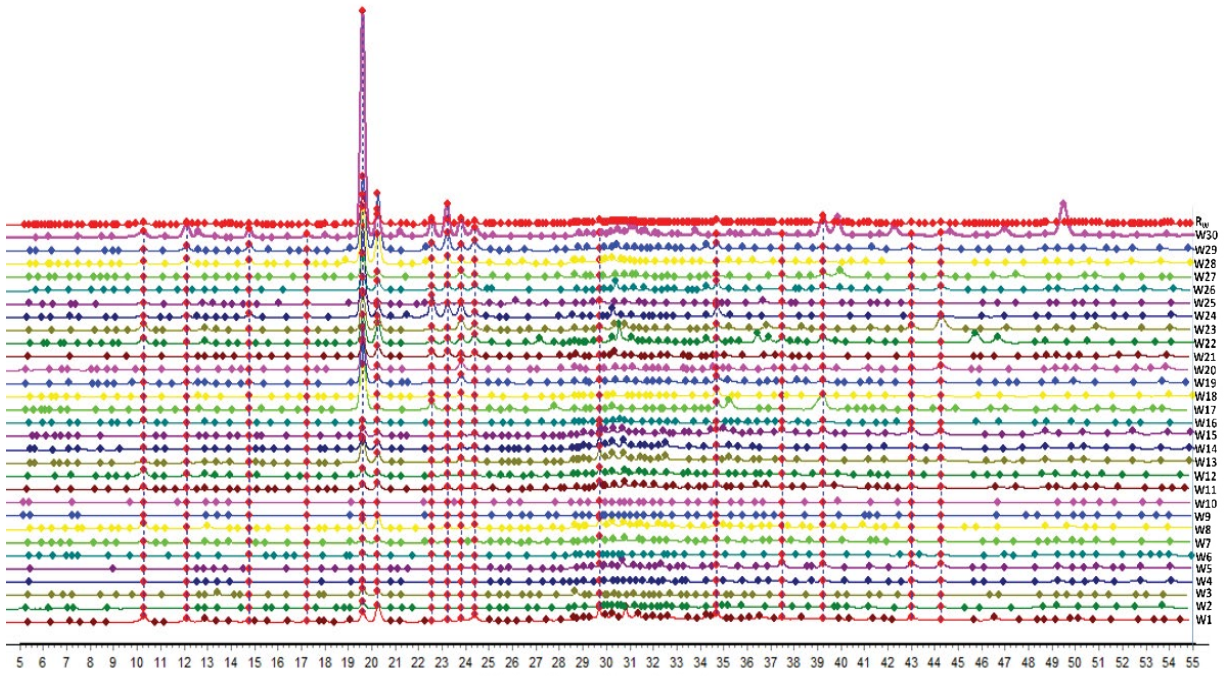

Figure 4: The HPLC chromatograms of W1-W30 and characteristic chromatogram (R_W) of 30 samples of wild agarwood. After multipoint correction, 16 common peaks were automatically matched. The first red chromatogram (R_w) was the characteristic chromatogram

HPLC chromatograms established the characteristic chromatogram (Figure 5b). In the 9 characteristic peaks, the CPW1-CPW7 (at 18.5-35.0 min) was the same with cultivated agarwood CPC1-CPC7. The reasons why presented these differences between cultivated and wild agarwood may be derived from the formation time difference. Cultivated agarwood is nearly formed in several months and wild agarwood requires several years or even decades to be produced, which results in the metabolic difference of chemical compositions [32].

Comparison of wild and cultivated agarwood

The analysis results indicated that the chromatograms of wild and cultivated agarwood had both of similarities and differences. The similarities consisted of a few identical chemistry compositions, e.g. CP1-CP7 (the abbreviation of characteristic peaks due to the same of CPC1-CPC7 and CPW1-CPW7). However, there were obvious differences between wild and cultivated agarwood, which could be used to differentiate these two types of agarwood. Based on the cultivated and wild agarwood characteristic chromatograms (Figures 3b and 5b), their characteristic peaks presented variance. Wild agarwood contained CPW8 and CPW9; however, the CPC8-CPC10 did not exist or not be obviously.

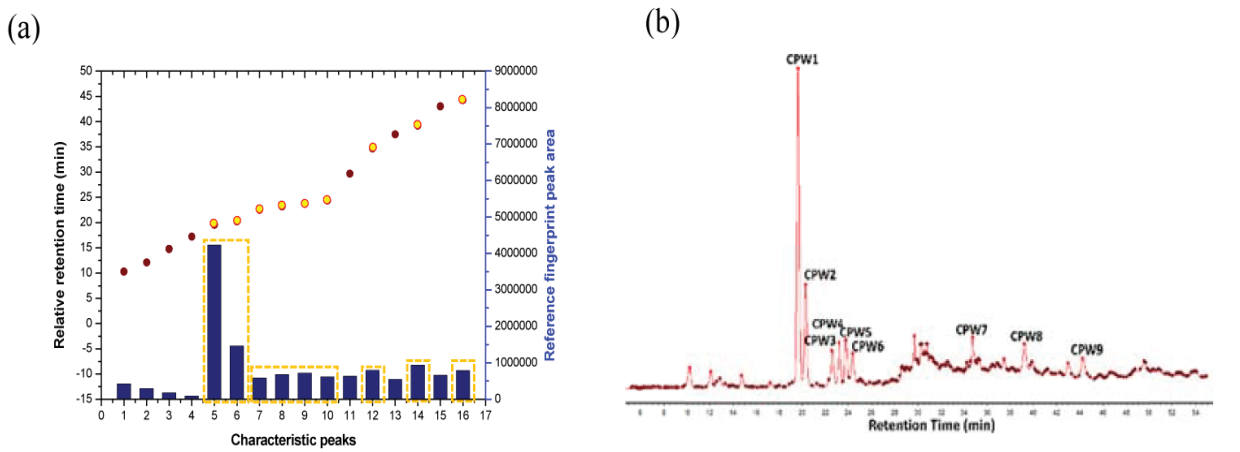

Figure 5: The data analyses of 16 characteristic peaks of wild agarwood and characteristic chromatogram. (a) 9 obvious common chromatographic peaks (orange dashed frame) were chosen based on the peak areas and RSD optimization data. The yellow dots were their corresponding relative retention time. (b) The wild agarwood characteristic chromatogram was established using analysis software of 30 wild samples

In order to further clarify the differences of wild and cultivated agarwood using HPLC chromatograms, we analyzed the characteristic peak area percentage, including 41 common peaks in cultivated agarwood and 16 in wild agarwood. The peak area percentage was calculated the ratio of peak area versus full peak area of whole chromatogram. The average percentage of 41 cultivated agarwood common peaks ranged from 0.1%-14.22%, RSD was 25.5%-91.9%. CPC1-CPC10 area percentages ranged from 2.61% to 14.22%, therein, the CPC1 and CPC10 were the top two peaks by comparison of area percentages. They were respective 14.22% and 7.73%. The total of CPC1-CPC10 peak area percentage was higher to 52.9% (Table 3a). However, the average percentage of 16 wild agarwood was 0.45%-11.39% and RSD was 47.3%-111.5%. The total of CPW1-CPW9 was only 31.5% (Table 3b). And they ranged from 1.80% to 11.39%. Except CPW1 exceptionally high (11.39%), other peaks was homogeneous.

Based on the results mentioned above, the characteristic peaks area percentages of wild and cultivated agarwood were compared: 1) Both CPC1-CPC2 and CPW1-CPW2 were the most obvious characteristic peaks in cultivated and wild agarwood, specially, the CP1 was the most important common characteristic peak, and it had the maximum peak area (more than 10%) for all samples. 2) CP1-CP7 were all contained in cultivated and wild agarwood. However, the total average percentage of these 7 peaks in cultivated agarwood was 35.52%, but was only 26.05% in wild agarwood. This indicated that the main chemical components during 18.5-35.0 min of wild agarwood were decreasing. 3) For cultivated agarwood, CPC8, CPC9 and CPC10 were existence in the HPLC chromatograms, and the area percentage was higher than most of the other peaks. Specially, CPC10 was the most peak area percentage except CPC1, but it was even not a characteristic peak in wild samples, which became a comparatively strong feature to separate wild samples from cultivated ones. Based on previous literatures [19,23,30], resin formation time might have a greater impact on the chemical compounds, which resulted in the large difference at 35.0-55.0 min. The characteristic peak difference could be considered as a potential standard for distinguishing agarwood types.

PCA analysis and sample validation

To further verify the accuracy of characteristic peaks chosen from cultivated and wild agarwood, the PCA analysis according to characteristic peak area was applied. In statistical analysis, PCA is performed to provide a visualization result of classification and differentiation. PCA scores plot can discriminate the different classes of samples. To obtain statistical information, the Unscrambler X10.0 software was used to perform PCA according to cross-validation. The characteristic peak areas of 10 cultivated (C1-C10), 30 wild agarwood (W1-W30), 2 cultivated validation samples (SVC1-SVC2) and 5 wild validation samples (SVW1-SVW5) were used to perform PCA. Figure 6 showed the PCA result of cultivated and wild agarwood. And the inset green dotted box was the magnification image. 47 samples were obviously separated into two types according to the different characteristic peak areas. The PC1/PC2 plot described 92.6% of the total variance (PC1~57.6% and PC2~35.0%). The C1-C10 and SVC1-SVC2 were clustered into the same group (in orange region) even they originated from different inducement methods. On the contrary, the W1-W30 and SCW1-SCW5 were clustered into one group. However, seen from the wild clustering region, the wild group was more scattered, especially, W30 deviated the group center. It was consistent with the lower similarity index of wild agarwood. But the validation samples (SVW1-SVW5) were totally in the wild group.

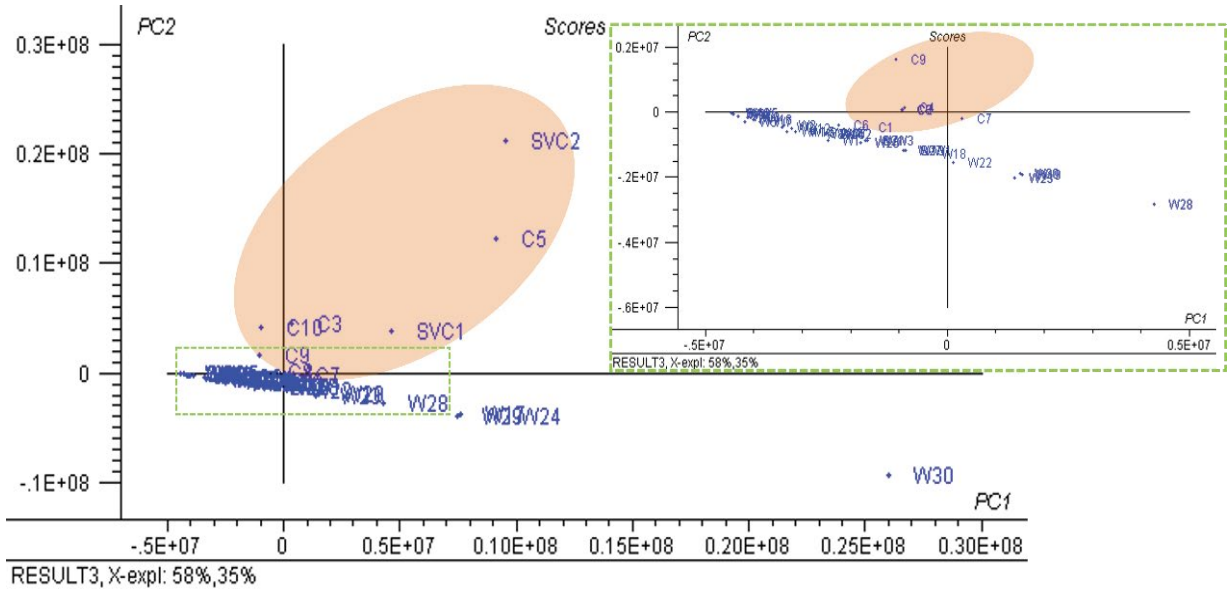

Figure 6: The PCA analysis of 10 cultivated (C1-C10), 30 wild agarwood (W1-W30), 2 cultivated validation samples (SVC1-SVC2) and 5 wild validation samples (SVW1-SVW5). The inset image with the green dotted box was the magnification image. The cultivated and wild agarwood were obviously clustered into two domains

CONCLUSION

The HPLC characteristic chromatograms of cultivated and wild agarwood were established using 10 cultivated and 30 wild agarwood samples. By comparing the retention time and peak areas of agarwood samples, we further established the 10 cultivated and 9 wild characteristic peaks to differentiate the two types of agarwood. In addition, statistical analyses of characteristic peak area and peak area percentage were provided to further demonstrate the metabolic changes between cultivated and wild agarwood. According to HPLC in conjunction with multivariate analysis, the chemical components during 35.0-55.0 min of chromatogram presented obvious differentiation between cultivated and wild agarwood, which showed that the wild group could be obviously identified from cultivated one. This result demonstrated that characteristic peaks could be used for differentiation of cultivated and wild agarwood. The PCA analysis and sample validation further verified the feasibility of characteristic chromatograms and characteristic peaks. The HPLC characteristic chromatograms and characteristic peaks help to differentiate the cultivated and wild agarwood and provide a new development of cultivated agarwood trade and protect wild agarwood resource.

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